Contents lists available at ScienceDirect

Journal of Acute Disease

journal homepage: www.jadweb.org

Document heading doi: 10.1016/S2221-6189(13)60128-6

Development of quality control parameters for the standardization of fruit of *Ficus racemosa* Linn. (M)

Ramandeep Singh[1], Ashraf Ali[1], G. Jeyabalan[1], Satinder Kakar[2], Alok Semwal[3]

[1]*Department of Pharmacy, Sunrise University, Alwar, Rajasthan, India*

[2]*Department of Pharmacy, Doon Valley Institute of Pharmacy & Medicine, Karnal (Haryana), India*

[3]*Department of Pharmacy, Himachal Institute of Pharmacy, Paonta Sahib (H.P), India*

ARTICLE INFO

Article history:
Received 9 June 2013
Received in revised form 19 July 2013
Accepted 30 July 2013
Available online 20 September 2013

Keywords:
Standardization
Phytochemical analysis
Ficus racemosa Linn.
TLC

ABSTRACT

Objective: To develop a novel Standardization technique, which can pave the way for rapid determination of different phytoconstitutents of *Ficus racemosa* (*F. racemosa*) Linn. (Moraceae). From extensive literature survey it was revealed that no reports were available on, standardization parameters of fruits of *F. racemosa* Linn. **Methods:** Phytochemical test, TLC analysis, Foreign matter, Ash values, Swelling index, Foaming index, Extractive value, Moisture content, Microbiological analysis, Crude fibre content, amino acid content, aflatoxin, toxic element analysis and analysis of pesticide residue were performed in the present investigation for the quality control of the drug. **Results:** Thus it was thought worthwhile to explore this plant on the basis of it's standardization parameters. Alkaloids, steroid, flavanoids and tannins were found to be present in *F. racemosa* Linn. extracts. Ash value, insoluble ash value, soluble ash value, crude fibre content, crude fat, Swelling index and Foaming index were found to be 3.96%w/w, 9.29%w/w, 7.45%w/w, 16.65%, (4.39±0.14), 1 cm, 10.30%w/w. The study will provide referential information for the correct identification of the crude drug. **Conclusions:** These physicochemical data and phytochemical analysis of different extracts of *F. racemosa* Linn. is useful for further studies for pharmacological screening. In future this study will be helpful for qualitative & quantitative analysis of phytoconstituents for isolation of newer molecule from *F. racemosa* Linn.

1. Introduction

1.1. Ficus racemosa Linn.

Ficus racemosa (*F. racemosa*) Linn. (FR) (Moraceae) is commonly known as Gular. Gular fig, Cluster fig or Country fig, which is considered sacred, has golden coloured exudates and black bark.

This is native to Australia, South-East Asia and the Indian subcontinent. It is unusual in this plant that its figs grow on or close to the tree trunk. It is one of the herbs mentioned in all ancient scriptures of Ayurveda. It has various synonyms like yajnanga, yajniya, yajnayoga, yajnyasara etc. suggesting its use in ritual sacrifice.

The plant grows all over India in many forests and hills. It is frequently found around the water streams and is also cultivated[1,2].

1.2. Taxonomic classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Rosales
Family: Moraceae
Genus: *Ficus*
Species: *F. racemosa*

*Corresponding author: Ramandeep Singh, Department of Pharmacy, Sunrise University, Alwar (Rajasthan) India.
Tel: +91-9736922900
E-mail: ramandeep_pharma@yahoo.com

Synonyms: Ficus glomerata Roxb.

1.3. Vernacular names

Sanskrit: Sadaphala, Assamese: Jangedumuru, Yagyadimru Bengali: Jagnadumur, Yagnadumur, English: Cluster Fig. Country fig. Gujrati: Umbro, Umerdo Hindi: Gulara, Gular Kashmiri: Rumbal Marathi: Atti, Gular, Umber Oriya: Jajnadimbri, Dimbiri Punjabi: Kath Gular, Gular Tamil: Atti Telugu: Atti, Medi Urdu: Gular[1].

1.4. Description

Leaves are dark green, glabrous and shining, 7-10 cm long with tapering tips, having three veins, ovate, ovate-lanceolate or elliptic, sub acute, entire and petiolate with entire margin, rounded base.

