International Journal of Pharma and Bio Sciences V1(2)2010

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION ON ROOT OF *CADABA FARINOSA* FORSK.

UMESH B. TELRANDHE*[1], S. HEMALATHA[2] AND ANUJ MODI[1]

[1] Department of Pharmacognosy, ADINA Institute of Pharmaceutical Sciences, Sagar, (India)
[2] Vels college of pharmacy, Pallavaram, Chennai, (India)

***Corresponding author** umed_057@yahoo.co.in

ABSTRACT

According to ethanomedical information plant *Cadaba farinosa*, Forsk is commonly known as Indian Cadaba, has been widely treating various ailments such as in diabetes, anthelmintic, purgative and anti-inflammatory. Present work is related to pharmacognostic investigation of fresh, powdered and anatomical sections of root of C*adaba fa*rinosa, Forsk were carried out to determine its macromorphological, micromorphological and chemomicromorphological profiles. These findings will be useful towards establishing pharmacognostic standards on identification, purity and quality.

KEYWORDS

Cadaba farinosa, Pharmacognostic evaluation, Physicochemical Analysis.

INTRODUCTION

Cadaba farinosa, Forsk (Capparidaceae) is unarmed shrub or tree growing up to near about 1.0 -2.0 meters in height. Older stems are smooth and purplish in color while young stems are pubescent and yellowish brown in color. Leaves are 12-25mm long and 8-12mm wide with simple entire, elliptical oblong, ovate in shape. Flowers are corymbose type, are dirty white in color. Fruits are cylindrical in shape and glabrous or pubescent while seeds are strait, surrounded by orange red aril. Wood of tree is white in color and yellow on aging[1,2]. Root, leaves, flower buds and fruit of the plant are used in traditional medicine. Roots are used in anthelmintic, emmenagogue, utrine obstruction, dysentery and female fertility. Leaves are used in amenorrohea, dysmenorrheal, purgative, rheumatism, uterine obstruction and in the preparation of medicated oil. Flower buds have stimulant, purgative, antipologistic and anthelmintic property. Fruit has edible property while ash of plant is rubbed into skin to relieve the general body pain[3,4]. Pharmacognostic and phytochemical studies on the root has not been reported. The contribution besides proving these data corroborates the claim.

MATERIAL AND METHOD

1. *Collection and Authentication of Plant Material*

The plant specimens for the proposed study were collected from Shri Venkateswara University, Tirupati, Andhra Pradesh., India, during the month of July 2008. It was authenticated by Dr. P. Jayaraman,

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION ON ROOT OF *CADABA FARINOSA* FORSK.

Director, Plant Anatomy Research Centre, Chennai. A voucher specimen no. PARC/2008/239.

2. *Chemicals*

All the solvents and chemicals used were purchased from Merck chemical (LR grade), India.

3. *Macroscopic Examination*

The root part of *Cadaba farinosa* was studied individually for its morphological characters such as shape, colour, dimension, upright or creeping, smooth or ridged and hairs present or not, etc[5].

4. *Microscopic Evaluation*

The crude root was subjected for microscopical evaluation. The paraffin embedded specimens were sectioned with the help of rotary microtome (10-12µm thick) was prepared with the help of an instrument called rotary microtome. Dewaxing of the section was done by customary procedure[6]**.** The sections were stained with toludine blue (0.25 % having a pH of 4.7) as per method suggested by O'Brien. Since Toludine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, blue to the protein bodies and violet color to cortex and calcium oxalate crystals etc. Wherever necessary sections were also stained with safranin, fast-green and Iodine (for starch). The photomicrographs in different magnifications of all necessary cells and tissues were taken with NIKON Cool Pix 8400 digital camera and Nikon labphoto-2 microscopic units. For normal observations bright field were used. For the study of crystals, starch grains and lignified cells, polarized light was employed[7].

5. ***Determination of Physical Constants***

(i) *Ash Values*

Different Ash values like total ash, water soluble ash and acid insoluble ash were determined as per standard procedure mentioned in Indian pharmacopoeia[8].

(ii*) Extractive Values*

Different extractive values like water-soluble extractive and alcohol-soluble extractive value were determined as per standard procedure mentioned in Indian pharmacopoeia [8].

