Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Suppl 1, 2014

Full Proceeding Paper

ANALYTICAL AND CHEMICAL STANDARDISATION STUDIES ON *TERMINALIA CATAPPA* BARK

P. VENKATALAKSHMI[1], P.BRINDHA[2], R.P.SARALLA[2]

[1]Department of Biochemistry, S. T. E. T. Women's College, Mannargudi, [2]Centre for Advanced Research in Indian System of Medicine, SASTRA University, Thirumalaisamudhram, Thanjavur, Tamilnadu, India. Email: brindha@carism.sastra.edu

Received: 17 Nov 2013, Revised and Accepted: 09 Dec 2013

ABSTRACT

Since century's traditional medicines has become an integral part of the human society. In the last few decades there has been an exponential growth in the field of phytotherapeutics. Lack of standardization and validation reduces the international recognition and market potentials of herbal medicines. In the recent years considerable research has been focused towards the standardization and validation aspects. In the present study, attempts are made to evaluate chemical standards for *Terminalia catappa* bark which has been reported to possess activities like anticancer, anti-HIV reverse transcriptase, hepato-protective, anti-inflammatory, anti hepatitis, anti diabetic and aphrodisiac activity. The aqueous extract of the bark is subjected to physicochemical and phytochemical standardization studies. Identification of active principles is achieved through analytical standardization techniques such as HPTLC finger printing and GC-MS analysis. The data of the results obtained will be presented and discussed. Such studies will give valuable insights regarding the production of standard herbal products and also increase their market potentials,

Keywords: Analytical standardization, GC-MS, HPTLC, *Terminalia catappa.*

INTRODUCTION

Plants produce wide array of bioactive principles and constitute a rich source of medicines [1]. Ayurvedic and other complementary systems of medicine prescribe traditional herbal therapies to treat various human ailments. At least one form of unconventional therapy including herbal medicine is used by all developed countries [2, 3]. The need to control and assure the quality of the herbal medicine through systematic scientific studies including chemical standardization, biological assays and validated clinical trials is gaining tremendous importance, as a large majority of population of developed as well as developing countries relies on these medicines.

Different times of harvesting and storage conditions, processing and formulation methods produce variations in the nature of phytoconstituents which necessitates the quality assurance of herbal products [5, 6]. Guidelines on various aspects of quality control of medicinal plants such as identification; water content, assay of active ingredients, inorganic impurities or heavy metals, microbial limits, mycotoxins, pesticides, etc. for standardization and validation of herbal preparation have been drafted by The United States Food and Drug Administration (USFDA) and the European Agency for the Evaluation of Medicinal Products (EMEA). Amongst these, the chemical standardization of a herbal formulation with respect to its major phyto constituents has emerged as the most important parameters. Numerous separation methods such as high- performance liquid chromatography (HPLC), high- performance thin layer chromatography (HPTLC), gas chromatography (GS) and capillary electrophoresis (CE) [7,8]and analytical methods such as UV-VIS spectrofluorimetric, diode array detection (DAD) and mass spectrometric detection (MS) are employed for herbal drug analysis and standardization [9]. International Union of Pure and Applied Chemistry (IUPAC) has also published a report on efficacy, safety, standardization and documentation of herbal medicines [10]. Keeping in view of the significance of standardization, in the present paper Terminalia catappa bark is studied from chemical standardization point of view as this drug source is common in south India with rich therapeutic potentials such as anticancer, anti inflammatory, antioxidant and hepatoprotective.

Terminalia catappa Linn. belongs to the family Combretaceae which is found wild in the Andaman islands and in the Malay Peninsula along coastal forests. It is extensively grown as tree in India and Myanmar as an avenue tree. Tropical almond, Indian almond, Sea almond, Umbrella tree, Talisay tree are the common English names of the tree. The tree has corky, light fruit and the nut. The fully ripe fruit is edible. The nuts taste almost like almond but underutilized by humans. However, fruits are eaten by birds and bats. The fruit pulp is sweet, acrid, cooling astringent to the bowels and is useful in biliousness and bronchitis [11]. The juice of young leaves is used in South India to prepare an ointment for leprosy, scabies and other cutaneous diseases [12]. The bark is rich in tannins. The root bark is given in dysentery and diarrhea [13]. The potential medicinal use of this tree has not been fully studied and present work is undertaken to contribute towards the quality control studies.

