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DEVELOPMENT OF QUALITY CONTROL PARAMETERS FOR THE STANDARDIZATION OF BARK OF *FICUS TSIELA* AND *FICUS TOMENTOSA*

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ABSTRACT

Quality control parameters for the standardization of herbal drugs and its formulation are essential in order to assess the quality of drugs. The present paper reports quality control parameters for the standardization of bark of *Ficus tsiela* and *Ficus tomentosa* the herbal drugs. Here we calculate and discussed about Pharmacognostical parameters such as Macroscopical study, Foreign matter, Extractive values, Moisture content, Ash values, Fluorescence analysis etc. and preliminary Phytochemical studies. These parameters and others standardization parameters are required for authentication of any herbal drug and its formulation. The information given by this study will be of used for further pharmacological and therapeutically evaluation of the species and will assist in standardization for quality, purity and identification of drug sample.

KEYWORDS: *Ficus tsiela, Ficus tomentosa,* Fluorescence analysis**,** Moisture content, Extractive values, Ash values, HPTLC.

1. INTRODUCTION

The term “herbal drugs” denotes plants or plant parts that have been converted into Phytopharmaceuticals by means of simple processes involving harvesting, drying and storage

(EMEA, 1998; WHO, 1998, 1996, Watson, 1999). Standardization of herbal drugs and its formulations is essential in order to assess of quality drugs, based on the concentration of their active principles, Physical, Chemical, Phyto-chemical, and *In-vitro, In-vivo* parameters. The quality assessment of herbal formulations is of paramount importance in order to justify their acceptability in modern system of medicine (Satheesh Madhavi et al., 2011). There are different parameters for stability of herbal drugs and its formulations, which includes Pharmacognostic parameters, Physico-chemical parameters, Phyto-chemical parameters, microbiological assay, chromatographic analysis, pharmacologic analysis etc (Mukherjee, 2002a, b, c, Mukherjee et al., 1998).

1.1 Pharmacognostic evaluation

It includes color, odor, taste, texture, size, shape, microscopical characters, and histological parameters etc (Khandelwal, 1991).

1.2 Physico-chemical parameters

It includes foreign matter, total ash, acid-insoluble ash, swelling and foaming index, assay, successive extractive values, moisture content, viscosity, pH, disintegration time, friability, hardness, flow capacity, flocculation, sedimentation, alcohol content etc (Kokate, 1986).

1.3 Chemical parameters

It includes limit tests, chemical tests etc.

1.4 Chromatographic and spectroscopic analysis

It includes TLC, HPLC, HPTLC, GC, UV, IR, FT-IR, LC-MS, GC-MS, Fluorimetry etc (Harborne, 1998).

TLC is the common fingerprint technique for herbal analysis. The herbal compounds can easily identified by TLC (Xie, 2006). In this technique the authentication of various species, evaluation of stability and consistency of their preparations from different manufacturers were studied (Soni and Naved, 2010). HPTLC is the common and more accurate fingerprinting technique, mainly used to analyze the compounds which are having low or moderate polarities. HPTLC technique is widely used in the pharmaceutical industry for process development, identification and detection of adulterants, substituent in the herbal products and also helps in the identification of pesticide content, mycotoxins and in quality control of herb and health products (Kumar, 2010).

1.Experimental methods

The plant materials were collected from the botanical garden of National Botanical Research Institute (NBRI) Lucknow, India in the month of April to May and were authenticated by Dr. A.K.S. Rawat, Head of Pharmacognosy & Ethnopharmacology Division, National Botanical Research Institute (NBRI) Lucknow India.

The fresh materials were preserved in 70% alcohol for microscopic evaluation. Plant materials were properly dried in shade and powdered. Parameters used to fulfill the objective of the study include various methods such as phytochemical test, TLC analysis, Foreign matter, Ash values, Fluorescence analysis, Extractive value and Moisture content etc. Macroscopical study of *Ficus tsiela* and *Ficus tomentosa* barks was performed organoleptically, which is shown in (Table 1).

