Published on: 1st February 2011

COMPARATIVE STANDARDIZATION PARAMETERS *OF FICUS CARICA* LINN. AND *FICUS RELIGIOSA* LINN.

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ABSTRACT:

The quality control of crude drugs and herbal formulations is of paramount importance in justifying their acceptability in modern system of medicine. But one of the major problems faced by the herbal drug industry is non availability of rigid quality control profile for herbal material and their formulations. The present work was conceived to standardize the leaves of *Ficus carica* and *Ficus religiosa*. Various standardization parameters such as morphological features, physicochemical evaluation, preliminary phytochemical screening, TLC, UV spectroscopy are reported in present paper.

KEY WORDS: Standardization, *Ficus carica*, *Ficus religiosa*, Quality control.

INTRODUCTION:

India is one of among the most popular country in the world, where traditional medicine system is practiced in primary health care. Medicinal plants are used in the treatment of much life threatening disease[1]. In almost all the traditional system of medicine, the quality control aspect has been considered from its inspection. However, in modern concept it require necessary changes in their approach by that way concrete method of quality control in terms development of modern methodologies. Thus, today quality assurance is thrust area for the evaluation of traditional used medicinal plants and herbal formulation[2].

Standardization of drug means confirmation of its identity and determination of its quality and purity. Herbal medicines, however are not necessary always safe simply because they are natural. Herbal product has been enjoying renaissance among the customers throughout the world. The quality of herbal medicine i.e. the profile of the constituents in the final product has implication in efficacy and safety. Due to complex nature and inherent variability of the constituents of plant based drugs, it is difficult to establish quality control parameter and modern analytical technique are expected to help in circumventing this problem[3].

The quality control of crude drugs and herbal formulations is of paramount importance in justifying their acceptability in modern system of medicine. But one of the major problems faced by the herbal drug industry is non availability of rigid quality control profile for herbal material and their formulations. Quality controls of synthetic drug offer no problems with very well defined parameters of analysis. In contrast, herbal products represent a number of unique problems when quality aspects are considered. These are because of the nature of the herbal ingredients present therein, which are complex mixtures of different secondary metabolites that can vary considerably depending on environmental and generic factors. Furthermore, the constituents responsible for the claimed therapeutic effects are frequently unknown or only partly explained. These complex positions of quality aspects of herbal drugs are further complicated by the use of combination of herbal ingredients as are being used in traditional practice[4].

Ficus carica Linn *and Ficus religiosa* Linn belong to family Moraceae are medicinally important plants, wildly grown in some parts of our country and used in the treatment of various disease and disorders of human ailments by tribal and rural people of our country[5]. So, far no any systematic work was carried out on the comparative study of *Ficus carica* Linn and *Ficus religiosa* Linn based on the standardization parameter therefore, these plants were selected for present investigations.

MATERIALS AND METHODS:

Collection and authentication of plant material

The leaves of selected plants were collected in the months of August 2010 from the botanical garden of UIPS and authenticated by Dr. S. N. Dwivedi, Prof. and Head, Department of Botany, Janata PG College, APS, University, Rewa, M.P-India and a voucher specimen FC/05/23, FR/05/59 were deposited in our department. The leaves were later air-dried, powdered and stored in an air-tight container for further use.

Pharmacognostical evaluation

Macroscopic characters: Various morphological features of the leaves of *Ficus carica* Linn *and Ficus religiosa* Linn *were* studied[6].

Physico-chemical evaluation: The dried leaves of *Ficus carica* Linn. *and Ficus religiosa* Linn., were subjected to standard procedure for the determination of various physicochemical parameters.

Extraction of Plant Material: 10g of the leaf powder of both the plants was extracted with water and ethanol in soxhlet apparatus[6]. The different extracts were tested for the presence of

steroids, reducing sugars, carbohydrates, triterpenoids, alkaloids, phenolic compounds, saponins, protein glycosides, tannins and flavonoids.

Limit Test: The limit test of chloride and sulphate for both the aqueous and ethanolic extract was performed as per method described[7].

Preliminary Phytochemical Screening: Preliminary phytochemical analysis of the various extracts of the leaf powder in both the solvents has been performed[8].