Fruits are in cluster on woody branches, with innumerable tiny grain like seeds. Green when raw and red when ripe. Fruits have small worms within them. The fruit receptacles 2-5 cm in diameter, pyriform, in large clusters, arising from main trunk or large branches.

Bark is reddish and smoky. Seeds-The seeds are tiny, innumerable, grain-like, the outer surface of the bark consists of easily removable translucent flakes grayish to rusty brown, uniformly hard and non-brittle.

Flower have basal bracts 3, ovate triangular; male, female and gall flowers together in one receptacle, the male flower forming a zone near the mouth, the fertile female flowers forming a layers near the walls of the receptacle, and the gall flowers a more internal layer.

1.5. Habitat

It is found throughout India from sea level to 1 200 m altitude on hilltop.

1.6. Cultivation

The plant is propagated by using cuttings of stem and root suckers. Seeds can also be used for propagation. The flowers are pollinated by very small wasps. It has evergreen leaves, if it is close to a water source. Otherwise it sheds its leaves in January.

Figs have been traditionally used by children to play. Thin sticks can be joined by inserting them in gular figs to make interesting shapes[3-6].

1.7. Pharmacognostical characteristics

Macroscopical: *F. racemosa* can grow over 40 feet tall and 20 to 40 feet wide (Figure 1).

Figure 1. Fruit, bark, leaves and roots of *F. racemosa*.

The tree is medium to moderate sized deciduous. The rich green foliage provides a good shade. The leaves are dark green, 7.5-10.0 cm long, glabrous; receptacles small sub globose or piriform, in large clusters from old nodes of main trunk. The fruits receptacles are 2-5 cm in diameter, pyriform, in large clusters, arising from main trunk or large branches. The fruits resemble the figs and are green when raw, turning orange, dull reddish or dark crimson on ripening.

The fruit of *F. racemosa* is ¾ inch to 2 inches long, circular and grows directly on the trunk. The seeds are tiny, innumerable and grain-like. Outer surface of the bark consists of easily removable translucent flakes grayish to rusty brown, uniformly hard and non-brittle[3,7]. Bark is reddish grey or grayish green, soft surface, uneven and often cracked, 0.5-1.8 cm thick, on rubbing white papery flakes come out from the outer surface, inner surface light brown, fracture fibrous, taste mucilaginous without any characteristic odour. Unlike the banyan, it has no aerial roots.

Those looking for the flower of gular should know that the fig is actually a compartment carrying hundreds of flowers[2,8]. Texture is homogeneously leathery[1,9].

The roots of *F. racemosa* are long, brownish in colour. It's having characteristic odour and slightly bitter in taste. The

roots are irregular in shape[3,7].

2. Material and methods

2.1. Plant material

The Plant material *F. racemosa* Linn. (fruits) were collected from F. R. I, Dehradun (U.K.), India. The plant was authenticated by botanist Dr. R. M. Painuli, Department of Botany H. N. B. Garhwal (A Central University) Srinagar Garhwal, Uttarakhand India.

2.2. Preparation of plant extract

The plant material fruits were air dried ground to moderately fine powder and soxhlet extracted with increasing polarity solvent (Petroleum ether, chloroform, acetone, methanolic and water)[10]. Each extract was evaporated to dryness under reduce pressure using rotary evaporator.

The coarse powder of fruit were subjected to successive hot continuous extraction with various solvent each time before extracting with next solvent the powdered material will be air dried. The various concentrated extracts were stored in air tight container for further studies.

2.3. Nutritional and mineral assay

The fruits of *F. racemosa* Linn. was analyzed for moisture content, ash value, fat, fiber as per method reported in AOAC. Total nitrogen was analyzed by microkjeldhal method and for crude protein the value was multiplied by 6.25.

Total carbohydrates were obtained by subtracting the value moisture, crude protein, crude fat, crude fiber and ash from 100%.

The total energy value equal to addition of fat, protein and sugars calorie, each gram of fat give 9 kcal, protein and sugar give 4 kcal energy. The minerals analyzed were potassium using atomic absorption spectrophotometer, calcium and phosphorus by flame photometer. Ascorbic acid in fruits was estimated[11-14].

2.4. Successive value

Accurately weighed 500 g coarse and air dried drug material were subjected to hot successive continuous extraction in soxhlet apparatus with different solvents with increase in polarity petroleum ether, chloroform, acetone, methanol, and finally with water.

The extracts were filtered in each step concentrated and the solvent was removed by vacuum distillation.