(iii) *Foreign Organic Matter and Moisture Content*

Foreign organic matter was determined from the weight of the drug taken and moisture content was determined by loss on drying method in terms of percent w/w as per standard procedure mentioned in Indian Pharmacopoeia 1996[8].

(iv) *Reaction of Powdered Drug with Different Reagents*

Powdered drug was treated with different reagents and colored shown by that treatment is noted down[9].

(v) *Fluorescence Analysis*

Fluorescence characteristic of powdered drug with different reagent were observed under day light and U.V. light after drug treatment with different reagents[9].

(iv)*Qualitative Phytochemical Screening*

Freshly prepared hydroalcholic (50:50) extracts of root was tested for the presence of phytochemical constituents by using reported methods[10, 11, 12].

6. *Estimation of Phytoconstituents*

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION ON ROOT OF *CADABA FARINOSA* FORSK.

(i) Estimation of Carbohydrate

50 ml of alkaline cupric tartrate TS was pipetted into a 400ml beaker, 48ml of water was added, mixed. 2ml of above mixture that have been diluted quantitavely with water, upto 5.0% concentration. The solution was heated to boil, and continued boiling for 2 minutes. The hot solution was filtered through a tared porcelain filtering crucible; the precipitate was washed with water maintained at 60^0C, then with 10 ml of alcohol. Dried at 105^0C to constant weight. A blank determination was performed, and made any necessary correction. The corrected weight for the precipitate was compared with dextrose (carbohydrate) of known concentration[13].

(ii) *Estimation of Fat*

3.0 g of powdered drug was dissolved in 100ml water, transfer to a separating funnel, acidified with sulphuric acid and extracted with successive quantities i.e. 50, 40, and 30 ml of ether, was mixed in a separating funnel and washed with water until the washings were free from mineral acid. The ether solution was transferred to a tared flask; the ether was removed and dried the residue of fatty acids to constant weight at 80°[13].

(iii*) Estimation of Total Protein*

The total protein content was estimated by Lowry method [14].

(iv) *Estimation of Total Alkaloids*

The alcoholic extract of plant sample was treated with 0.1NHCl and aqueous acidified layer thus obtained was partitioned with $CHCl_3$ in separating funnel. The chloroform layer is rejected. The aqueous layer was basified with ammonium hydroxide and partitioned with chloroform again. The chloroform layer was concentrated and tested for alkaloids with alkaloidal reagents[15].

(v) *Estimation of Total Flavonoids*

Total flavonoid content in plant was estimated by spectrometric method. Dried powdered plant material (10 gm) was extracted by continuous mixing in 100 ml of 70% ethanol for 24 hr at room temperature. After filtration, ethanol was evaporated untill only water remained. Water phase was subsequently extracted with ethyl acetate. The extract was dried over anhydrous sodium sulphate, filtered and concentrated under vacuum up to a concentration of 1gm/ml of extract. They were further diluted with ethyl acetate to obtain 0.01gm/ml solutions used in the experiments. About 10ml of the solution was transferred into a 25ml volumetric flask, to this 1 ml of 2% $AlCl_3$ was added and the solution was make upto 25ml with methanol-acetic acid and was kept aside for 30min, the absorbance was measured at 390nm against the same solution without $AlCl_3$ being blank. Luteolin was used to construct the calibration curve in the concentration range 1.0-10.0 µg/ml. Result was calculated by using caliberation curve method[16].

7. *Inorganic Mineral Analysis*

Determination of Copper, Selenium, Iron, Phosphorous, Magnesium and Chromium by Atomic absorption spectroscopy

Determination of heavy metals was carried out by using "Perkin Elmer" atomic absorption spectrophotometer by calibration curve method. Nitric acid and perchloric acid used in the preparation of sample solution to be examined and also were used in same concentration in standard solution preparation. After calibration of the instrument, each standard was introduced into the flame three times and the steady reading was recorded. A calibration curve was

prepared by plotting the mean of each group of three readings against the concentration. The sample solution prepared above was then introduced into the flame, the reading recorded. Using the mean of the three readings, the concentration of the element was determined from the calibration curve[17].