MATERIALS AND METHODS

Collection of plant material

The bark pieces of *Terminalia catappa* were collected from Mannargudi, Tamilnadu in the month of January 2013. Identification and authentication was done at the Department of CARISM, SASTRA University Thanjavur. The collected plant materials were cleaned, shade dried and coarsely powdered. These powdered materials were used for further physicochemical, phyto-chemical and fluorescence analysis. The procedures recommended in the Indian pharmacopoeia were followed for the determination of Total ash, Water soluble ash, Acid insoluble ash, Sulphated ash and loss on drying at 110^{o}C .

Qualitative Analysis

Extraction of plant materials - Cold extraction method

Nearly 500 g of shade dried, coarsely powdered material was subsequently extracted with various organic solvents in the order of increasing polarity and the extracts were subjected to preliminary phytochemical screening. All chemicals and solvents used for different studies were of analytical grade.

Preliminary Phytochemical Analysis

Preliminary phytochemical analysis for determining the presence of phytochemicals was carried out according to the standard procedures [14].

Fluorescence Analysis

Fluorescence of the drug was observed under day and UV light (254nm) using various solvent extracts of the drug. The powder was treated with neutral solvents like Hexane, Benzene, Chloroform, Ethyl acetate, Alcohol, Acetone and acids like 1N Hydrochloric acid, 50%Sulphuric acid and alkaline solutions like aqueous and alcoholic 1N NaOH [15].

Quantitative Analysis

Estimation of Carbohydrate, Phenol, Ascorbic acid, Amino acid, Protein are carried out according to standard procedures.

Analytical Standardization

Analytical techniques such as HPTLC and GC-MS were carried out to determine the genuineness of the drug according to the routine procedures.

RESULTS

Table 1: Physico chemical analysis

S. No.	Parameters	%W/W
1	Foreign matter	1.3
2	Loss on drying	5.3
3	Total ash	7.3
4	Water soluble ash	7.45
5	Acid soluble ash	2.03

Table 2: Successive Extractive values

S. No.	Solvents	%W/W
1	Hexane	0.318
2	Chloroform	0.564
3	Ethyl acetate	1.08

Table 3: Extractive values as per IP

S. No.	Solvents	%W/W
1	Alcohol	14.2
2	Water	20

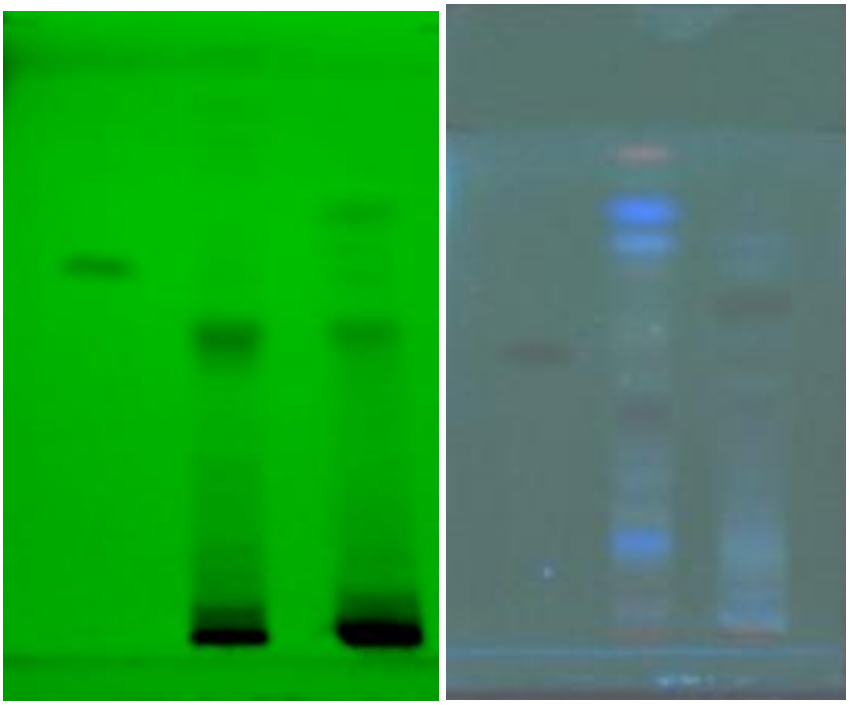
Table 4: Preliminary phytochemical Analysis