The extracts were dried in the vacuum dessicator and the residues were weighed[15]. Which contain maximum chemical compound are these categories as depend upon solvent nature and types.

2.5. Detection of chemical compound through TLC

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. Thin layer chromatography is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material usually silica gel, aluminium oxide, or cellulose. This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action.

Thin Layer Chromatographic plates are prepared by spreading silica gel G on glass plate using distill water as solvent these plates are activated in oven at 110 ℃ for half hour. All five extracts are applied separately and run in different solvent system of varying polarity. These plates are developed in Iodine chamber, UV chamber and spraying reagent for different spot of constituent chemical[16].

2.6. Phytochemical analysis

Preliminary phytochemical analysis extract was prepared by weighing and the dried powdered fruits, were subjected to hot successive continuous extraction with different solvents as per the polarity petroleum ether, chloroform, acetone, methanol, ethanol and finally with water.

The extracts were filtered in each step concentrated and the solvent was removed by vacuum distillation. The extracts were dried over desiccators and the residues were weighed. The presence or absence of the primary and secondary phytoconstituents were detected by using standard methods[17].

2.7. Analysis of toxic elements

Analysis of toxic elements and essential minerals was carried out by Atomic Absorption Spectroscopy (Perkin Elmer-400, carrier gas-Argon, flow rate- 2 mL/3 min) by following method.

Accurately weighed 500 mg of air-dried powder was taken in round bottom flask. To this 5 mL of conc. nitric acid was added and refluxed for half an hour in a hot plate at 60-80 ℃. It was then cooled, 5 mL of conc. nitric acid was added and warmed on water bath. 2 mL of 30% hydrogen peroxide solution was added to the above mixture and warmed till clear solution

was obtained. It was then cooled, filtered through Whatman-42 filter paper diluted with deionised water and made upto 100 mL in volumetric flask.

2.8. Analysis of pesticide residue

Analysis pesticide residue was carried out by Gas Chromatography-Mass spectra (GC-MS)(Instrument - Agilent, Detector - Mass selective detector, column specification - DB5MS, carrier gas - Helium, flow rate - 1ml/min, column length - 30 m, internal diameter - 0.25 mm, column thickness - 0.25 μm).

2.9. Sample preparation

Accurately weighed 25 g of coarsely powered air-dried material is taken in a conical flask. 65 mL of acetonitrile and 35 mL of deionised water is added to it. Shake well and allow it to stand for two hours with constant shaking. Filter through whatman-41 filter paper and collect the filtrate in separating funnel. Add 3×65 mL of petroleum ether (Boiling point of petroleum ether 60-80 ℃).

Shake vigorously and collect the solvent layer in the round bottom flask. Evaporate to dryness on the water bath. Then add 1 mL of acetonitrile to the residue. Inject this sample in the Gas Chromatography-Mass Spectra (GCMS).

2.10. Determination of aflatoxins

Aflatoxins were determined by Kobra cell technique using Agilent HPLC instrument as per the method ASTA. (Lachrome Merck HPLC D7000 series, detector - fluorescent detector, column specification - ODS - 3 V, column thickness & length - 5 μ & 150 m, internal diameter - 4.6 mm, flow rate - 1 mL/min)

2.11. Procedure

50 g of powdered plant material was transferred to a glass stoppered conical flask. 100 mL of methanol and water (4:1) was added and vigorously shaken for 15 min in dark condition by wrapping the flask with an aluminium foil and filtered. The filtrate (30 mL) was passed through AFLA preparative column. The flow rate was 1 mL/min. The column was washed with 20 mL distilled water and then eluted with 2 mL of methanol followed by 1 mL of water and the elute was collected. 100 μL of the sample was injected into HPLC. The mobile phase employed was water: acetonitrile: methanol (3:1:1) along with 0.109 g potassium bromide and 0.35 mL of 4 M nitric acid. The aflatoxin present in the sample was converted to the bromide derivatives in the Kobra cell. The fluorescence detector with 362 nm excitation and 455 nm emissions detected the bromide derivatives. 10 μL of standard aflatoxin containing 9.6 ppb of B1 and G1 each, 2.88 ppb of B2 and 2.81 ppb of G2 was injected for quantitative estimation. The eluting order was of B1, B2, G1, and G2. The area of the graph obtained in both sample and standard was calculated to give the total residual aflatoxin present[18].

$$\text{Aflatoxin} = \frac{\text{Sample area}}{\text{Standard area}} \times \text{Standard concentration} \times \text{Sample dilution}$$

Table 1

Phytochemical investigation of various extracts of *F. racemosa* Linn. fruits.

