Pharmacognostic, Phytochemical and Optimization Studies of Hydroalcoholic Extract of *Terminalia catappa* Linn. Dried Fruit

Saharan Anjali, Dureja Harish and Dhiman Anju*
Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak- 124401, INDIA
*anju.dhiman@mdurohtak.ac.in

Abstract
The dried fruits of Terminalia catappa are well known for their virtuous medicinal value. The various phytoconstituents found in this plant are reported to have phytochemicals such as carbohydrates, alkaloids, glycosides, saponins, flavonoids, tannins and phenolic compounds. The present research work was performed to evaluate the pharmacognostic studies such as phytochemical standardization of the whole dried fruits of Terminalia catappa Linn. Morphological evaluation, moisture content determination, extractive values, ash values such as sulphated ash value, water-soluble ash, acid insoluble ash, total ash were determined.

To ensure the safety of the constituents and plant, heavy metal analysis test was performed using atomic absorption spectrometry. Based on the above reports of extractive value, the hydroalcoholic solvent was quantified. The extraction was carried out using the Box Behnken design of optimization with 3 variables using the microwave-assisted method of extraction. Quantification of the optimized extracts was performed using high-performance liquid chromatography. Based on these findings, this species can be further explored in terms of pharmacological investigation and standardization for quality, purity and sample identification.

Keywords: *Terminalia catappa*, morphological, hydroalcoholic, microscopic, macroscopic, calcium oxalate crystals.

Introduction

Plant-derived substances are the crucial part of the current running industries due to their tremendous benefits over allopathic medicines such as toxicity, poisoning. This leads to sudden amplification of herbal drug manufacturers. Herbal species are the treasure of various nutraceuticals, food supplements, pharmaceutical intermediates and act as chemical precursors for synthetic drugs[11]. The prime use of plant and derived substances is to isolate their phytoconstituents which were either directly used as drugs such as morphine, vinblastine or to create some new bioactive compounds using phytoconstituent as a precursor e.g. glimperamide, nabilone, verapamil etc[8]. For the identification of plants, pharmacognostic studies are the most reliable techniques over modern techniques.

Terminalia catappa Linn. (Combretaceae) is also called a tropical almond which is found in warmer parts of India[5]. *T. catappa* L. is a combretaceaeous plant whose leaves and fruits are widely used as traditional medicine in various parts of the world for its therapeutic efficiency such as antibacterial, antidiabetic, antifungal and antioxidant[6]. The fruits of *T. catappa* developed on the basal part of the flower spike[14]. The colour of ripped fruit was reddish-brown. Fruit size varies in length from 2.5 to 10 cm. The fruit is a sessile, laterally compressed, ovate and smooth-skinned drupe[3].

Material and Methods

The proposed whole dried fruit of *T. catappa* L for the research study was collected from Sri Ramalinga Sowdambigai (SRS) College, Tamil Nadu, India. The collected whole dried fruit was taxonomically authenticated from the National Institute of Science Communication and Information Resources (NISCAIR), Delhi. The voucher specimen no. is NISCAIR/RHMD/Consult/2018/3224-5. The fruits were rinsed thoroughly in distilled water and dried at a low temperature. The processed whole dried fruits of *T. catappa* L. were further investigated.

Chemicals and Reagents: Analytical grade nitric acid and hydrochloric acid (Sigma and Merck grade) were used. The chemical purchased from E. Merck, Germany is of analytical grade. Various chemical compounds such as absolute alcohol, phloroglucinol, acetic acid, chloral hydrate, sulphuric acid, sodium hydroxide, nitric acid, ferric chloride, distilled water, concentrated hydroalcoholic acid and chloroform are used for the various evaluation profiles such as in phytochemical analysis[10].

Physicochemical characterization: Physicochemical estimation of extractive values in various solvents was carried out such as in methanol, acetone, ether, chloroform, aqueous, ethanol and hydroalcoholic. These extractive values were used to determine the polarity of compounds. Ash values are indicative of purity determined as per the standard procedure as total ash, acid insoluble ash and water-soluble ash[4].

Extraction of the whole dried fruit of *T. catappa* L.: The extraction of the drug was carried out using 3 variable conditions. The microwave-assisted method of extraction was used and resulting formulation combinations are evaluated and found to be with the extraction time ranges from 5 seconds to 30 seconds. The solute-solvent ratio varies

from 10 to 90 percentages and power varies from 200 Watt to 800 Watt. The microwave oven with specifications as (LG MC2149BB) having power rages from 800W, 640W, 480W, 320W and 160W was used to give maximum flexibility and control for extraction[2].