2.1 Powder study

Macroscopical study on powder of *Ficus tsiela* and *Ficus tomentosa* barks was performed organoleptically (Table 2).

2.1.1 Fluorescence analysis

Fluorescence analysis is a sensitive method and enables precise and accurate determination of parameters of the samples. Fluorescence analysis of bark powder of all the two *Ficus* species was observed in day light and in UV light (254 nm & 365 nm). The drug powder was treated with different solvents in different test tubes. The solvents used were 1N sodium hydroxide (aqueous), Conc. Hydrochloric acid, Conc. H_2SO_4, Conc. HNO_3, Acetic acid, iodine, 50% potassium hydroxide and 1N sodium hydroxide (alcoholic). They were subjected to fluorescence analysis (Table 3) in day light and in UV light (Kritkar and Basu 1999a, b, Kumar and Augusti, 1989, Mousa et al., 1994).

2.2 Physicochemical analysis

2.2.1 Foreign matter

The amount of foreign matter shall not be more than the percentage prescribed in the monograph. 250 g of the original sample was weighed accurately and spread out in a thin layer. The sample was inspected with the unaided eye or with the use of a magnifying lens (6X or 10X) and the foreign organic matter was separated manually as completely as possible and weighed. The percentage of foreign organic matter (Table 4) was weighed and determined with reference to the weight of the drug taken (Mukherjee, 2002a, c).

2.2.2 Moisture content

For the evaluation drug sample moisture content is the precise and accurate technique. About 2-5 g of the prepared air dried material was accurately weighed in a previously dried and tared flat weighing bottle. The sample was distributed evenly and was placed in the drying chamber (Oven). Drying was carried out by heating to 100-105°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 till 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 and percent moisture content (Table 5) was determined (Mukherjee et al., 1998).

2.2.3 Ash values

Ash content of the crude drug is generally taken to be the residue remaining after incineration. It represents the inorganic salts naturally occurring in the drug and adhering to it, but may also include inorganic matter added for the purpose of adulteration.

Total ash is the residue remaining after incineration. Acid insoluble ash is the part of the total ash, which is insoluble in dilute hydrochloric acid. Water-soluble ash is the part of total ash, which is soluble in hot water (Mukherjee, 2002a, c, Mukherjee et al., 1998, Nitin et al., 2008).

2.2.4 Total ash

About 2 g of the powdered drug was accurately weighed in a tared silica crucible. The powdered drug was spread as a fine layer at the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 450°C until free from carbon. The crucible was cooled and weighed. The procedure was repeated till a constant weight was observed. The percentage of the total ash (Table 6) was calculated in triplicate with reference to the air dried drug.

2.2.5 Acid insoluble ash

The ash obtained as described in the determination of total ash was boiled with 25 ml of hydrochloric acid for 5 min. The insoluble ash was collected on an ash less filter paper by filtration and it was washed with hot water. The insoluble ash was transferred into a tared silica crucible, ignited, cooled and weighed. The procedure was repeated till a constant

weight was observed. The percentage of acid insoluble ash (Table 6) was calculated with reference to the air-dried drug.

2.2.6 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 tared silica crucible and ignited at a temperature not exceeding 450°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 (Table 6) was calculated with reference to air-dried drug.

2.2.7 Extractive value

Extractive value is a measure of the content of the drug extracted by solvents (water, ethanol, hexane, ether etc). Extractive value is unless and otherwise prescribed, carried out by maceration. Previously 4 g of air-dried powdered material was taken in a glass stoppered flask and macerated with 100 ml of chloroform water (1:99). It was shaken frequently for 6 h and then allowed to stand for 18 h. It was filtered rapidly taking precautions against loss of the solvent. 25 ml of filtrate was evaporated to dryness in a tared flat-bottomed petri-dish, dried at 105°C, cooled in desiccators and weighed. Same procedure was repeated for alcohol soluble extractive and for hexane soluble extractive. The percentage of water-soluble, alcohol soluble, hexane soluble extractives (Table 7) was calculated with reference to air-dried drug. Successive solvent extractive value (Table 8) of *Ficus tsiela* and *Ficus tomentosa* barks was also calculated.

