



BOTANICAL IDENTIFICATION AND PHYSICOCHEMICAL INVESTIGATION OF LEAF OF NILI-NIRGUNDI (*JUSTICIA GENDARUSSA*)

Patel Sonal^a, Kapadia Nayana^b, Shah Bakula^b and Shah Mamta^b

a. Department of Pharmacognosy, K.B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India.

b. Department of Pharmacognosy, L.M. College of Pharmacy, Navrangpura, Ahmedabad-380009, Gujarat, India.

*Corresponding author's E-mail: sonal999patel@gmail.com

Accepted on: 23-05-2011; Finalized on: 25-08-2011.

ABSTRACT

The recent global resurgence of interest in herbal medicines has led to an increase in the demand for them. Commercialization of the manufacture of these medicines to meet this increasing demand has resulted in a decline in their quality, primarily due to a lack of adequate regulations pertaining to this sector of medicine. The need of the hour is to evolve a systematic approach for authentication of herbal plant and to develop well-designed methodologies for the standardization of herbal plant. Therefore, the present work deals with pharmacognostical study of a well-known folklore remedy for chronic rheumatism "*Justicia gendarussa* Burm". The pharmacognostical studies are carried out in terms of morphology, qualitative and quantitative microscopy, powder study, ash values, extractive value and loss on drying. Hence, the present attempt was undertaken to investigate the pharmacognostical study of *J. gendarussa*.

Keywords: *Justicia gendarussa*, Physicochemical, Acanthaceae.

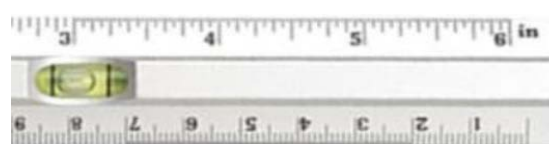
INTRODUCTION

India has a rich cultural heritage of traditional medicines. The crude drugs being always available easily in abundance, comparatively cheaper, with negligible side effects and have frequently been prescribed to patients of all age groups. The multiple therapeutic action and uses of these drugs are sufficiently described in classical literature on indigenous medicines in many medicinal plant books. Various species of Genus *Justicia* are being used traditionally for wide varieties of ethnomedicinal purposes. *Justicia gendarussa* belongs to family *Acanthaceae* is commonly known as Nili-Nirgundi¹. The present plant is up to one meter height and found in tropical and subtropical parts of Asia and in India at seashore area like Valsad, Surat and Hills like Khasi hills, Pavagarh. *J. gendarussa* herb is cultivated in Indian gardens for its attractive foliage and flowers^{2,3}. Flowers of *J. gendarussa* are white with purplish ting when fresh. Ethnobotanically, decoction of leaf of *J. gendarussa* is a popular remedy for chronic rheumatism^{5,7}. Leaves of this plant are also used in fever, cough, jaundice, thrush, arthritis, cephalgia, hemiplegia, facial paralysis, otalgia, hemicrania and bronchitis liver disorders^{8,9}. A Literature survey and screening of scientific data revealed that drugs have not been investigated for their systematic standardization, botany and chemistry. The present investigation include pharmacognostical study of *J. gendarussa* is therefore taken up to establish certain botanical standards which would help in crude drug identification as well as in checking adulteration, if any. Further, the study will greatly help in quality assurance of herbal drugs.

MATERIALS AND METHODS



Herb of *Justicia gendarussa*



Morphology of leaf

Figure 1: Morphology of *Justicia gendarussa*

Leaf of *Justicia gendarussa* were collected from Anand farm and nursery Gandhinagar, Gujarat. The plant was authenticated at Department of Botany, School of science, Gandhinagar, Gujarat. The voucher specimen was kept at department of Pharmacognosy K.B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat. The fresh sample of leaf was subjected to morphological and microscopical study and after drying leaves were powdered and passed through 60 mesh size and stored in an airtight container for further use. Macerates were prepared by the Schulz maceration method⁷. Photomicrographs were shot for histological observation (Labomed). Powder of dried leaf was used for chemical analysis. Physicochemical studies of the powdered drug such as ash value, extractive value, loss on drying and crude fiber content were performed according to the WHO guidelines.^{9, 10} Extract of dried leaf powder of *Justicia gendarussa* was prepared by using soxhlet extractor in successive manor with petroleum ether, methanol and water on polarity basis. The dried extracts were then stored in airtight containers until usage. This three extract was analyzed for the presence of alkaloids¹¹, flavanoids^{12,13}, saponin^{14,15}, carbohydrates¹⁶, steroids¹⁷, triterpenoids¹⁸, carotenoids, amino acids, tannins^{19,20}, phenolics^{21,22}, coumarines^{23,24} and anthraquinones²⁵ using standard procedure.