RESULTS AND DISCUSSION

1: ***Macroscopic Evaluation***

Table 1.
Macroscopic Evaluation

Evaluation Parameter	Results
Colour	Yellowish brown
Odour	Odour less
Taste	Light bitter
Shape	Cylindrical
Size	Up to 3mm long
Facture	Small groove and irregular fissures on surface

2. ***Microscopic Evaluation***

(i) Periderm and Cortex

The root has thick superficial periderm which has shallow and irregular fissures and large exfoliated flakes. The periderm comprises of outer thick zone of phloem with dark contents and inner wide colourless phelloderm. The cortex is wide and parenchymatous with scattered masses of Sclerids. Secondary phloem is a narrow cylinder of sieve elements and phloem parenchyma (Figure 1).

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION ON ROOT OF *CADABA FARINOSA* FORSK.

T.S of the Root showing Periderm

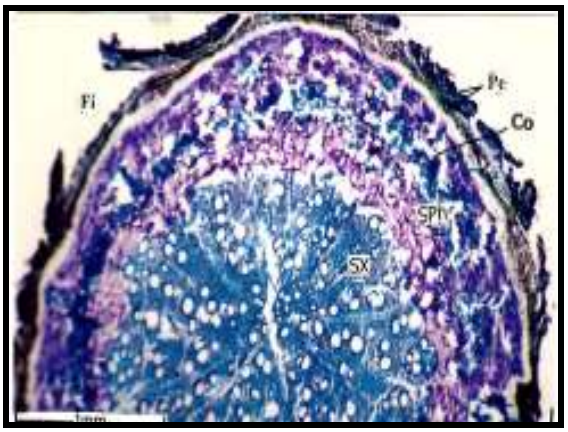

Figure 1

Co-Cortex; Fi- Fibres; Pe-Periderm; SPh-Secondary Phloem; SX-Secondary xylem

(ii) *Secondary xylem*

Secondary xylem is in the form of wide, solid, dense cylinder measuring 1.5 mm wide. It consists of vessels which are arranged in radiating wings, which are narrow in the center and gradually expanded towards the periphery (Figure 2). The vessels are thick walled, circular and solitary. The narrow vessels are 20µm wide while the wider vessels are 50 µm in diameter. The xylem rays are wide and dilated. They have dense accumulation of starch grains. The xylem fibers are thick walled with heavy lignifications. The lumen of the fibers ranges from 5-10µm wide (Figure.3).

Central Portion of the Secondary Xylem enlarged

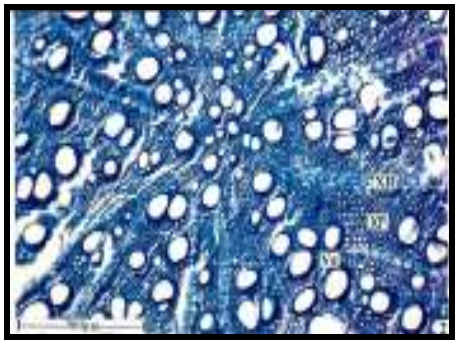

Figure 2 ***Shows enlarged view of central portion secondary xylem***

Outer Portion of the Secondary xylem enlarged

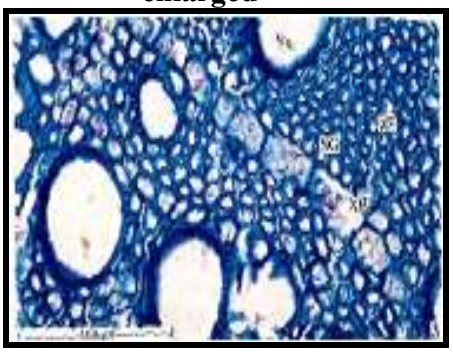

Figure 3 ***Shows enlarged view of outer portion of secondary xylem***

[Ve-Vessel; XF-Xylem fibres; XR-Xylem ray SG-Starch Grain]

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION ON ROOT OF *CADABA FARINOSA* FORSK.

(iii) ***Periderm***

The periderm has outer zone of dark, compressed, tabular phellem cells, fissured at several places. Inner to the phellem is equally wide phelloderm which is undulate or wavy in outline. The phelloderm cells are thin walled and hyaline (Figure.4).