2.3 Phytochemical analysis

Preliminary screening tests are useful in the detection of bioactive principles and subsequently may lead to drugs discovery and development. In the present study, several phytochemical constituents were evaluated qualitatively (Table 9) and following chemical tests were carried out for ethanolic extracts of various *Ficus* species to compare them on the basis of presence and absence of various phytochemical constituents qualitatively (Khandelwal, 1991, Kokate, 1986, Harborne, 1998, Vinha, 2012, Sofowara, 1993, Trease and Evans, 1989).

2.4 HPTLC Analysis

To determine the chemical components in methanolic extract of *Ficus tsiela* and *Ficus tomentosa* High Performance Thin Layer Chromatography (HPTLC) was a precise and accurate method.

A densitometry HPTLC analysis was performed for the development of characteristics fingerprint profile of the sample, which may be used as marker for quality evaluation and standardization of the drug (Harborne, 1998).

2.4.1 Preparation of methanolic extracts

Accurately weighed 2.0 gm of the coarse powder of two *Ficus* species, were extracted separately with methanol (4x25 ml) under reflux (30 min each time) on water bath. The combined extracts were filtered and concentrated on freeze drier and prepared 10 mg/ml solution with analytical grade methanol.

2.4.2 Standard preparation

A stock solution of concentration 1 mg/ml was prepared for each reference standard.

2.4.3 Methodology

Plates were developed to a distance of 80 mm, with toluene: ethyl acetate (80:20v/v**)** as mobile phase in a Camag twin-trough chamber (20cm x10cm) previously saturated with mobile phase vapour for 10 min. Room temperature was 28°C. After removal of plates from chamber completely dried with dryer and then sprayed with anisaldehyde-sulphuric acid solution, followed by heating at 110ºC for 15 min. Lupeol and β-Sitosterol (Fig. 1 and 2) was simultaneously quantified (Table 10) by using CAMAG TLC Scanner model-3 equipped with win CATS [version 3.2.1] Software. Results were HPTLC fingerprinting profile of *Ficus tsiela* and *Ficus tomentosa* (Fig. 3). HPTLC densitometric scan data at 600nm of reference compounds (β-sitosterol, lupeol) and test samples (Fig. 4 and 5)**.**

2. RESULT AND DISCUSSION

In the present study it was resulted that the physicochemical parameters such as organoleptic (Tables 1 and 2), Fluorescence analysis (Tables 3) Foreign matter study (Table 4) moisture content (Table 5), water-soluble, alcohol-soluble, and ether-soluble extractive values (Tables 7 and 8), water-soluble ash, acid-insoluble ash (Table 6), Qualitative chemical tests (Table 9) characteristics can be efficiently used for standardization of herbal drug and its formulation.

The results obtained from the study could be utilized as a reference for setting limits for the reference standards for the quality control and quality assurance of these drugs.

HPTLC analysis was done both for qualitative as well as quantitative purposes. HPTLC plates showed the presence of β-sitosterol and lupeol in the methanolic extract (Table 10) of two Ficus species under the investigation. A densitometry HPTLC analysis was also performed for the development of characteristics fingerprint profile (fig.3, 3.1 and 3.2). The concentration of β-sitosterol was found to be maximum in *Ficus tsiela* and the conc. of lupeol was found to be maximum in *Ficus tomentosa* (Table 10).