Quantitative Standards

Determination of phenolics²⁶

To 1 ml of the methanolic extract of leaf of *J. gendarussa*, 10 ml of distilled water and 1.5 ml of diluted (1:2) Folin ciocalteu reagent were added and the mixture was kept a side for 5 min. After adding 4 ml of 20 % w/v Na₂CO₃ solution the final volume was adjusted to 25 ml using distilled water. The absorbance was measured at 765 nm at an interval of 30 min up to 2 h using distilled water as a blank. The total phenol content was measured using following formula: $C = A \times 282.6 - 8.451$ where, A= Absorbance.

Determination of Saponin²⁷

According to the results obtained from foaming test and high foaming index of leafs of *J. gendarussa*. Further study was carried out for estimation of total saponin content. Pour the decoction into 10 stoppard test-tubes (height 16cm, diameter 16mm) in successive portions of 1ml, 2ml, 3ml etc. up to 10ml, and adjust the volume of the liquid in each tube with water to 10ml. Stopper the tubes and shake them for 15sec, two shakes per second. Allow standing for 15 min and measuring the height of the foam. Froth number is calculated.

Estimation of flavonoids (by AlCl₃ method)²⁸

Preparation of extract

0.1g of air-dried leaf powder was extracted with 100 ml methanol by maceration for 24 h and filtered. The final volume of the filtrate was adjusted to 100 ml using methanol. 1 ml of this extract was diluted up to 10 ml

with methanol and was used for the estimation of flavonoids.

Method

To 3 ml of the methanolic extract 3 ml of methanolic AlCl₃ was added. After 10 min, the absorbance was read at 430 nm. Results were expressed in g/ 100g of dry matter with respect to Rutin serves as a standard.

Estimation of total alkaloids:^{29,30}

Preparation of extract

10g coarsely powdered material (leaf) was extracted with 25 ml of 2%v/v aqueous acetic acid and ethanolic acetic acid separately at 100° for 10 min. The procedure was repeated 2 times. The extracts were mixed and diluted to 100 ml with 2%v/v acetic acid and ethanolic acetic acid respectively.

Procedure for Assay of alkaloids

5 ml amount of the extract was taken and the pH was maintained at 2- 2.5 with dilute HCL. 2 ml amount of Dragendorff's reagent (DR) was added to it, and the precipitate formed was centrifuged. The centrifugate was checked for complete precipitation by adding DR. After that the precipitates were washed with alcohol. The filtrate was discarded and the residue was then treated with 2 ml disodium sulfide solution. The brownish black precipitate was dissolved in 2 ml concentrated nitric acid, with warming if necessary. This solution was diluted to 10 ml in a standard flask with distilled water; 1 ml was then pipetted out, and 5 ml thiourea solution was added to it. The absorbance was measured at 435 nm against the blank containing nitric acid and thiourea. The factor is obtained from the standard curve, which is a constant for different concentrations.

Estimation of triterpenic acids³¹

Preparation of extract

0.5g of air-dried leaf powder (60#) was extracted with 50ml methanol by maceration for 24h and filtered. The final volume of the filtrate was adjusted to 50ml using methanol.

25 ml of the methanolic extract was evaporated to dryness and then extracted with chloroform containing 0.05%v/v acetic acid. Then it was further extracted with 10%w/v sodium carbonate. Separate the aqueous layer and acidify with formic acid or acetic acid. After that it was further extracted with chloroform and organic layer was evaporated to dryness. Then it was reconstituted in 50 ml alcohol and was used for triterpenic acid estimation.

Method

50 ml of alcoholic solution was titrated against 0.1 N sodium hydroxide (NaOH) using phenolphthalein as an indicator.

Factor: 1 mol 0.1 N NaOH = 1 mol of triterpenic acid.