S. No.	Test	Powder	Aqueous	Ethyl acetate	Hexane
1	Saponin	-	-	-	-
2	Tannin	+	+	+	-
3	Sterol	-	-	-	-
4	Terpene	-	-	-	-
5	Flavonoid	-	-	-	-
6	Coumarin	+	-	+	+
7	Quinone	+	+	+	-
8	Lignin	+	+	+	-
9	Alkaloid	+	+	+	+

Table 5: Quantitative Analysis

S. No.	Parameters	Amount (mg/g)
1	Proteins	36.3
2	Carbohydrates	100
3	Phenol	33
4	Total free amino acids	10
5	Ascorbic acid	50

Photo documentation under UV



AT 254nm **At 366nm**

Fig. 1: HPTLC fingerprinting profile of *Terminalia catappa* bark extract

TLC details of Gallic acid

Track 1 -10µl of Standard (Gallic acid)

Track 2-25µl of Sample (Ethanol Extract)

Track 2-25µl of Sample (Aqueous Extract)

Evaluation: A band (Rf 0.50) Corresponding to Gallic acid is visible in both standard solution (Track 1) and test solution tracks, (Track 2 and 3)

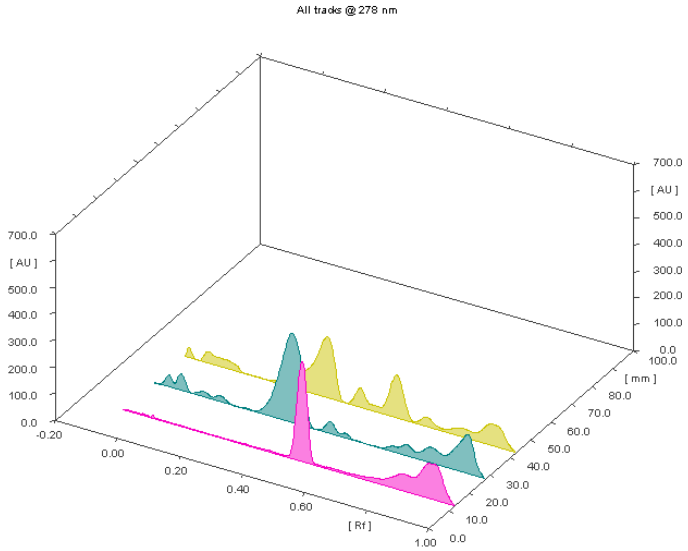
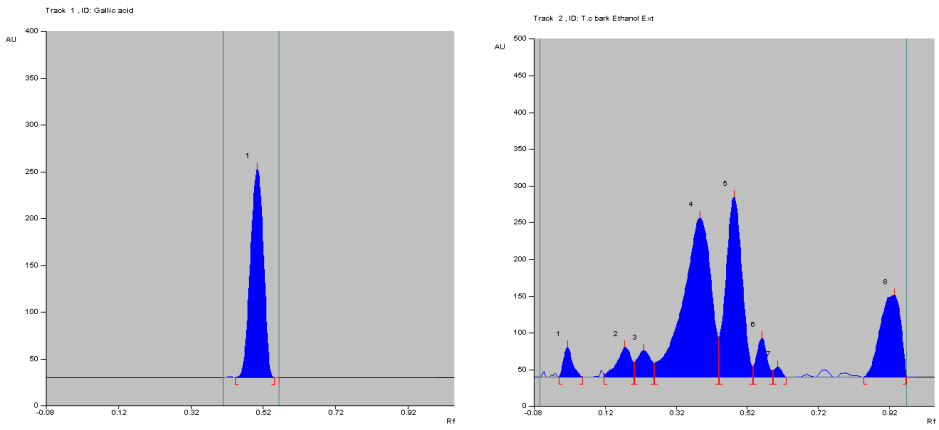
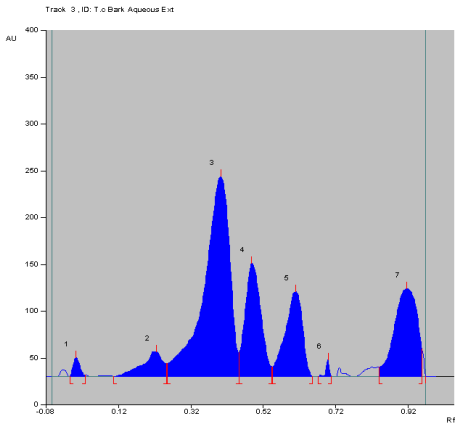