Optimization: The design of the experiment of Box Behnken design was applied on 3 variables and response was calculated in terms of percentage yield. It was reported that the power of 500 Watt at a temperature of 17.50 and solute-solvent ratio of 50 results in maximum yield percentage.

Methods of determination of ash value and moisture content:

Moisture content: The moisture content of the whole dried fruits of *T. catappa* L. was evaluated via drying method. The resultant moisture sontenet is the difference between initial volume and final volume.

Total ash value: Total ash value was determined using the 3gm of powdered drug sample which was incinerated gradually by the rise in temperature/heat. This step was repeated until free carbon was not formed. The resulting sample was allowed to cool and placed in a desiccator. Finally, the percentage of ash was determined.

Acid insoluble ash: It was determined from the total ash by boiling that for an interval of 5 minutes in 25 ml of dilute hydrochloric acid. The sample was filtered, ignited in a crucible and then placed in the desiccator. The weight of the final residues was determined.

Sulphated ash value: The sulphated ash test was used to determine the amount of residual substance which did not volatilize from a sample when the sample is ignited in the presence of sulphuric acid[12].

Determination of Heavy Metal: The nitric acid digestion method was used for the determination of heavy metals. In this method, 1 gram of dried fruit was packed in 250 ml digestion tube having 10 ml of nitric acid. The sample was heated for 15 minutes at 90 ºC to get completely dissolved. The solution is allowed to cool later. 5ml of perchloric acid was incorporated in it with a temperature rise up to 150 °C. The sample is allowed to boil at this temperature until a transparent solution was obtained. Further 5ml of nitric acid was successively added in 3 intervals each at regular time intervals.

This process was repeated until the volume of the sample was reduced to 1ml with a transparent appearance. Walls and tubes of the digestion equipment were thoroughly washed with distilled water during the digestion process to prevent the loss of the samples[9]. The net solution was filtered using Whatmann filter paper no. 42. After that, distilled water was used to bring the final volume up to 100ml, which was then put into the volumetric flask. The resultant filtrate was used for the detection of metals using an Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) metal analyzer.

HPTLC Fingerprinting for identification: HPTLC (Qaulity assessment) tool was used for the fingerprinting of phytoconstituents present in the given sample of extract.

Sample Preparation: 100mg of hydroalcoholic extract was dissolved in 1ml of ethanol and centrifuged for 5 minutes to prepare the sample. This solution was utilised as an HPTLC analysis test solution. From the prepared sample, 2 ml aliquot of the test solution and 2 ml of a reference solution of ellagic acid were utilised.

Developing Solvent System: The solvent system as Toluene: Chloroform: Ethyl Acetate: Formic Acid:: 2:6:6:2 gave the satisfactory outcomes of the result.

Sample Application: Samples were applied on percolated silica gel sheets with a band variation at 8mm in length and 2µg, 5 µg in concentration with the help of Linomat 5 applicator attached to CAMAG HPTLC system. The using HPTLC was installed with WIN CATS software.

Chromatogram Development: The chromatograms were developed in twin glass 20 x 10 cm saturated in solvent for 5 minutes.

Results and Discussion

Pharmacognostic studies were performed for the determination of various parameters.

Morphological Evaluation: Organoleptic evaluation of *Terminalia catappa* L. have been carried out according to the standard of the World Health Organization (WHO) standards of quality control methods of herbal medicines[13]. The different parameters such as colour, size, odour, shape and taste of the whole fruits of T. *catappa* were observed. The colour was found to be reddish-brown with some abrasions on their surface. The odour and taste were found to be characteristic.

Morphological characteristics: Fresh fruits of *T. catappa* L. fruits are shown in figure 1A and dried ripe fruit of *T. catappa* are shown in figure 1B.

Microscopic Evaluation:

Preparation of sections: The transverse section of the dried fruit of *T. catappa* L. represents the presence of parenchymal cells along with epidermis and collenchyma cells. The stone cells are also present in regular shape. The presence of prismatic calcium oxalate crystals with patted thickening of xylem vessels and parenchymal cells is shown in figure 2 and 3 respectively.

Physicochemical Analysis: WHO quality methods were used for the screening of various physicochemical parameters.

1 A

1 B

Figure 1: (1 A) Fresh fruits of *T. catappa* (1 B) Ripe fruits of *T. catappa* L.