Estimation of carbohydrates^{32,33}**(A) Estimation of sugar (Phenol sulphuric acid method)****Preparation of test solution**

1g of powder was added to the 20 ml 80%v/v ethanol containing centrifuging tube, which was tightly closed and kept in boiling water bath for 20 min and subsequently cooled to room temperature. The supernatant was collected in a 100ml beaker after

Method

Prepared 10, 20, 30...90 μ /ml concentration of standard glucose solution (100 μ /ml). Take 9 test tubes, to the each test tube 2 ml standard glucose solution of different concentrations, to this 1ml of 5 %w/v phenol solution and mixed well in test tubes. Test sample was prepared in the same way. Blanks were prepared with 1ml of water instead of sugar solution. The yellow orange color developed was stable for several hours. Absorbance was measured at 482nm.

The amount of sugar was determined by reference to a standard curve previously prepared for glucose being assayed.

(B) Estimation of starch: (Anthrone colorimetric method)**Preparation of test solution**

To the residue left after sugar extraction, 10 ml of distilled water was added, while stirring, 13 ml of 52%v/v perchloric acid was added. The mixture was kept cold (4°) all the time. The content was stirred for about 5min continuously with a glass rod and occasionally thereafter for 15 min. After that 20 ml of distilled water was added, stirred and contents were centrifuged at 10,000 g for 10 min.

Standard Glucose solution was prepared (100mg/100 ml).

Method

Prepared 12.5, 25, 37.5, 50, 62.5, 75, 87.5, 100 μ /ml concentrations of standard glucose solution (500 μ /ml). Take 9 test tubes, to the each test tube added 3 ml standard glucose solution of different concentrations, then cooled in an ice bath and 10ml of fresh anthrone reagent (0.2% w/v in 95%v/v cold H₂SO₄).

The color intensity of the solution was read at 620nm using spectrophotometer (Shimadzu 1600). A standard curve was prepared using different concentration of standard glucose solution to estimate starch content.

Estimation of carotenoids³:**Preparation of extract**

10g of fresh leaves grind and was extracted with 20ml \times 5 *n*-hexane by maceration for 24 h and filtered. The final volume of the filtrate was adjusted to 100ml using *n*-hexane.

Method

Take 3 ml of the *n*-hexane extract and determine the density of solution as soon as possible with spectrophotometer at 436 nm. Results were expressed in g/ 100g of matter with respect to β – carotene (1mg/ml) serves as a standard.

RESULTS**Macroscopy**

Leaf is simple, opposite, decussate, exstipulate, petiolate small, lanceolate, 7.5 to12.5 cm long, crenate, obtuse, glabrous, main nerves about 8 pairs, midrib and main nerves prominent violet colored on the lower surface, dark violet above and pale green beneath, except Valsad sample, which was lemon green in color (Figure-1).

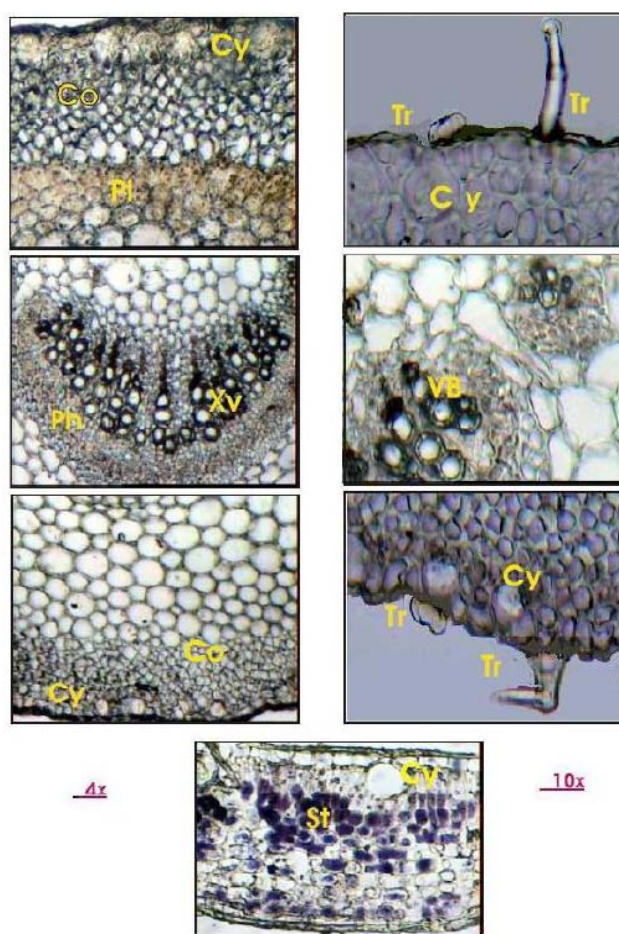
Microscopy

Figure 2: Transverse section of leaf of *J. gendarussa*

T.S of leaf shows midrib and lamina portion.