Table 1: Macroscopical study of *Ficus tsiela* and *Ficus tomentosa* barks

Parameters	*Ficus tsiela*	*Ficus tomentosa*
Size	0.2 - 0.5 cm.	0.4 – 0.8 cm.
Shape	Curved	Curved
Texture	Rough	Rough
Surface	Outer surface rough due to presence of warts, lenticels are transverse, inner surface slightly smooth	Outer surface rough due to presence of warts, lenticels are present, inner surface smooth with very fine lines
Fracture	Short to medium, splintery	Fibrous, short to medium
Color	Outer surface blackish brown, inner surface reddish brown	Outer surface ash brown, inner surface yellowish cream
Odor	Astringent	Characteristic
Taste	Tasteless	Slightly bitter

Table 2: Organoleptic study of *Ficus tsiela* and *Ficus tomentosa* barks powder

Parameters	*Ficus tsiela*	*Ficus tomentosa*
Color	dark brown	Brownish cream
Odor	Astringent	Characteristic
Taste	Tasteless	Slightly bitter

Table 3: Fluorescence analysis in day light and UV light at 254 & 365 nm *Ficus tsiela* barks

S. No.	Reagents used	*F. tsiela*		*F. tomentosa*		365 nm
		Day light	254 nm	Day light	254 nm	
1.	Powder as such	Brown	Muddy green	Cream	Muddy green	Black
2.	Powder+1NAq. NaOH	Chocolate brown	Coffee	Yellowish brown	Muddy green	Black
3.	Powder+Meth. NaOH	Chocolate brown	Greenish black	Yellowish brown	Muddy green	Black
4.	Powder + 1N HCl	Orange Brown	Greenish black	Orange brown	Greenish black	Black
5.	Powder +50% H_2SO_4	Coke	Chocolate	Brown	Brownish green	Black
6.	Powder+ 50% HNO_3	Dark orange	Dark green	Cream	Muddy green	Black
7.	Powder + H_2SO_4	Coke	Black	Black	Coke	Black
8.	Powder + 50% KOH	Orange brown	Greenish	Light orange	Muddy green	Black

			black			
9.	Powder + Glacial acetic acid	Reddish brown	Muddy green	Cream	Muddy green	Black
10.	Powder + Methanol	Reddish brown	Muddy green	Cream	Muddy green	Black
11.	Powder + Acetone	Brown	Muddy green	Cream	Muddy green	Black
12.	Powder+ethyl alcohol	Reddish brown	Blackish green	Cream	Muddy green	Black
13.	Powder + Alc. $FeCl_3$	Light green	Dark green	Light green	Light green	Black
14.	Powder+Iodine water	Dark brown	Blackish green	Muddy brown	Dark muddy green	Black

Table 4: Foreign matter study of *Ficus tsiela* and *Ficus tomentosa* barks

Ficus species	**Foreign matter (%)**	**Average (%)**
Ficus tsiela	0.35 - 0.45	0.40
Ficus tomentosa	0.01 - 0.03	0.02

Table 5: Moisture content of Ficus tsiela and Ficus tomentosa barks

***Ficus* Species**	**Moisture Content (%)**
F. tsiela	$\frac{2-1.878}{2} \times 100 = 6.1$
F. tomentosa	$\frac{2-1.892}{2} \times 100 = 5.4$

Table 6: Ash value of *Ficus tsiela* and *Ficus tomentosa* barks

***Ficus* species**	**Total Ash (%)**	**Average (%)**	**Acid insoluble Ash (%)**	**Average (%)**	**Water soluble Ash (%)**	**Average (%)**
F. tsiela	10.20-10.30	10.23	0.90 - 0.95	0.92	1.24-1.26	1.25
F. tomentosa	22.55-22.65	22.60	2.00 - 2.05	2.02	3.22-3.29	3.25

Table 7: Extractive values *Ficus tsiela* and *Ficus tomentosa* barks

***Ficus* species**	**Hexane soluble (%)**	**Average (%)**	**Ethanol soluble (%)**	**Average (%)**	**Water soluble (%)**	**Average (%)**
F. tsiela	2.50-5.00	3.25	14.00-19.50	16.75	15.5-16.5	16.08
F. tomentosa	1.50-2.50	1.91	2.00-3.00	2.5	5.00-6.00	5.33