Thin layer chromatography: The TLC plate was prepared by silica gel G. The plate was then get activated in hot air oven at 110^0c for 30 min. Solvent system was prepared from water and ethanol in (9:1): Then the spot was made on the plate Then the plate was kept in the solvent system until it reaches to 75% of the plate. The plate was kept in iodine chamber and spot was examined[6]. Calculation was done and R_f value was calculated.

Spectrophotometric method

UV Spectrophotometry: 100 mg of the leaf extract (*Ficus carica* Linn & *Ficus religiosa* Linn) was dissolved in 100ml of distilled water. The concentration of this solution is 1000 µg/ml. Further substock solution of 100 µg/ml was prepared from the stock solution by pipetting out 10 ml of the solution from the stock solution in 100 ml volumeteric flask . It was diluted to 100 ml with water. Then from the sub stock solution different dilution was prepared,5 µg/ ml,10 µg/ml,15 µg/ml, 20 µg/ml,25 µg/ml,30 µg/ml.Then 25µg/ml solution (*Ficus carica* Linn & *Ficus religiosa* Linn) was taken λ_{max} of *Ficus carica* Linn was found at 216nm and *Ficus religiosa* Linn was found at 220 nm. Then different sample solutions of *Ficus carica* Linn & *Ficus religiosa* Linn was observed in UV spectrometer at λ_{max} 216nm & 220mm[6].

RESULTS AND DISCUSSION:

The plant *Ficus carica* Linn. *and Ficus religosa* Linn. is an indigenous plants grown wildly in many parts of our country and was chosen for the present investigation. The plant belongs to the family Moraceae. The scanty availability of information on this plant facilitates the study on it. The attempt was made to study the comparative standardization of both the plants. The morphological features of roots and leaves of both the plants *Ficus carica* Linn. and *Ficus religosa* Linn. were studied. The morphological features of the leaves was given in table 1.

Fig. 5. *Ficuscarica* Linn **Fig. 6. *Ficus religiosa* Linn**

The comparative physicochemical analysis of leaves powder of *Ficus carica* Linn and *Ficus religiosa* Linn were carried out. In this study ash values (total ash, acid insoluble ash and water soluble ash), swelling index and LOD were determined and the results was given in table 2.

The dried and coarse powdered of leaves of *Ficus carica* Linn (FC) and *Ficus religiosa* Linn (FR) were extracted with ethanol and water(Table 3).

The both extract (aqueous and ethanolic) of the plant of *Ficus carica* Linn. and *Ficus religosa* Linn. were subjected to phytochemical screening which reveal the presence of various pharmacological active constituents. The results were given in table 4.

The pH of both the extract was determined by 1% w/v solution in same solvent. The results for all the extract was given in table 5

The limit test of chloride and sulphate were determined with aqueous extract of both the plants and the results were given in table 6.

The solubility of both the extract was carried out and the data was given in table 7.

The TLC of both the aqueous and ethanolic extract was performed with different solvent and the R_f value was calculated. The calculated R_f value was given presented in table 8.

The UV analysis of the aqueous extract of *Ficus carica* Linn and *Ficus religiosa* Linn was performed at λ_{max} 216nm & λ_{max} 220nm. The data obtained along with the curve which was obtained linear is given in table 9.

Therefore, it was concluded from the present investigation that the selected species of Ficus contains various active phytoconstituents which was confirmed by preliminary phyctochemical screening. Hence, detailed screening may be formed to isolate active moiety so, that it may be scientifically proved to access the pharmacological responses of the plant to ascertain its folklore uses.

Thus, the all these standardization parameters can be used as a diagnostic tool for the correct identification of the selected species. Hence, these standardization parameters are useful in detecting the adulterants if any in these plants and will lead to efficacy and purity of the selected plants.

ACKNOWLEDGEMENTS:

The authors are thankful to the Principal, UIPS for providing the necessary facilities in the College. Sincerely thanks to Dr. S.N. Dwivedi, Principal Investigator, Department of Botany, Janata College, A.P.S. University (M.P.) for their valuable support.