The midrib shows a planoconvex outline in the basal and biconvex in middle and apical regions. It shows a single layered epidermis covered externally with cuticle and presence of both covering (uniserrate and unicellular or multicellular) and glandular trichomes (Tr) (having unicellular stalk and multicellular head). Collenchyma (Co) is well developed, which is present bellow the upper epidermis and above the lower epidermis. In the middle region, there are three vascular bundles (VB), which are

collateral and open. The central one is large and arc shaped.

Lamina is dorsiventral type, differentiated into palisade and spongy parenchyma. Upper epidermis (Ep) is single layered, covered with a cuticle. It shows the presence of glandular trichomes with unicellular stalk and multicellular head and cystoliths (Cy). Lower epidermises is similar to upper, but on the lower surface mostly diacytic and few anisocytic type of stomata are present. The palisade cells are two layered in the lamina and becomes single layered in the midrib (Figure- 2, 3).

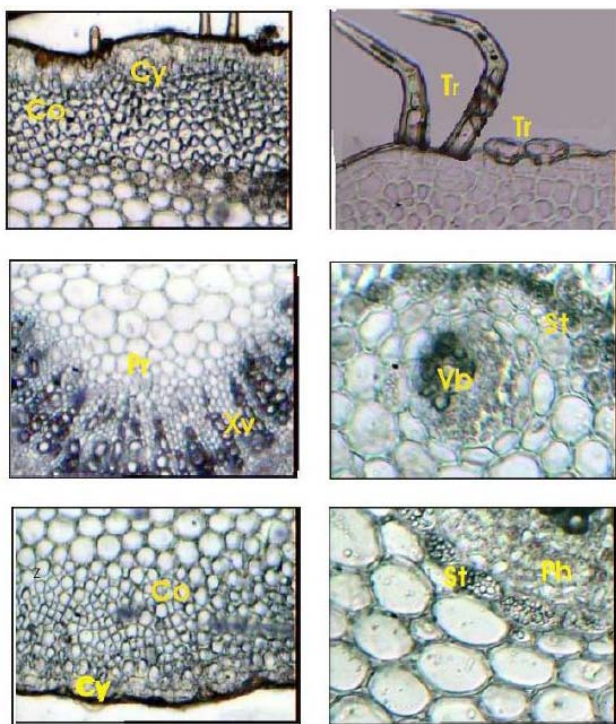


Figure 3: Transverse section of petiole of leaf of *J. gendarussa*

Powder study

The diagnostic characters are shown in Figure- 4.

Presence of fragments of upper and lower epidermis showing epidermal cells, stomata, cystoliths, and glandular trichomes. The epidermal cells are irregularly shaped having wavy and anticlinal walls. Stomata are diacytic and anisocytic type; the former are more in number, with abundant glandular trichomes.

Lower epidermal cells are polygonal and with glandular trichomes of which few are with unicellular or multicellular stalk and multicellular head.

Simple covering trichomes comparatively are less in number; cystoliths are oval to oblong with warty surface and starch grains are simple. (Table-1)

Physico-chemical standards like ash values, extractive values and loss on drying was showed in Table- 2.