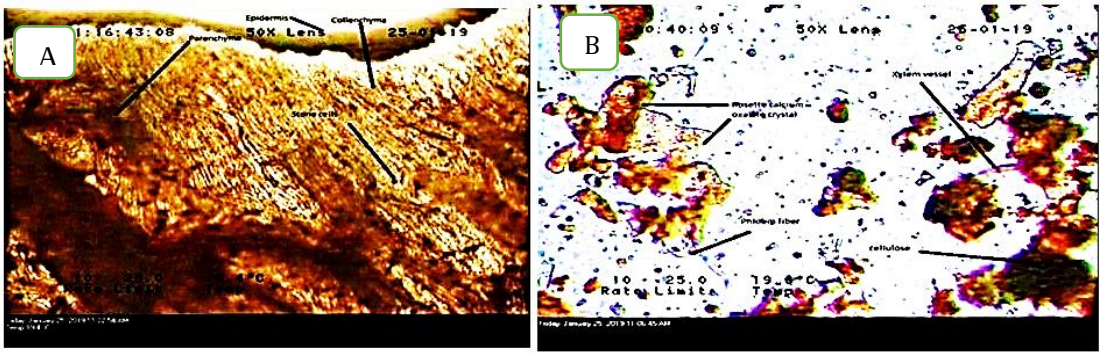


Figure 2: Transverse section (A) of *T. catappa* L. dried fruit and (B) powdered microscopy of *T. catappa* L. dried fruit

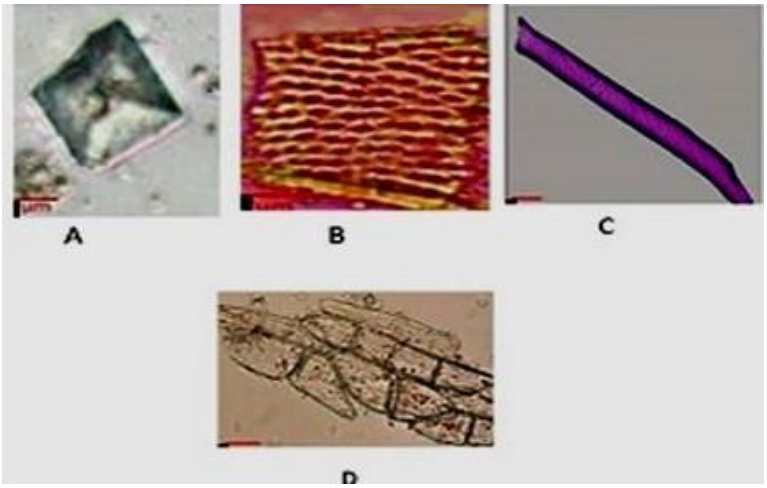


Figure 3: A- Calcium oxalate crystals. B and C- Xylem vessels with pitted thickening and D- Parenchymal cells

Table 1
Physicochemical parameters of *Terminalia catappa* L.

Physicochemical Parameters	Values (%w/w)
Moisture content (Loss on drying)	12.6
Total ash	11.766
Acid insoluble ash	8.92
Water soluble ash	2.853
Sulphated Ash Value	8.07

Physicochemical parameters: The moisture content of the dried fruits of *T. catappa* was reported to be 12.6 (w/w). Ash value is the prime most factor that was used to determine the presence of earthy matter along with impurities[7]. The ash values of the dried fruits of *T. catappa* L are mentioned in table 2. The total ash value was found to be the highest among all others. A decrease in ash value was observed due to the presence of less quantity of siliceous matter. The various physicochemical parameters are detailed in table 1.

Extracts preparation and preliminary phytochemical analysis: The extractive value of the (dried) fruits of *T. catappa* L. was carried out using various solvents in increasing orders of their polarity. The extractive values are prime indicators for the determination of exhausted or adulterated drugs. The extractive values of the crude powder of *Terminalia catappa* L. dried fruit in different solvent systems are mentioned in table 2.

The above mentioned value of extractive value in different solvents reveals that the extraction of phytoconstituents was found to be maximum in hydroalcoholic solvents used for further investigation. The preliminary phytochemical screening of the *T. catappa* L. fruit hydroalcoholic extract was performed using various identification tests for the confirmation of different phytoconstituents as mentioned in table 3.

Heavy Metal Analysis: For ecological and nutritional reasons, heavy metal toxicity is a major source of concern. It is critical to determine the concentration of minerals and heavy metals in herbal treatments to ensure their biosafety[1]. For the determination of heavy metals in the drug, nitric acid digestion method was used. The maximum permissible limits of all the carcinogenic metals are listed in table 4.