Sr. No.	Test	FRPE	FRCE	FRAE	FRME	FRWE
1	Alkaloides					
I.	Mayer's reagent	-	-	-	-	-
II.	Hager's reagent	-	+	-	-	-
III.	Wagner's reagent	-	+	-	-	-
2	Flavanoids					
I.	Shinoda test	-	-	-	-	-
II.	Lead acetate test	-	-	-	+	+
III.	Alkaline test	-	-	-	+	+
3	Steroids					
I.	Salkowaski test	+	+	-	-	-
II.	Leibermann' test	+	-	-	-	-
4	Tannins & phenolic compounds					
I.	Test with iron salt	-	-	+	+	-
II.	Chlorogenic acid test	-	-	+	+	_
III.	Lead acetate	-	+	+	+	-
IV.	Acetic acid	-	-	-	+	-
V.	Dilute HNO_3	-	-	-	-	+
VI.	Bromine H_2O	-	-	+	-	+
VII.	Dilute iodine	+	+	+	-	+
VIII.	Potassium dichromate	-	-	-	-	-
5	Amino acids					
I.	Ninhydrin test	-	-	-	-	-
6	Proteins					
I.	Biuret test	-	-	-	-	+
II.	Precipitation test	-	-	-	+	+
7	Fat & Oil					
I.	Solubility test	+	-	-	-	-
II.	Filter paper test	+	-	-	-	-

+ Present; - Absent; FRPE: *F. racemosa* Pet. Ether extract; FRCE: *F. racemosa* Chloroform extract; FRAE: *F. racemosa* Acetone extract; FRME: *F. racemosa* Methanolic extract; FRWE: *F. racemosa* Water extract.

3. Results

Plants are important source of potentially bioactive constituents for the development of new chemotherapeutic agents.

The first step towards this goal is the phytochemical screening, amino acid screening, nutritional profile, extractive value, TLC, Aflatoxin and toxic element analysis. The results of Phytochemical screening, amino acid screening, nutritional profile, extractive value, TLC analysis, Aflatoxin, toxic element analysis and analysis of pesticide residue are shown in Table 1, 2, 3, 4, 5, 6, 7 and 8.

Table 2

Qualitative estimation of *F. racemosa* fruit amino acid screening.

Sr. No.	Amino acid test	*F. racemosa* Linn. fruit
1.	L– Hydroxy proline	–
2.	DL Serine	+
3.	DL–Alanine	+
4.	DL–Tryptopham	–
5.	DL Iso–leucine	+
6.	DL Valine	+
7.	DL–Nor–leucine	+
8.	L–Cystein hydroxyl	+
9.	L–Ornithin	–
10.	DL–2–Aminobutyric acid	+
11.	DL – Aspartic acid	+
12.	Glycine	–
13.	3–C–3–4Dihydroxy phenyl	–
14.	L–Glutamic acid	+
15.	L – Tyrosine	+
16.	DL – Threonine	+
17.	L –Proline	–
18.	L– Arginine	+
19.	L – Leucine	+
20.	L –Lysine monochloride	+
21.	DL – Methionine	+
22.	L– Histidine	–
23.	L –Cystein hydroxychloride	+
24.	DL – β –Phenyl alanine	–

+ Present; – Absent.

4. Discussion

Phytochemicals have been used for the treatment and prevention of various health ailments from time immemorial. a large percentage of the drugs prescribed worldwide are derived from plants and 121 such active compounds are in use. Who's essential medicine list contain large number of drug from plant origin. Physicochemical standards were generally used for deciding the identity, purity and strength of the drug source. These parameters were also used to detect the adulterants if any present in the plant materials[19,20].

Physical parameters such as Moisture content, Ash value, insoluble ash, soluble ash, crude fibre, crude fat, total nitrogen, total protein, carbohydrate, Organic matter, Preliminary phytochemical analysis, Thin layer chromatography analysis and heavy metal detection can be used as reliable aid for detecting adulteration.

These are simple, but reliable standards will be useful to a layperson in using the drug as a home remedy. Effective formulations have to be developed using indigenous medicinal plants, with proper pharmacological experiments and clinical trials.