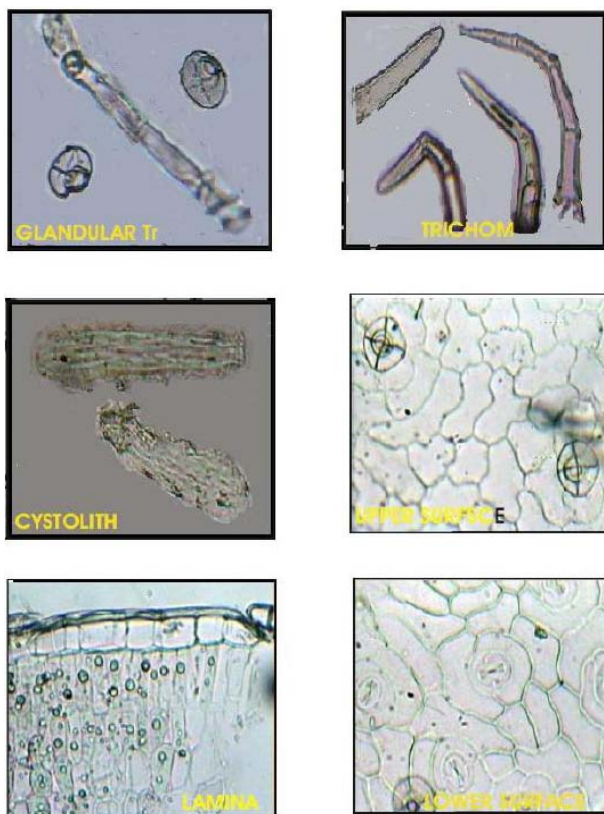


Figure 4: Power study of leaf of *J. gendarussa*

Table 1: Quantitative Microscopy of leaf of *J.Gendarussa*

S. No	Parameters	Measurement
1.	Cystolith Mid rib Lamina length width	27-36 μ 2-46 μ 44-56.4 μ 60-104 μ
2.	Stomatal index (lower)	19.6 \pm 0.980
3.	Palisade ratio	04.9 \pm 0.245
4.	Vein islets	09.5 \pm 0.475
5.	Vein termination	12.0 \pm 0.600
6.	Trichomes	
	Simple	84-182 μ
	Knee shape	78.5-126.8-168

Standard deviation (SD) = \pm SD; Number of readings (N) =3

Table 2: Physico-chemical parameters of leaf of *J.Gendarussa*

Quality Parameters	Samples % w/w
Ash value	
a. Total ash value	14.1 \pm 0.705
b. Acid insoluble ash	0.74 \pm 0.037
c. water soluble ash	4.84 \pm 0.242
Extractive value	
a. Water soluble extractive	16.52 \pm 0.626
b. Alcohol Soluble extractive	12.42 \pm 0.821
c. Acetone soluble extractive	03.48 \pm 0.174
d. Chloroform extractive	00.98 \pm 0.049
e. Petroleum ether extractive	01.60 \pm 0.08
Loss on drying	13.7% \pm 0.34

Standard deviation (SD) = \pm SD; Number of readings (N) =3

Phytochemical screening shows the presence of alkaloid, phenolics, steroids, triterpenoids, carotenoids, saponins in different extract as per Table- 3.

Table 3: Phyto-chemical screening of different extracts of leaf of *J.gendarussa*

Phytoconstituents	Tests	Petroleum ether extract	Methanolic extract	Water extract
Alkaloid	Dragondroff's reagent test	-ve	+ve	+ve
Flavonoid	Shinoda test	-ve	+ve	-ve
	Fluorescence test	-ve	+ve	-ve
Phenolics	With FeCl ₃	-ve	+ve	+ve
	With Folin ciocalteu reagent	-ve	+ve	+ve
Carbohydrates	Molisch's test	-ve	+ve	+ve
	Fehling test	-ve	+ve	+ve
Steroids and triterpenoids	Lieberman burchard test	+ve	-ve	-ve
	Salkowski reaction	+ve	-ve	-ve
Carotenoids	Antimony trichloride	+ve	-ve	-ve
	Sulphuric acid	+ve	-ve	-ve
	Hydrochloric acid	+ve	-ve	-ve
Tannins	Test with gelatin	-ve	-ve	-ve
	Test with lead acetate	-ve	-ve	-ve
Saponins	Froth test	-ve	+ve	+ve
	Haemolytic zone test	-ve	+ve	+ve
Coumarins	With ammonia	-ve	-ve	-ve
	With hydroxylamine hydrochloride	-ve	-ve	-ve
Anthraquinone glycoside	Borntrager's test	-ve	-ve	-ve
	Modified borntrager's test	-ve	-ve	-ve

Standard deviation (SD) = \pm SD; Number of readings (N) =3

The leaf was found to be rich in carotenoids (5.8-7.88%W/W), phenolics (1.82-2.21%w/w), alkaloids (1.38-1.62%w/w), flavonoids (1.93-2.03%w/w) and carbohydrates (8.31-8.74), triterpenic acids (0.128-0.199% w/w) as shows in Table-4.