Table 2
Extractive values of the dried fruits of *Terminalia catappa* L. in different solvents

Extractive values	Values (% w/w)
Methanolic extract	0.4
Ethanolic extract	0.8
Acetone	0.10
Chloroform	0.07
Ether	0.02
Hydro alcoholic	0.82
Aqueous	0.63

Table 3
Preliminary phytochemical screening of *T. catappa* L. hydroalcoholic extract

Phytoconstituents	Method	Results
Flavonoids	Lead acetate test	Negative
Alkaloids	Mayer's test	Positive
Carbohydrates	Fehling test	Negative
Reducing Sugars	Benedict test	Positive
Monosaccharaides	Barfoed's test	Positive
Hexose sugars	Salwinoff's test	Positive
Iodine solution test	Iodine test	Positive
For steroid	Salowski test	Positive
For cardiac glycosides	Legal's test	Positive
	Keller kilani test	Positive
Proteins	Biuret	Negative
	Milon's test	Negative
Amino acids	Cysteine test	Positive
Phenolic and tannins	Ferric chloride test	Positive
	Dilute iodine test	Positive

Table 4
Permissible limits of Heavy metal present in hydroalcoholic fruit extract of *T. Catappa* L.

Heavy Metals	Concentration (ppm)	Permissible limits in (ppm-parts per million)
Cadmium	not detected	0.20-0.30
Mercury	0.273	0.20-1.0
Lead	0.347	0.20-10.0
Arsenic	Not detected	0.20-3.0

The presence of these heavy metals will lead to impairment in the functioning of the central nervous system, liver, lungs, heart, kidney, brain etc. which results in hypertension, abdominal pain, skin eruptions, intestinal ulcers etc. Heavy metals such as mercury, lead, arsenic and cadmium are toxic and have shown mutagenic effects even in very low concentrations. The presence of lead will lead to abdominal pain, vomiting, severe anaemia and hemoglobinuria. The heavy metal content of *T. catappa* L. dried fruits was found to be within the permissible limits and reported to be safe.

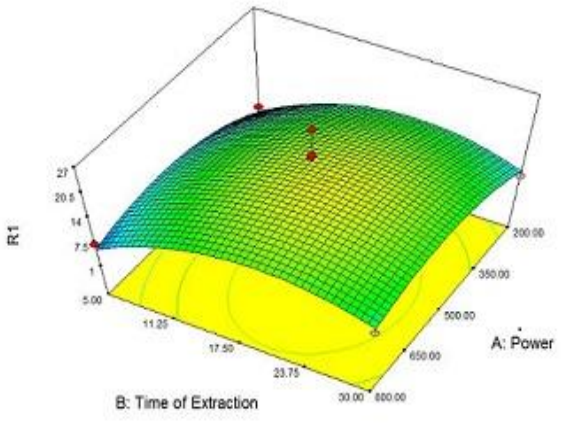
Extraction of dried fruits of T. *catappa* L.: The extraction of the dried fruits of *T. catappa* L. was carried out with hydroalcoholic solvent using the microwave-assisted

method of extraction. The influence of yield was monitored using 3 variables and outcomes are reported in the form of percentage yield. The design of full factorial designs of box Behnken was used to calculate the resulting yields. Possible variables with their outcomes in the form of percentage yield of the design of experiments of *T. catappa* L. fruit extracted in hydroalcoholic solvent are summarized in table 5.

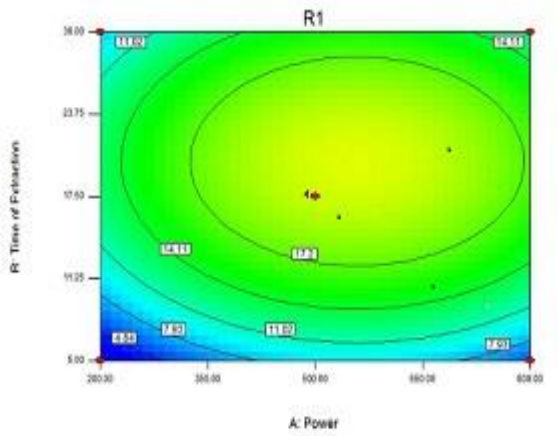
Contour plots of the resulting optimized hydroalcholic extract of dried fruits of *T. catappa* L. are discussed in figure 4.