Outer sector – Periderm of root bark

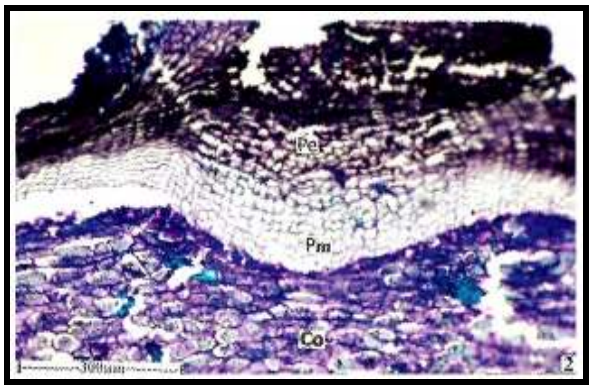

Figure 4

T.S of Root bark showing enlarged view of Periderm [Co-Cortex; Pe-Periderm; Pm-Phellem]

(iv) ***Sclerids and Secondary Phloem***

The Sclerids are hexagonal or elongated or rectangular, scattered in the cortical zone are large masses of levaehy Sclerids. They have thick liquefied walls and narrow canal like pits. Secondary phloem is 300 µm wide. It consists of narrow ray and regular radial files of sieve-elements and parenchyma cells (Figure 5).

Root bark Inner Sector - Sclerids and Secondary Phloem

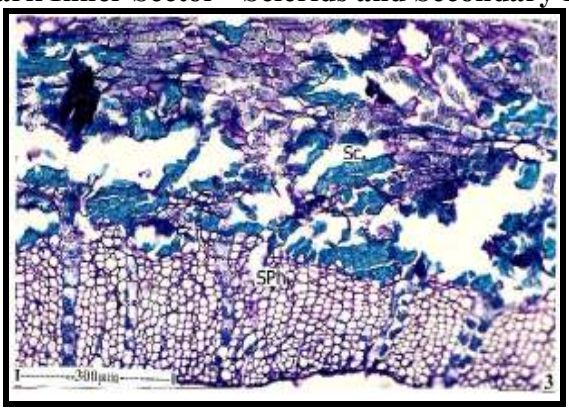

Figure 5

T.S of root bark showing sclerid and secondary phloem [Sc-Sclerids ; SPh-Secondary Phloem]

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION ON ROOT OF *CADABA FARINOSA* FORSK.

(v) ***Sieve Elements and Non Collapsed Phloem***

The sieve elements are rectangular in outline; the companion cells are on the lateral side of the Sieve element. The sieve elements are 20-25µm wide in tangential plane (Figure 6)

Sieve Elements and Non Collapsed Phloem

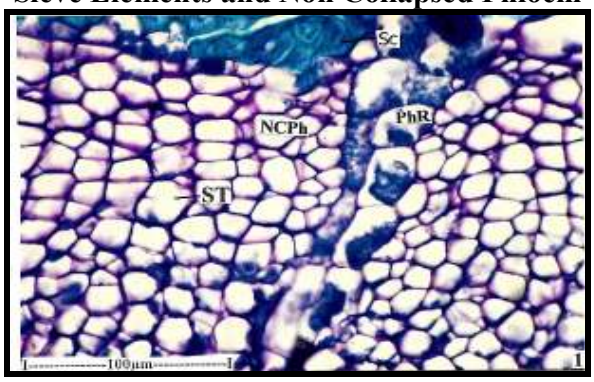

Figure.6

T.S. of Root Showing Sieve Elements and Non Collapsed Phloem [NCPh-Non-Collapsed phloem; PhR-Phloem ray; Sc-Sclereids; ST-Sieve]

(vi) ***Starch grains***

The cortex has tangentially elongated, compact thin walled parenchyma cells, starch grains are abundant in the cortical parenchyma. Prismatic crystals are also sparsely seen in the cortical cells (Figure 7).