Fig. 2: 3D display of chromatogram at 278nm



Peak display(10µl of standard) Peak display (10µl of Sample Ethanolic Extract)



Peak display(10µl of Sample Aqueous Extract)

Fig. 3: Peak displays of Standard gallic acid and ethanolic, aqueous extracts of *Terminalia catappa* bark

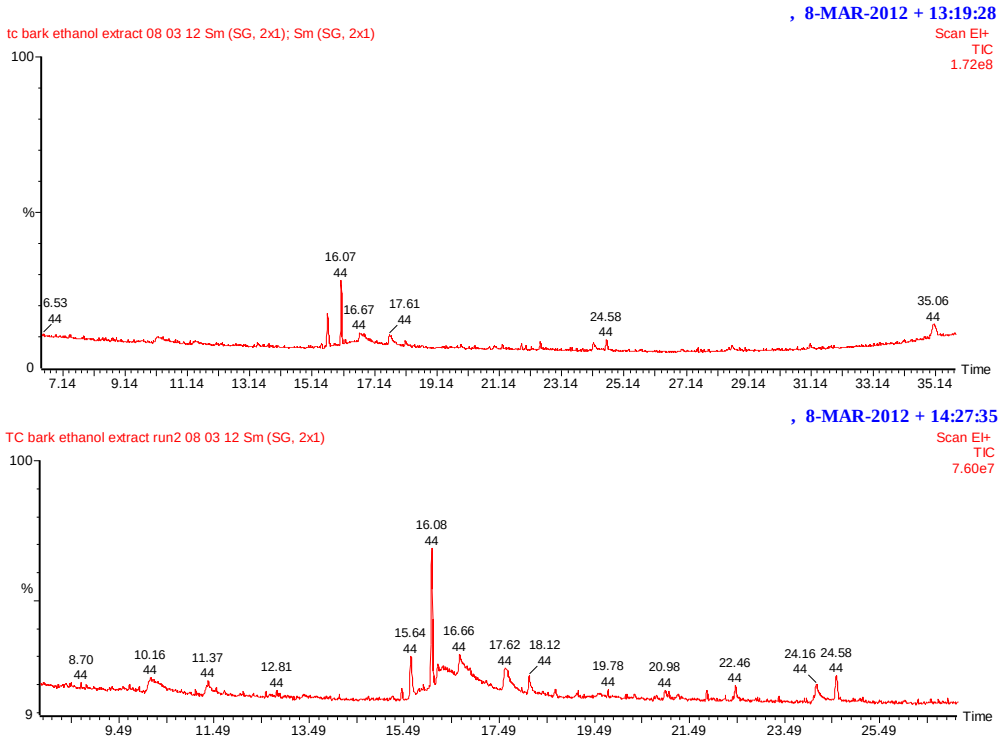


Fig. 3: GC-MS profile of ethanolic extract of *Terminalia catappa* bark

Table 12: GCMS profile of ethanolic extract of *Terminalia catappa* bark

S. No.	Peak Name	Retention time	Peak area	%Peak area
1	Name: Ethane, 1,1-diethoxy-Formula: C6H14O2 MW: 118	3.40	2447367	24.1649
2	Name: 1,3,5-Cycloheptatriene Formula: C7H8 MW: 92	4.01	2153280	21.2611
3	Name: Phenol, 4-methoxy-, acetate Formula: C9H10O3 MW: 166	10.17	601179	5.9359
4	Name: Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)- Formula: C15H24 MW: 204	15.44	110576	1.0918
5	Name: Benzene, 1,1'-(1-methylethylidene)bis[4-methoxy- Formula: C17H20O2 MW: 256	15.63	587500	5.8009
6	Name: Caryophyllene Formula: C15H24 MW: 204	16.07	1398856	13.8121
7	Name: D-Allose Formula: C6H12O6 MW: 180	17.61	497836	4.9156
8	Name: Tridecanoic acid Formula: C13H26O2 MW: 214	18.12	325899	3.2179
9	Name: 2,6,10-Trimethylundeca-1,3-diene Formula: C14H26 MW: 194	21.86	107091	1.0574
10	Name: n-Hexadecanoic acid Formula: C16H32O2 MW: 256 CAS#:	24.16	610150	6.0245
11	Name: Eicosanoic acid, ethyl ester Formula: C22H44O2 MW: 340	24.58	329043	3.2489
12	Name: 6-Nitroundec-5-ene Formula: C11H21NO2 MW: 199	28.61	350997	3.4657
13	Name: 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester Formula: C16H22O4 MW: 278	35.04	608002	6.0033

DISCUSSION

Several concerns regarding the safety and quality of the herbal medicines have become mandatory because of the tremendous increase in the usage of medicinal plants globally.The safety and efficacy profiles of numerous plants worked out through animal studies and clinical trials is very expensive and time consuming. Reverse pharmacology can be used to identify bioactive constituents from herbal formulations. Hence in the present work, attempts are made to determine the chemical standards for an interesting herbal drug *Terminalia catappa bark* which finds use in gastro-intestinal problems.