Table 8: Successive solvent extractive value of *Ficus tsiela* and *Ficus tomentosa* barks

Ficus species	**Hexane Extractive (%)**	**Chloroform Extractive (%)**	**Acetone Extractive (%)**	**Ethanol extractive (%)**	**Water Extractive (%)**
F. tsiela	2.12	0.74	2.68	7.06	10.66
F. tomentosa	1.56	1.92	1.58	2.68	4.16

Table 9: Qualitative chemical tests of ethanolic extracts of *Ficus tsiela* and *Ficus tomentosa* barks

Test	*Ficus tomentosa*	*Ficus tsiela*
Steroids	-	-
Triterpenoids	+	+
Saponin	-	-
Flavonoids	+	-
Tannin	+	+
Resin	-	+
Alkaloids	-	-
Glycosides	-	-
Carbohydrate	+	+
Reducing sugar	+	+
Fixed oils & fats	-	-
Protein & amino acids	+	+

+ For Present, - For Absent

Table 10: Quantitative Analysis of *Ficus tsiela* and *Ficus tomentosa* barks

Ficus Species	β-sitosterol (%)	Lupeol (%)
Ficus tsiela	0.078	0.025
Ficus tomentosa	0.044	0.047

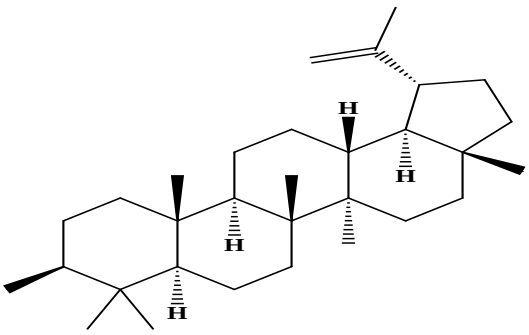


Fig. 1. Lupeol

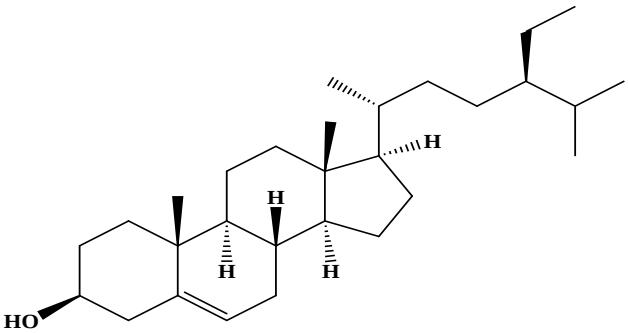
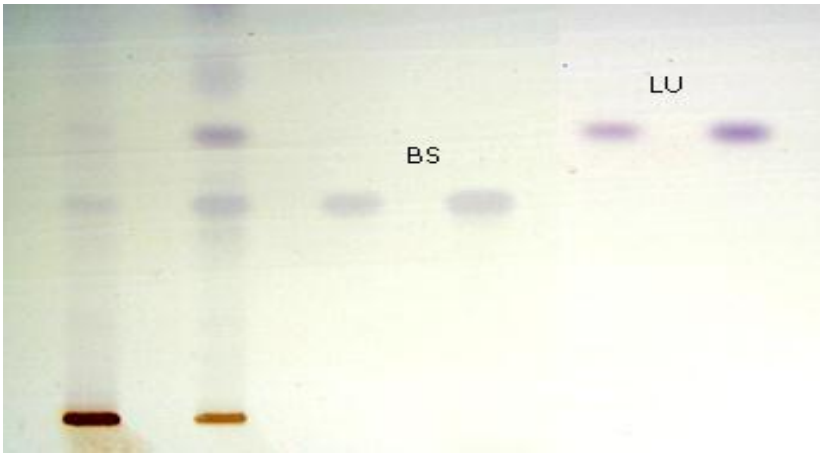