In these contour plots, effect of different variables such as solvent, power and extraction time has been reported. The Box Behnken Design was implemented to optimize the extract obtained using microwave-assisted extraction of the dried fruits of *T. catappa.*

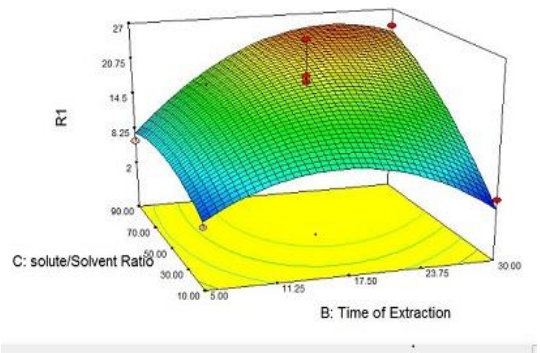
In this method, 3 different varibales wer used such as ethanol concentration, microwave irradiation power and extraction time. These controlled variables were further studied for possible combinations of these variables using the design of expert in table 5.



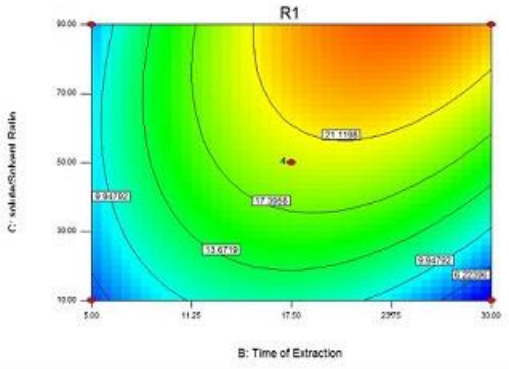
4(a) - 3D contour plot of interaction between the time of extraction and power



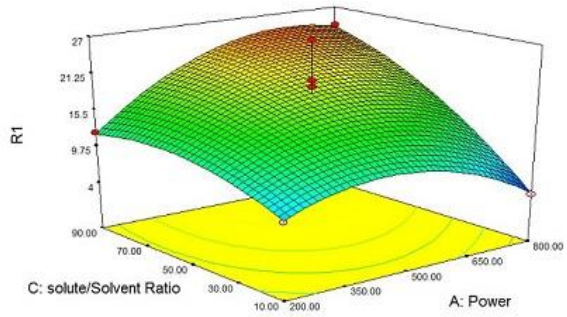
4(b) - 2 D contour plot of interaction between the time of extraction and power



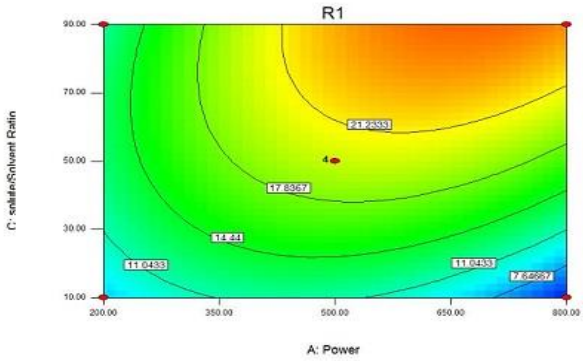
4(c) - 3D contour plot of interaction between the solute-solvent ratio and time



4(d)- 2D contour plot of interaction between the solute-solvent ratio and time



4(e) - 3D contour plot of interaction between the solute-solvent ratio and power



4(f) – 2 D contour plot of interaction between the solute-solvent ratio and power

Figure 4: Contour plots of optimized hydroalcoholic extract of whole dried fruit of *T. Catappa* L.

Table 5
Possible combinations of variables using Design of Expert

Power	Time of extraction	Solute: Solvent	Response % yield
200	30	50	10
500	17.5	50	13
500	30	10	18
500	17.5	50	24
800	17.5	90	21
500	17.5	50	28
200	5	50	7
800	30	50	11
800	17.5	10	8
500	17.5	90	23
800	5	50	15
500	30	90	14
200	17.5	90	10
200	17.5	10	8
500	5	90	6
500	5	10	4