The manufacture of plant products should be governed by standards of safety and efficacy. In future, these characters are also used to check the genuine nature of the crude drug, thus it plays an important role in preventing the possible steps of adulteration.

The fruit, of *F. racemosa* Linn. contain phytoconstituents like alkaloids, steroids, fats & fixed oil, flavonoids & tannins. So finally we concluded that these physicochemical data and phytochemical analysis of different extracts of *F. racemosa* Linn. is useful for further studies of Pharmacological parameters. More detailed study must be done for further isolation leading to the pure compounds.

Table 3

Nutritional value of *F. racemosa* Linn. fruit.

Sr. No.	Nutrients	Value
1.	Moisture (%)	46.20 ± 0.14
2.	Ash (%)	3.96 ± 0.15
3.	Insoluble ash (%)	9.29 ± 0.10
4.	Soluble ash (%)	7.45 ± 0.10
5.	Crude fibre (%)	16.65 ± 0.10
6.	Crude fat (%)	4.39 ± 0.14
7.	Total nitrogen (%)	0.75 ± 0.08
8.	Total protein (%)	4.06 ± 0.06
9.	Carbohydrate (%)	19.78 ± 0.10
10.	Organic matter (%)	94.85 ± 0.15
11.	Na (mg/100 g)	0.72 ± 0.12
12.	Ca (mg/100 g)	1.45 ± 0.13
13.	K (mg/100 g)	1.48 ± 0.10
14.	Mg (mg/100 g)	0.90 ± 0.13
15.	P (mg/100 g)	1.86 ± 0.02
16.	Fe (mg/100 g)	0.02 ± 0.02

Table 4

Extractive values of *F. racemosa* Linn. fruit.

Sr. No.	Method of extraction	Values of three replicates (%w/w)	Mean (% w/w) ±SEM
1	Cold maceration:		
	1) Water soluble	(19.05, 20.35 & 19.80)	19.73 ± 0.10
	2) Alcohol soluble	(55.85, 52.63 & 52.86)	53.45 ± 0.25
2	Hot extraction:		
	1) Pet. Ether soluble	(0.90, 1.37 & 1.02)	1.09 ± 0.05
	2) Chloroform soluble	(3.30, 2.86 & 3.10)	3.15 ± 0.20
	3) Acetone soluble	(4.70, 5.25 & 5.26)	5.07 ± 0.34
	4) Methanol soluble	(54.40, 51.86 & 53.13)	53.13 ± 0.50
	5) Water soluble	(25.75, 26.49 & 26.54)	26.26 ± 0.92

Table 5

Observations of thin layer chromatographic (TLC) studies of fruit of *F. racemosa* Linn.

Sr. No	Extract	Mobile phase	No. of spot	R_f value
1	Pet. Ether Extract	(C:M:W) 60:30:10	2	0.49, 0.67
2	Chloroform Extract	(C:M:W) 60:30:10	1	0.73
3	Acetone Extract	(C:M:W) 90:5:5	4	0.26, 0.34, 0.63, 0.86
4	Methanolic Extract	(C:M:W) 65:25:10	6	0.26, 0.38, 0.47, 0.56, 0.73, 0.87
5	Water Extract	(C:M:W) 60:30:10	2	0.52, 0.64

Table 6

Analysis of aflatoxins of fruits of *F. racemosa*.

Sr.No.	Aflatoxins	*F. racemosa*
1.	Aflatoxin B1	BDL (DL: 1.0 ppb)
2.	Aflatoxin B2	BDL (DL: 0.5 ppb)
3.	Aflatoxin G1	BDL (DL: 1.0 ppb)
4.	Aflatoxin G2	BDL (DL: 0.5 ppb)

BDL: Below Detectable Limit, DL: Detectable Limit.

Table 7

Toxic element analysis of fruits of *F. racemosa*.

Sr. No.	Heavy metals	*F. racemosa* (ppm)	Permissible limit
1.	Cadmium	0.002 1	10.0 ppm (WHO)
2.	Lead	0.024 6	0.3 ppm (WHO)
3.	Mercury	0.002 0	10.0 ppm (FDA)
4.	Arsenic	0.000 1	1.0 ppm (FDA)

Table 8

Analysis of pesticide residues of fruits of *F. racemosa*.