Fig. 2. β-sitosterol



BS= β-sitosterol, LU= lupeol, *Ficus tsiela* (*F.ts*), *Ficus tomentosa* (*F.to*)

Fig. 3. HPTLC fingerprinting profile of *Ficus tsiela* and *Ficus tomentosa*

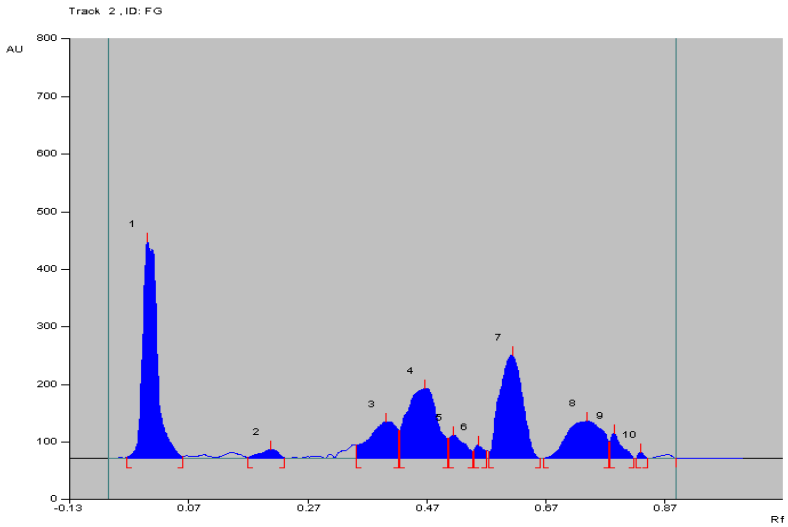


Fig. 4. HPTLC densitometric scan (at 600nm) of reference compounds (β-sitosterol, lupeol)

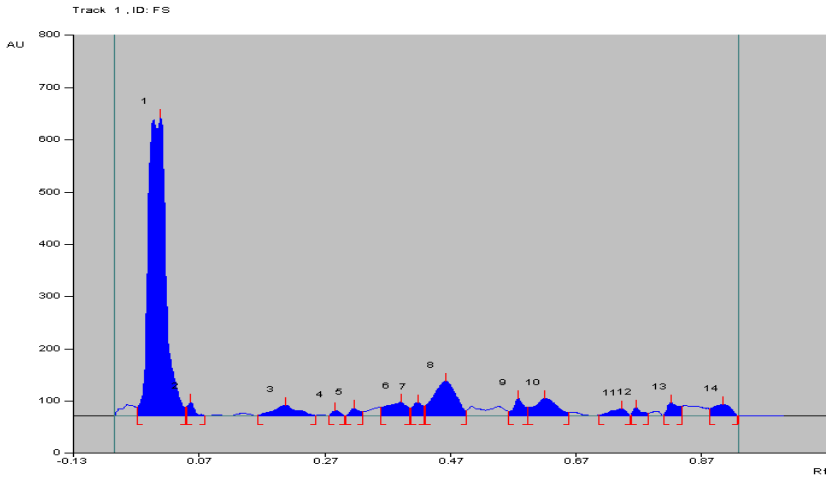


Fig. 5. HPTLC densitometric scan (at 600nm) of test samples

3. CONCLUSION

In conclusion, above parameters revealed that the result obtained provide a support for the use of *Ficus tsiela* and *Ficus tomentosa* barks in traditional medicine and suggest its future advance investigation. It also states that ficus species are rich source of naturally occurring antioxidants. These parameters can be utilized for quick identification of the bark of *Ficus tsiela* and *Ficus tomentosa* and will definitely help in the development of pharmaceutically useful formulations.

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