Table 4: Estimation of phytoconstituents of leaf of *J.Gendarussa*

Sr. No.	Phytoconstituents	% w/w value
1.	Carotenoids	7.88 \pm 0.394
2.	Alkaloids	1.62 \pm 0.081
3.	Phenolics	2.21 \pm 0.11
4.	Flavonoids	2.03 \pm 0.105
5.	Triterpenic acids	0.199 \pm 0.009
6.	Carbohydrates	
	Sugar	8.74 \pm 0.435
	Starch	5.85 \pm 0.292

DISCUSSION

The above studies provide information in respect of their identification, chemical constituents and physicochemical characters which may be useful for pharmacognostical study and standardization of herbal drugs of folk medicinal practice of present era and enrichment of traditional medicine. It will also determine therapeutic diagnostic tools for the scientists who are keen and sincere to evaluate the herbal medicine of indigenous resources.

Morphological identity of leaf of *J. gendarussa* is midrib and main nerves prominent violet colored on the lower surface and bilabiate flower which is characteristics of acanthaceae family. In microscopical study it shows presence of cystolith, diacytic stomata, Knee shaped trichomes and sessile glandular trichomes which, supports the fact that, this plant is belongs to acanthaceae family. Identifying character of leaf is

presence of cystolith in epidermis and starch grains in endodermis and lamina. Acid insoluble ash was lower because of the presence of cystolith in large number. Petroleum ether extract of is yellow to orange in color, that indicates petroleum ether extract having carotenoids.

Phytochemical screening indicated that the leaf of *Justicia gendarussa* was rich in carotenoids, with phenolics, alkaloids, carbohydrates, flavonoids (flavanone), sterols and triterpenoides as other important constituents. The carotenoids content was found to be 5.8-7.88%w/w, total phenol content 1.82-2.21%w/w. Alkaloidal content was found to be 1.38-1.62%w/w. Thus, the preliminary phytochemical tests are helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compounds. Therefore, the work on pharmacognostical characteristics and phytochemical screening of *J. gendarussa* provide useful information, which may help in authenticating the genuine plant along with nature of phytoconstituents present in it.

Present study is the first time report of standardization of *Justicia gendarussa* leaf.

REFERENCES

- Handa SS, Sharma A, Chakraborti KK, Evaluation of Indian herbal hepatoprotective drugs. *Fitoterapia* 57(5), 1986, 307-351.
- Doreswamy R and Sharma D, Plant drugs for liver disorder management. *Indian Drugs* 32(4), 1995, 139-154.
- Ashok Bendre and Ashok Kumar, A Text Book of Practical Botany. 6th edition, Rastogi Publication 2, 1996, pp. 16-21.