Sr. No.	Pesticides	*F. racemosa*
1.	a– HCH	ND
2.	b – HCH	ND
3.	g – HCH	ND
4.	o p′ – DDT	ND
5.	d – HCH	ND
6.	p p′ – DDT	ND
7.	o p – DDE	ND
8.	a– Endosulfan	ND
9.	b – Endosulfan	ND
10.	o p – DDD	ND
11.	p p – DDD	ND

Detection limit – 0.01 ppm, ND: Not detectable.

Conflict of interest statement

We declare that we have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

Acknowledgment

Authors′ expressed their deep sense of gratitude to the Director, Himachal Institute of Pharmacy, Paonta Sahib (H.P) for providing support to the study and other necessary facility to carry out research project.

References

[1] Paarakh PM. *Ficus racemosa* Linn.– An overview. *Nat Prod Radiance* 2009; **8**: 84–90.
[2] Ramandeep Singh, Ashraf Ali, Satinder Kaur, Alok Semwal. Ethanomedicinal & phytopharmacological potential of *Ficus racemosa* Linn. (Moraceae): A Review. *Universal J Pharm* 2013; **2**(1): 66–74.
[3] Joseph B, Raj SJ. Phytopharmacological and phytochemical properties of three *Ficus* species – an overview. *Int J Pharma Bio Sci* 2010; **1**: 246–253.

[4] Chopra RN, Chopra IC, Handa KI, Kapur LD. *Indigenous Drugs of India*. Calcutta: U.N. Dhur and Sons Pvt. Ltd; 1958, p. 674-675.
[5] Warrier PK. I*ndian Medicinal Plants-A Compendium of 500 species* (Vol. III). Chennai: Orient Longman Ltd; 1996, p. 34-35.
[6] Chopra RN, Chopra IC, Varma BS. *Supplement to glossary of indian medicinal plants*. New Delhi: CSIR; 1992, p. 29-30.
[7] Babu K, Hankar SG, Rai S. Comparative pharmacognostic studies on the barks of four *Ficus* species. *Turkish J Bot* 2010; **34**: 215-224.
[8] Kirtikar KR, Basu BD. *Indian Medicinal Plants*, (Vol. III). L.M. Basu (ed). Allahabad: 1998, p. 2327-2328.
[9] Waarrier PK, Nambiar VPK, Ramankutty C. *Indian Medicinal Plants*. (Vol. 1-5). Madras: Orient Longman Ltd; 1995, p. 157-159.
[10]Lin J, Opak War, Geheeb-Keller M. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and antimicrobial activities. *J Ethnopharmacol* 1999; **68**: 267-274.
[11]Iswaran V. *A laboratory handbook for agreeculural analysis*. New Delhi: Today and Tomorrow′s Prienters and Publisher; 1980, p. 209-222.
[12]Ward GM. *Chemical methods of plant analysis*. Canada: Department of Agriculture Publication: 1962, p. 19-20.
[13]Negi YS, Rawat MSM, Pant-Joshi G, Badoni S. Biochemical investigation of fruits of some common *Ficus* species. *J Food Sci Technol* 1992; **25**: 582-584.
[14]Jayaraman J. *Laboratory manual in biochemistry*. New Dehli, India: Wiley Estern Ltd; 1958, p. 56.
[15]World Health Organization. *Quality control methods for medicinal plant materials*. Geneva: World Health Organization; 1998, p. 10-24.
[16]P. K. Mohanty, Neha Chourasia. Preliminary phytochemical screening of *Cajanus cajan* Linn. *Asian J Pharm Tech* 2011; **1**(2): 49-52.
[17]Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, Nirali prakashan 33 edition P. No. 108-109, Nov. 2005.
[18]D. Ramasamy, Rampratap Meena, Shamshad Ahmed Khan. Comparative studies on fruits of two *Ficus* species using HPTLC and quality control methods. *J Pharm Res* 2011; **4**(11): 4106-4108.
[19]Dinesh K. Patel, Kanika Patel, S. P. Dhanabal. Development of quality control parameters for the standardization of *Gymnema sylvestre*. *J Acute Dis* 2012; **1**(2): 141-143.
[20]Amit Chawla, Payal Chawla, Neeru Vasudeva, Surendra K. Sharma, US Baghel. Pharmacognostic standardization of the stem of *Aerva persica* (Burm.f) Merrill (Amaranthaceae). *J Med Plants Res* 2013; **7**(11): 637-644.