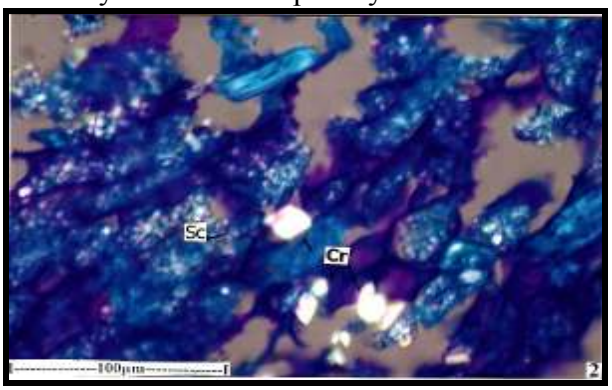

Figure 7

T.S. of Root Showing Crystals in the Cortical Tissue [Cr-Crystals; Sc-Sclerids;]

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION ON ROOT OF *CADABA FARINOSA* FORSK.

3. ***Determination of Physical Constants***

(i) Ash Values

Table 2.
Ash Values

Evaluation parameter	Yield (% w / w)
Total ash	6.0
Water- soluble ash	1.0
Acid insoluble ash	4.0

(ii) ***Extractive value***

Table 3.
Percentage Yield of Extract in Different Solvent

Extractive Value	% yield (w/w)
Alcohol soluble extractive value	25.65
Water soluble extractive value	73.6%

(iii) ***Foreign Organic Matter and Moisture Content***

Table 4.
Foreign Organic Matter and Moisture Content Values

Particulars	Results (% w / w)
Foreign organic matter	0.1%
Moisture content	9.0 %

(iv) ***Reaction of Powdered Drug with Different Reagents***

The behavior of powder with various reagents under ordinary light by naked eye is given in Table 5

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION ON ROOT OF *CADABA FARINOSA* FORSK.

Table 5.

Behavior of the Powder of Cadaba farinosa with Different Chemical Reagents

Treatment	Colour
Powder as such	Yellowish white
Powder + Conc.sulphuric acid	Brownish black
Powder + Conc. nitric acid	Reddish brown
Powder + Conc. Hydrochloric acid	Brownish yellow
Powder + 5% I_2	Brownish yellow
Powder + 5N NaOH	Pale yellow
Powder + glacial Acetic acid	Pale green
Powder + 80% H_2SO_4	Reddish brown

(v) ***Fluorescence Analysis***

The fluorescence analysis of powder with various reagents is observed under day light and U.V. light is given in Table 6**.**

Table 6.

Fluorescence Characteristics of Powdered Drug of Root Part of Cadaba farinosa

Reagents	Day light	UV Light
Drug Powder	Greenish Yellow	Green
Drug Powder +1M NaOH	Brownish green	Green
Drug Powder + alcoholic 1M NaOH	Greenish yellow	Pale Green
Drug powder + 1M HCl	Yellowish Brown	Faint yellow
Drug Powder + 50 % HNO_3	Greenish Yellow	Green
Drug powder + 5% $FeCl_3$	Brown	Green
Drug powder +80% H_2SO_4	Yellowish Brown	Dark Green
Drug powder + Water	Faint yellow	Yellowish green
Drug powder+Conc.H_2SO_4	Black	Yellowish green
Hydro alcoholic extract	Brownish yellow	Faint green

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION ON ROOT OF *CADABA FARINOSA* FORSK.

(iv) ***Qualitative Phytochemical Screening***

Table 7.
Results of Phytochemical screening

Constituents	Tests	EF	AQ
Alkaloids	a) Mayer's reagent	+	+
	b) Dragendorff's reagent	+	+
	c) Hager's reagent	+	+
	d) Wagner's reagent	+	+
Sugars and Carbohydrates	a) Molish's reagent	–	+
	b) Barfoed's test	–	+
	c) Fehling's solution test	–	+
	d) Benedict's test	–	+
Glycosides	a) Keller-Killiani test	–	+
	b) Borntrager's test	–	–
	c) Legal's test	+	–
	d) Baljet's test	–	–
Protein	a) Million's test	–	+
	b) Biuret test	–	+
	c) Xanthoproteic test	–	+
Amino acid	Ninhydrin test	–	+
Saponin	Foam test	–	+
Flavanoids	Shinoda test	+	+
Tannins	a) Ferric chloride solution	+	+
	b) Lead acetate test	+	+
	c) Gelatin solution test	–	+
	d) Bromine water	–	+
	e) Potassium dichromate	+	+

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION ON ROOT OF *CADABA FARINOSA* FORSK.