Table 6
ANOVA for Response Surface Quadratic Model
Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of squares	Df	Mean squares	F value	p-value (Prob>F)
Model	891.19	9	99.02	4.13	0.0491
A- Power	32.00	1	32.00	1.34	0.2917
B- Time of extraction	84.50	1	84.50	3.53	0.1095
C- Solute/ solvent ratio	264.50	1	264.50	11.04	0.0160
AB	0.000	1	0.000	0.000	1.0000
AC	64.00	1	64.00	2.67	1.1533
BC	81.00	1	81.00	3.38	0.1156
0	105.06	1	105.06	4.39	0.0811
A^2	232.56	1	232.56	9.71	0.0207
B^2	27.56	1	27.56	1.15	0.3247
C^2	143.75	6	23.96		
Residual	13.00	3	4.33	0.099	0.9551
Lack of fit	143.75	1	43.58		
Pure Error	130.75	1			
Cor total	1034.94	15			

The response using the surface quadratic model in terms of standard error, the sum of squares and F-ratio is summarized (Table 6). The value of 4.13 indicates that the model is significant. The error due to noise is around 2.49%. Experimental data was used to fit the extract yield as a function of time used in extraction, concentration of ethanol and power used. The "Lack of Fit F-value" of 0.09 depicts that Lack of Fit is not significant relative to the pure error.

The yield of extract as a function of extraction duration, ethanol concentration and applied power is evaluated using the experimental data. The optimum regression equation of ellagic acid is as follows:

$$Y\ (\%\ yield) = 12.39 + 0.046X_1 + 1.51X_2 - 0.016X_3 + 0.00X_1X_2 + 3.33X_1X_3 + 9.00X_2X_3 - 5.694X_1^2 - 0.048\ X_2^2 - 1.64\ X_3$$

where X_1 is irradiation power, X_2 is extraction time, X_3 is solute solvent concentration. The Design-Expert 8.0.7.1 was used to obtain the optimal conditions. The obtained optimized values are used for the calculations of the

percentage biases and errors. It was reported that the theoretical value was found to be 26.6 and the practical value was found to be 27.5.

Percentage Bias = 28-26.6= 1.1
Percentage Error = (1.1/26.6)x100= 4.13.

The above results demonstrated that quantitative estimation of the active constituents in the optimized extract is quantified using the high-performance thin-layer chromatography (HPTLC) method.

Detection of Spots: The plates were presented under an ultraviolet chamber and chromatograms were sprayed with dinitrogen and scanned using a densitometer at 254 nm and 366 nm. The Rf values obtained and fingerprint data were recorded by WIN CATS software. The fingerprinting of the optimized dried fruit extract of *T. catappa* L. is represented in figure 6. In this, A represents pure ellagic acid, B represents the test sample (*T. catappa* fruit hydroalcoholic extract) with dilution up to 2 %. C represents the dilution of the extract up to 3% and D represents the dilution of the optimized extract up to 4%.

Figures 6 and 7 represent the pure ellagic acid which was prepared using 2 mg of the ellagic acid diluted to 100 ml. The area under the curve was observed to be 5925.6. The area under curve B was found to be 104.22. The area under curve C was found to be 847.0 and the area under curve D was found to be 104.3. The average recovery of the ellagic acid in *T. catappa* L. dried fruits is 80.43w/w.