4. Kirtikar CR and Basu BD, Indian Medicinal Plants, F. L. M. Basu, Allahabad, 2, 1935, pp.1861.
5. Metcalf CR, Chalk L. Anatomy of the Dicotyledons. Clarendon Press, Oxford, 2, 1950, 1014-1024.
6. Chava AR and Oza GM, The Flora of Pavagadh. M. S. University Publications Sales Unit, Baroda, India, Botanical Memoirs, 1966, pp. 183.
7. Henry Collett and Botting W, Flowering Plants of Simla: The Neighborhood. Flora of Simlensis, Thacker, Spink and Co. London, 1921, pp. 369.
8. ANONYMOUS. The Wealth of India: Raw Materials, Council of Scientific and Industrial Research, New Delhi, 5(H-K), 1959, pp. 312.
9. Kirtikar CR and Basu BD, Indian Medicinal Plants, F. L. M. Basu, Allahabad, 2, 1935, pp. 1896-1898.
10. Anonymous. WHO Guidelines, 1st ed. Delhi, A.I.T.B.S. Publishers and distributors, 2002, pp. 40-43.
11. Geissman A, Modern Methods of Plant Analysis, Peach K and Tracy MV ed. Heidelberg, Berlin, Springer Verlag, 3,1955, pp. 471.
12. List PH and Horhammer L, Hager Hand buch der pharmazeutischem praxis. Berlin, Springer Verlag Band, 1, 1967, pp. 256.
13. Geissman A, Peach K and Tracey MV, Morden methods of plant analysis. Springer Verlang, Berlin, Gottingen, Heidelberg, 3, 1955, pp. 473.
14. Fishcher R, Praktikum der Pharmakognosic, 3rd ed. Berlin, Springer Verlag, 1952, pp. 362.
15. Evans WC and Evans D, Trease and Evan's Pharmacognosy, 15th ed. London, W.B. Saunders company Ltd., 2002, pp. 193.
16. Griffin WJ, Owen WR and Perkin JE, A Phytochemical survey of eastern Australian plants for saponins. *Planta Medica*, 16(1), 1968, 75-81.
17. Simes JH, Tracey JG, Webb LJ and Dunstan WJ. An Australian Phytochemical survey - Saponins in Eastern Australian Flowering Plants. Australia common wealth scientific Industrial Research organization Bulletin, 1959, 281.
18. Wilson JA and Merill HB. Analysis of Leather and Material used in making it, 1st ed. New York, The Mcgraw Hill Book Co. Inc., 1931, pp. 290-293.
19. Freudenberg K and Weinger K. The Chemistry of Flavonoid Compounds. Geissman A ed. Oxford, Pregamon Press. 1962, pp. 211.
20. Robinson T, The Organic Constituents of Higher Plants, their Chemistry and Interrelationships. Minneapolis 15 Minn., Burgers publishing company, 1964, pp.64.
21. Clerk JD, Descamps A and Vander Meersch E. Colorimetric Method for Determining Tannin. Bulletin Association Anciens etud. Brass, University Louvain, 43(4), 1947, 68-76.
22. Harborne JB, Phytochemical Methods, 2nd ed. London, Champan and Hall Ltd. 1973, pp. 42.
23. Feigl F, Identification of individual organic compound. In: Spot Tests in Organic Analysis. 4th ed. London, Elsevier Publishing Company, 1956, pp.237.
24. Feigle F, Identification of individual organic compound. In: Spot Tests in Organic Analysis, 4th ed. London, Elsevier Publishing Company, 1956, pp. 419-421.
25. Kokate CK, Purohit AP and Gokhale SB, Pharmacognosy, 12th ed. Nirali prakashan, 1999, pp. 145-155.
26. Anonymous. WHO Guidelines, 1st ed. Delhi, A.I.T.B.S. Publishers and distributors, 2002, pp. 45-46.
27. List PH, Horhammer L, Hager Hand Buch der Pharmazeutischem Praxis. Berlin, Springer Verlag Band, 1, 1967, pp. 447.
28. Singleton VL and Rossi JA, Colorimetry of total phenolics with phosphomolybdic phosphotungustic acid reagent. *American J Enology Viticulture*, 16(3), 1965, 144 -58.
29. Baharam T, Gressier B, Trotin F, Brunet C, Dine T and Pinkash M, Oxygen spicies scavenging activity of phenolic extracts from Hawthornfresh plant organsand pharmaceutical preparations. *Drug Research*, 46, 1996, 1086-1089.
30. Sreevidya Narsimhan and Mehrotra Shanta, Spectrophotometric method for estimation of Alkaloids. *Journal of AOAC International* 86(6), 2003, 1124-1127.
31. Khoda V, Kasar Rp and Ladda KS, A modified method of estimation of alkaloids in *R. serpentina* by colourimetric method. *Indian Drugs*, 43(3), 2006, 252-254.
32. Gupta RK, Gupta VK, Gupta VN and Atal CK, Estimation of total tritrpeneacids. *Indian Drugs*, 1984, 523-525.
33. Gurusamy Chinnasamy and Arya kumar Bal, Seasonal changes in carbohydrates of perennial root nodules of Beach Pea. *J Plant Physiology*, 2003, pp. 1-8.
34. Hodge JE and Hofreiter BT, Determinations of Reducing sugars and carbohydrates. Analysis, chapter-4. In: Roy LW, Wolfrom ML eds. *Methods in Carbohydrate Chemistry*, Vol I. London, Academic Press, 1962, pp.388-390.
35. Zechmeister L and Petracek, Estimation of Carotenoids. *Journal of American Chemical Sociey*.74.:1952, 282.