The chances of variations in the phytoconstituents of crude drugs/raw materials of plant origin occur due to varied climatic conditions, geographical distribution, source and seasons of collection. Therefore, the only solution to place the Indian system of medicines on global market, is to standardize the herbal products.

Keeping in view of these aspects, the present study was carried out to evaluate the bark of *Terminalia catappa* from chemical and analytical standardization point of view.Physico chemical constants determined for the bark of *Terminalia catappa* (Table 1) revealed the genuineness and purity of the plant drug. Preliminary phytochemical analysis revealed the presence of major secondary metabolites such as Tannins, Alkaloids, Lignins, Quinone and Coumarins (Table 4). Biochemical analysis revealed the presence of proteins (36.3mg/g), Carbohydrates (100mg/g), Phenol (33mg/g), Total free aminoacids (10mg/g) and Ascorbic acid (50mg/g). This data suggested the rich nutritive potential of the selected plant drug. HPTLC analysis confirmed the presence of gallic acid (R_f 0.50) and GC-MS analysis revealed the presence of 13 components. To conclude present work depicted presence of interesting phytochemicals, nutraceutical elements and molecules which could very will be used as marker molecules for the identification of useful parts of bark and could act as chemical standards for the selected herbal drug.

ACKNOWLEDGEMENT

Authors extend a deep sense of gratitude to Hon'ble Vice Chancellor, SASTRA University, Thirumalaisamudhram for providing necessary infrastructure and the Management, S.T.E.T.Women's College,Mannargudi for permitting us to do the work at SASTRA University, Thirumalaisamudhram.

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