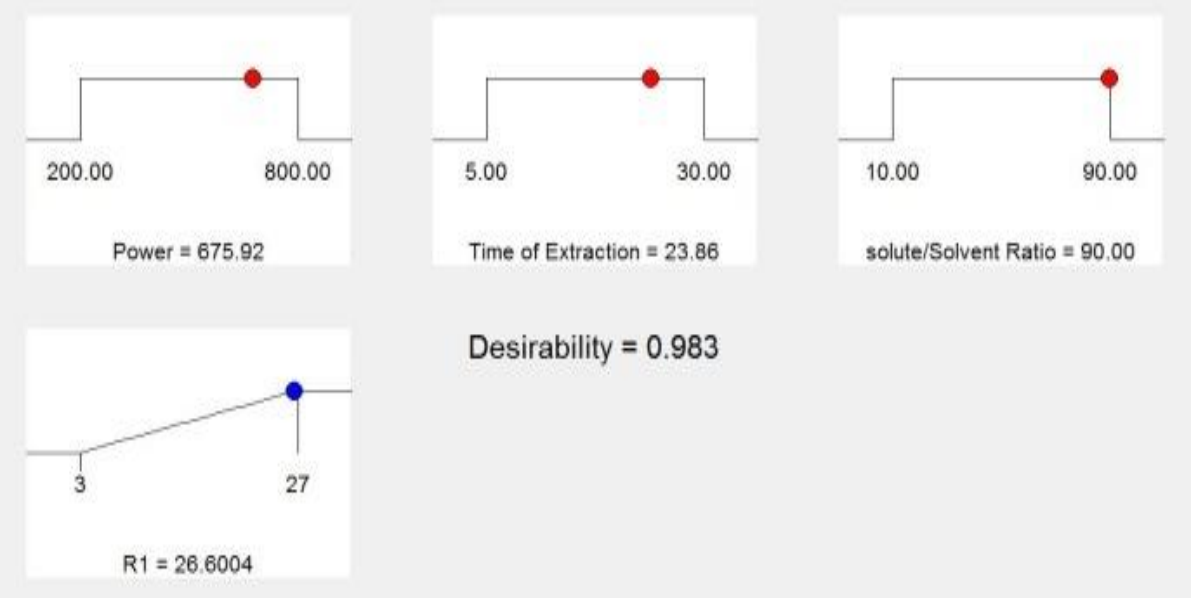


Figure 5: Optimization of hydroalcoholic extract of *T. Catappa L.*

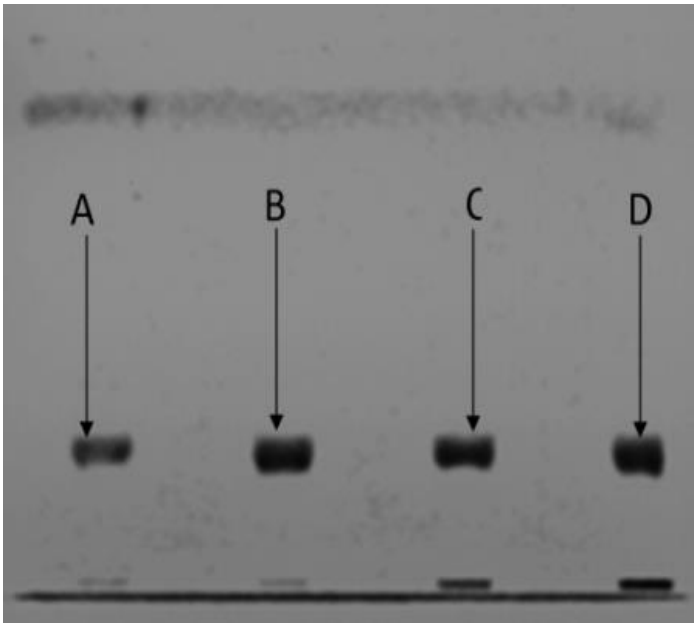


Figure 6: TLC fingerprinting of the different concentrations of the *T. catappa* L. fruit hydroalcoholic extract

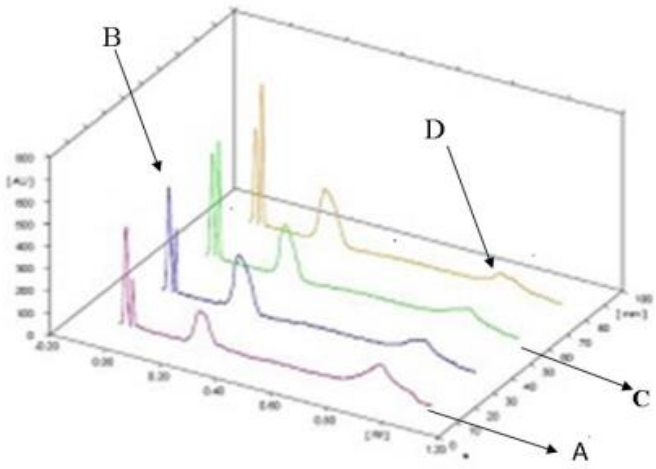


Figure 7: 3 D chromatogram showing peaks of *T. catappa* L. fruit hydroalcoholic extract in different concentrations

Conclusion

The present study determines pharmacognostic and phytochemical studies of dried fruits of *T. catappa* L. of the crude drug for the identification and standardization. The morphological evaluation of *T. catappa* reported that fruits are reddish-brown with abrasions on their surface. Parenchymal cells, stone cells, prismatic calcium oxalate crystals with patted thickening of xylem vessels and phloem fibers are found in the transverse and powder microscopy of the dried fruit of *T. catappa* L. which distinguish it from the other species of *Terminalia*. The extractive value, moisture content and ash values were also determined used for the standardization of the *T. catappa* L. dried fruits.