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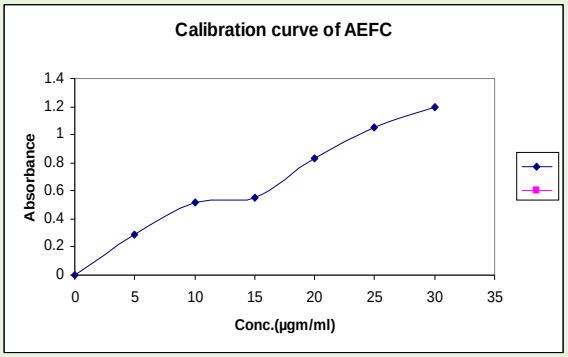
Graph: 1 Calibration curve of AEFC



Graph: 2 Calibration curve of AEFR

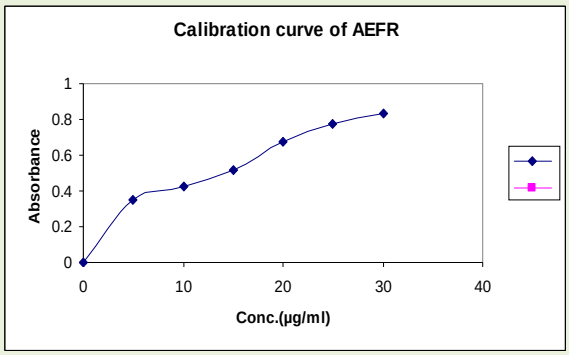


Table 1 : Comparative morphological features of leaves of *Ficus carica* Linn and *Ficus religiosa* Linn.

Parameter	LFC	LFR
Colour	Upper :Dark green Lower :light green	**Upper :Dark green** **Lower :light green**
Odour	Odorless	**Odorless**
Taste	Bitter	**Bitter**
Size	Length :26cm Width :24cm	**Length :13cm** **Width :8cm**
Texture	Upper: rough Lower: rough	**Upper:rough** **Lower:smooth**
Shape of Lamina	Deltoid	**Deltoid**
Margin	Dentate	**Waxy**
Apex	Acute	**Acuminate**
Nature of leaf	Simple	**Simple**
Duration of leaf	Persistent	**Persistent**
Base	Symmetrical	**Cordate**
Venation	Reticulate,unicostate(pinnate)	**Reticulate,unicostate(pinnate)**
Petiole	Petiolated	**Petiolated**
Midrib	Present	**Present**
Phyllotaxy	Alternate	**Alternate**
Surface appearance	**Pubescent**	**Pubescent**

Abbr.: LFC=Leaves of *Ficus carica* Linn, LFR= Leaves of *Ficus religiosa* Linn

Table 2: Comparative physicochemical parameters of leaves of *Ficus carica* Linn. and *Ficus religosa* Linn.

S./No.	Parameters	Values obtained	
		***Ficus carica* L.**	***Ficus religiosa* L.**
1.	Total Ash	5.6% w/w	**6.8%w/w**
2.	Water Soluble Ash	4.1% w/w	**3.9% w/w**
3.	Acid Insoluble Ash	0.4% w/w	**0.25%w/w**
4.	FOM	1.7%w/w	**2.3%w/w**
5.	Swelling Index	45.45%	**30%**
6.	**LOD**	**2.5%w/w**	**0.1%w/w**

Table 3: Comparative extractive values of leaves of *Ficus carica* Linn. and *Ficus religosa* Linn .

S./No.	Solvent	Estimated percentage (%w/w)		Color of extract	
		FC	FR	FC	**FR**
1.	Water	12.7193	5.2801	Greenish Brown	**Dark Brown**
2.	**Ethanol**	**6.3350**	**4.3489**	**Light Brown**	**Blackish Brown**

Table 4:Comparative preliminary phytochemical screening of leaves extracts of *Ficus carica* Linn. and *Ficus religosa* Linn .