Phenolic compounds	a) Ferric chloride solution b) Lead acetate solution	+ +	+ +
Terpenoid	Noller's test	_	_
Fixed oil and fats	a) Spot test	_	_
Gums and mucilage	Swelling test	_	+
Steroids	Salkowski reaction	+	_

EF: Ethyl acetate Extract, AQ: Aqueous extract, (+) Present, (-)Absent

(v) Estimation of Phytoconstituents

Each 100 grams of sample contains

Table 8.

Amount of Phytoconstituents Present in Per 100gm of Cadaba farinosa.

Phytoconstituent	**% w/w**
Fat	1.48
Protein	4.22
Carbohydrate	7.03
Total alkaloids	0.0786
Total flavanoids	0.2156

(vi) ***Inorganic Minerals Analysis***

Estimation of Copper, Selenium, Iron, Phosphorous, Magnesium and Chromium estimated by Atomic Absorption Spectroscopy

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION ON ROOT OF *CADABA FARINOSA* FORSK.

Table 9.
Estimation of metals/Heavy metals in dried material of Cadaba farinosa.

Name of the Inorganic Mineral	Amount in mg/ 100gm of extract
Phosphorous	9.58
Magnesium	0.787
Chromium	In traces
Copper	2.126
Selenium	Below detectable level
Iron	0.1009

CONCLUSION

In conclusion, the present study is related to pharmacognostical and phytochemical account of *cadaba farinosa,* Forsk. root provided useful information in regard to its correct identity and evaluation, and help to differentiate from the closely related other species of *cadaba farinosa,* Forsk. The other parameters observed are also useful for the future identification of the plant, and serves as a standard monograph for identification and evaluation of plant.

REFERENCES

1. Kirtikar K.R. and Basu B.D. Indian Medicinal Plants**,** International book Distributors: 259-261, (1999).
2. The Wealth of India-A Dictionary of Indian Raw Materials and Industrials products, C.S.I.R Publications, 1: 178-179, (1998).
3. Wallis TE, Ed. Textbook of Pharmacognosy. 5th Edn, CBS Publications: 121-127, (1985).
4. Nadkarni A.K., Indian Materia Medica, 3rd Edn. (1), Popular Prakashan: 225-226, (2004).
5. World Health Organization: Quality control methods for medicinal plant materials, WHO Library: 128-135, (1998).
6. Johansen DA., Ed. Plant Microtechnique. Mc Graw Hill Book Co.: 523-524, (1940).
7. O'Brien TP, Feder N and Mc Cull ME. Ed. Polychromatic staining of plant cell walls by toluidine blue-O Protoplasma; 59: 364-373, (1940).
8. Indian Pharmacopoeia, Government of India, Ministry of Health, Controller of Publications, Vol. II, 3rd Edn., 74, (1985).
9. Ansari M.M., Ahmad J., Ahmad A. and Ansari S.H., Pharmacognostic characterization and standardization of *Morus alba* stem bark. Journal of Medicinal and Aromatic Plant Sciences, 28, 31-36, (2006).
10. Khandelwal KR, Ed. Practical Pharmacognosy Technique and Experiments, 23rd Edn: 15-29, 149-56, (2005).
11. Kokate CK. Ed. Practical Pharmacognosy, 4th Edn., Vallabh Prakashan: 112-120, (1994).

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION ON ROOT OF *CADABA FARINOSA* FORSK.

12. Farnsworth NR., Biological and phytochemical screening of plants. J. Pharm. Sci. 55: 225-276, (1966).
13. Gabriel I. Giancaspro, Dietary Supplements: Botanicals, USP 27-NF 22, 2013.
14. Lowry O.H., Rosebrough N J. and Farr A L., Randall R J. Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem. 193, 265-275, (1951).
15. Ferguson N.M.A text book of pharmacognosy, Macmillian Company:191-195, (1956).
16. Kadifkova panovska T., Kulevanova S., Marina stefova**, (**2005) *In vitro* antioxidant activity of some *Teucrium* species (Lamiaceae). Acta Pharm, 55, 207-214.
17. Anonymous$_{a.}$ Indian Pharmacopoeia, Govt. of India, Ministry of Health and Family Welfare, Controller of Publications, A53-A55, (1996).