To determine the presence of foreign inorganic matter and adulteration, the ash value and extractive values were determined. The preliminary phytochemical screening of dried fruits of *T. catappa* was performed in the hydroalcoholic extract which confirms the presence of various phytoconstituents such as alkaloids, reducing sugars, monosaccharides, hexose sugars, iodine test, steroids, cardiac glycosides, amino acids and phenolic and tannins with the absence of flavonoids, carbohydrates and proteins. The heavy metal content determination reported that metals such as lead, cadmium, mercury and arsenic were found to be within the permissible limits.

Extraction was performed using the hydroalcoholic solvent system using the microwave-assisted method of extraction and the optimized extractive values were used quantitatively for the determination of the concentration of phytoconstituents using high-performance liquid chromatography. The optimized extractive values were used quantitatively for the determination of the concentration of phytoconstituents. The *T. catappa* L. may be further explored for its pharmacological potential based on its phytochemical constituents.

References

1. Chinaka C.N., Ezealisiji K.M. and Akpofure R.E., Phytochemical characterization of the leaf extracts of Terminalia catappa L. using Ultra violet-Visible, Fourier transform infrared and Gas chromatography-Mass Spectroscopic techniques, *J. Pharmacogn. Phtochem*, **7**, 2017-2023 **(2018)**

2. Karmakar K., Application of natural gum as a binder in modern drug delivery, *J. Anal. Pharm. Res*, **3**, 12-14 **(2016)**

3. Krishnaveni M., Kumari G.K., Kalaivani M. and Banu C.R., A Preliminary Study on *Terminalia catappa* L. Leaf, Stem, *Res J Pharm Technol*, **8**, 163-165 **(2015)**

4. Lin C.C., Chen Y.L., Lin J.M. and Ujiie T., Evaluation of the antioxidant and hepatoprotective activity of *Terminalia catappa*, *Am. J. Chin. Med*, **25**, 153-161 **(1997)**

5. Mohale D.S., Dewani A.P., Chandewar A.V., Khadse C.D., Tripathi A.S. and Agrawal S.S., Brief review on medicinal potential of *Terminalia catappa*, *J Herb Med*, **3**, 7-11 **(2009)**

6. Natranjan D., Rengarajan R.L., Radhakrishnan R., Allah E.F., Alqarawi A.A., Hashem A., Manikandan R. and Anand A.V., Phytotherapeutic efficacy of the medicinal plant *Terminalia catappa* L, *Saudi J. Biol. Sci.*, **26**, 985-988 **(2019)**

7. Rai V., Kakkar P., Khatoon S., Rawat A.K. and Mehrotra S., Heavy metal accumulation in some herbal drugs, *Pharm. Biol*, **39**, 384-387 **(2001)**

8. Rastogi S., Pandey M.K., Prakash J., Sharma A. and Singh G.N., Veterinary herbal medicines in India, *Pharmacogn Rev.*, **9**, 155-163 **(2015)**

9. Saini S.A., Dhiman A.N. and Nanda S.A., Pharmacognostical and phytochemical studies of *Piper betle* Linn. leaf, *Int. J. Pharm. Pharm. Sci*., **8**, 222-226 **(2016)**

10. Shalini S., Advantages and applications of nature excipients A review, *Asian J. Pharm. Sci.*, **2**, 30-39 **(2012)**

11. Shirwaikar A., Prabu S.L. and Kumar G.A., Herbal excipients in novel drug delivery systems, *Indian J. Pharm. Sci.*, **70**, 415- 422 **(2008)**

12. Singh V.K. and Chaudhary A.K., Ayurvedic natural excipients: An advance option for modern medicaments, *Int. J. Pharm. Sci*., **7**, 4743-4755 **(2016)**

13. Uddin A.H., Khalid R.S., Alaama M., Abdualkader A.M., Kasmuri A. and Abbas S.A., Comparative study of three digestion methods for elemental analysis in traditional medicine products using atomic absorption spectrometry, *Anal. Sci. Technol*., **7**, 1-7 **(2016)**

14. Wang H., Ko P., Chyau C., Mau J. and Kao M., Composition and antioxidative activity of essential oils from *Terminalia catappa* L. leaves, *J. Agric. Food chem*., **38**, 27-35 **(2000)**

15. Yongyu Z., Shujun S., Jianye D., Wenyu W., Huijuan C., Jianbing W. and Xiaojun G., Quality control method for herbal medicine-chemical fingerprint analysis, *J. Med. Plant Res*., **4**, 171-194 **(2011)**.

(Received 09th December 2021, accepted 05th February 2022)