Constituents	Test	AEFC	AEFCR	EEFC	EEFR
Alkaloids	Mayer's test	-	+	-	-
	Dragendroff' test	+	-	-	-
	Hager's test	+	-	-	+
	Wagner's test	+	-	-	+
Carbohydrates	Molisch's test	+	+	+	+
	Fehling's test	+	-	+	-
Glycosides	Brontrager's test				
	Legal's test	-	-	-	-
Fixed oil and fats	Spot test	-	-	-	-
	Soap formation test	+	+	+	+
Tannins	Fecl3	+	+	+	+
	Vanillin hydrochloride	-	+	-	+
	Alkaline reagent	+	-	+	-
Protein and amino acid	Million's test	+	+	-	-
	Ninhydrin test	-	-	+	+
	Biuret test	+	+	-	+
Flavanoids	With NaOH	-	-	-	-
	With H_2SO_4	+	+	-	-
Steroids and triterpenoids	Libermann's Burchard test	-	-	+	+
	Salkowski's test	+	+	+	+
Mucilage and gum	With 90% alcohol	-	-	-	-
Waxes	**With alc. KOH**	-	-	-	-

Abbr.: +=Present, - = Absent, AEFC= Aqueous extract of *Ficus carica,* AEFR= Aqueous extract of *Ficus religiosa,* EEFC= Ethanolic extract of *Ficus carica,* EEFR= Ethanolic extract of *Ficus religiosa*

Table 5. pH test of leaves extract of *Ficus carica* Linn, *and Ficus.religosa* Linn.

pH of *Ficus carica* Linn		pH of *Ficus religiosa* Linn	
AEFC	**EEFC**	**AEFR**	**EEFR**
7.4	**6.9**	**8**	**6.2**

Abbr.: AEFC= Aqueous extract of *Ficus carica,* AEFR= Aqueous extract of *Ficus religiosa,* EEFC= Ethanolic extract of *Ficus carica,* EEFR= Ethanolic extract of *Ficus religiosa*

Table 6: Comparative Limit test of aqueous leaves extract of *Ficus carica* Linn. and *Ficus religosa* Linn.

Limit test	AEFC	AEFR
Limit test of chloride	Pass	**Pass**
Limit test of sulphate	**Pass**	**Pass**

Abbr.: EFC= Aqueous Extract of *Ficus carica*, AEFR= Aqueous Extract of *Ficus religiosa*

Table 7: Comparative solubility profile of leaves extract of *Ficus carica* Linn. and *Ficus religosa* Linn.

Solvent	AEFC	AEFR	EEFC	EEFR
Water	Soluble	Slightly soluble	Soluble	**Partially soluble**
Toulene	Insoluble	Insoluble	Insoluble	**Insoluble**
Acetone	Insoluble	Insoluble	Insoluble	**Insoluble**
Hexane	Insoluble	Insoluble	Insoluble	**Insoluble**
Ethanol	Soluble after warming	Soluble	Freely soluble	**Freely soluble**
Methanol	Soluble	Soluble	Soluble	**Soluble**
DMSO	**Insoluble**	**Insoluble**	**Insoluble**	**Insoluble**

Abbr.: AEFC= Solubility of aqueous extract of *Ficus carica*, AEFR=Solubility of aqueous extract of *Ficus religiosa*, EEFC=Solubility of ethanolic extract of *Ficus carica*, EEFR=Solubility of ethanolic extract of *Ficus religiosa*

Table 8: Comparative TLC Profile of leaves extract of *Ficus carica* Linn. and *Ficus religosa* Linn.

Solvent system	AEFC	EEFC	AEFR	EEFR
water: ethanol (9:1)	**0.84**	**0.79**	**0.8**	**0.73**

Abbr.: AEFC= RF value of aqueous extract *Ficus carica*, EEFC=RF value of ethanolic extract of *Ficus carica*, AEFR=RF value of aqueous extract *Ficus religiosa*, EEFR=RF value of ethanolic extract *Ficus religiosa*

Table 9: Comparative UV analysis of aqueous leaves extract of *Ficus carica* Linn. and *Ficus religosa* Linn.

Dilutions (µg/ml)	AEFC (λ_{max} 216nm)	AEFR (λ_{max} 220nm)
5	0.286	0.349
10	0.516	0.423
15	0.550	0.516
20	0.830	0.674
25	1.048	0.778
30	1.197	0.832

Abbr.: AEFC= Aqueous extract of *Ficus carica*, AEFR= Aqueous extract of *Ficus religiosa*

Table 10: Table of regression analysis

S. No.	R^2 Value of AEFC	R^2 Value of AEFR
1	**0.9795**	**0.9890**