IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

"STANDARDIZATION OF AQUEOUS EXTRACT OF LEUCAS BIFLORA LEAVES"

KHUSHBU G. RAHANGDALE*, NARENDRA N. RAHANGDALE, SAGAR C. KAMBLE, KAMALA K. CHANDAK, MILIND J. UMEKAR

Department of Quality Assurance, Smt. Kishoritai Bhoyar College of Pharmacy, Kamptee, Nagpur-441002, Maharashtra, India.

ABSTRACT: World is endowed with a rich wealth of medicinal plants and they have been always principle form of medicine. Traditionally mature leaf decoction of *Leucas biflora* is used as eye drop twice a day in case of conjunctivitis. The mature leaves ground with leaves of Centella asiatica in a ratio of 2:1 and juice extracted from this mixture is applied directly to stop instance of bleeding from nose. Four to five leaves are also prescribed to chew with a leaf of Piper betel for the women who suffering from white discharge. The leaves of *Leucas biflora* was collected, identified and authenticated. The dried leaves of *L. biflora* pulverized to make coarse powder and decoction prepared and evaporated to get a dry residue. Aqueous extract have been standardized by evaluating organoleptic characters, macroscopically characters, physicochemical properties, screening of phytochemical constituents, total flavonoids content. Phytochemical screening shows the presence of carbohydrates, glycosides, flavonoids, tannins, Saponins and steroids. Quantitative determination of total flavonoids content in *L. biflora* aqueous extract was carried out using aluminium chloride colorimetric method. Quercetin was used as standard for calibration of flavonoids. The results indicate that the *L. biflora* plant is the rich source of high valve of phytochemical compounds.

Keywords: *Leucas biflora*, nasal bleeding, standardization, physicochemical characterization, Total flavonoid contents.

INTRODUCTION:

Medicinal plants are the local heritage with global importance. World is endowed with a rich wealth of medicinal plants. They have always been principle form of medicine in India and are becoming popular throughout the developed world. In traditional system of medicine like, Ayurveda, Siddha, Unani, Homeopathy various indigenous plants are used in the diagnosis, prevention and elimination of physical, mental or social imbalance since. The plant *Leucas biflora* belongs to family (Lamiaceae), leaves are traditionally used for the treatment of conjunctivitis and the leaves are also used to stop nose bleeding & white discharge in women[1].

The future of natural product (plants, animals and minerals) drug discovery will be more holistic, personalized and involving the wide use of ancient and modern therapeutic skills in a complimentary manner so that maximum benefits can be accrued to the patient and the community[2].

Decoction of *Leucas biflora* leaves is traditionally used for treatment of conjunctivitis, and the leaves are also used to stop nose bleeding & white discharge in women. The mature leaves ground with leaves of *Centella asiatica* in a ratio of 2:1 and juice extracted from this mixture is applied directly to stop instance of bleeding from nose (nose bleed). Four to five leaves are also prescribed to chew with a leaf of Piper betel for the women who suffering from white discharge. The main objectives of this study to develop the quality control parameters for aqueous extract of leaves and to find out important primary and secondary metabolites which are responsible for its therapeutic activities[3].

Description: It is a perennial herb with nodal roots, and many branches arising from a woody root-stock. Stem is square, much velvety with deflexed hairs at the ribs. Leaves are 0.7-3 cm long, 0.5-1.5 cm broad, ovate, toothed at margins. These are velvety on both the surfaces especially on the veins. Flowers are white, two-lipped, lower lip 4 lobed, spreading, velvety, upper lip concave, hairy outside borne in leaf axils. Fruits are Nutlets 1.5-2 mm long, flat at top.

MATERIALS AND METHODS:

Plant Material: Plant part (i.e. leaves) of *Leucas biflora* Linn. was collected from campus of Smt. Kishoritai Bhoyar College of Pharmacy, Kamptee, Nagpur, Maharashtra, India. In month of july. It was authenticated by Herbarium, Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University (RTMNU), Nagpur by Dr. N. M. Dongare, and Specimen Voucher no. 9998.

Materials: *Leucas biflora* leaves, Ethanol, Chloroform, Petroleum ether, Ethyl acetate, n-hexane, was obtained from Loba Chemicals Pvt. Ltd., Mumbai, India. Acetone was obtained from Merck Specialties Pvt. Ltd. Mumbai. Other chemicals used in the study were of analytical grade.

Methods: Maceration Extraction, TLC, Direct Compression.

EXTRACTION OF LEAVES OF *L. BIFLORA*:

Leaves were dried in shade, pulverized into coarse powder and about 500 g of powder were extracted with distilled water by Decoction method. The resulting filtrate was evaporated to dryness and the percentage yield were calculated and stored in clean container till further uses.

Organoleptic Evaluation of Leaf:

The macroscopic character are useful in quick identification of plant material and also serve as an important standardization parameter, Fresh leaf of *Leucas biflora* were evaluated for organoleptic characteristics like color, odor, taste, size, shape and texture.

Thin Layer Chromatography (TLC) Study[4-5]**:** Active extracts those having standardization were subjected to thin layer chromatography to find out the number of compounds present in them.

Ultraviolet-visible spectroscopy[6]**:** 5 mg of extract were weighed and added to 10 ml of volumetric flask. Then methanol was added to it and volume was made up to 10 ml. This solution was taken for spectrum in UV-Visible spectroscopy.

Physico-Chemical Characterization[7]**:**

Determination of Ash Content:

Total Ash Content:

Ash is the inorganic residue remaining after the water and organic matter had removed by heating in the presence of oxidizing agents.

Procedure: About 2 g accurately weighed leaf powder was taken in tarred silica crucible and incinerated at a temperature not exceeding 450ºC until free from carbon. It was then cooled and weighed. The percentage total ash was calculated.

Ash Value = Initial weight – Final weight/ Initial weight×100

Acid Insoluble Ash: Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present, especially as Sand and Siliceous earth.

Procedure: The ash obtained in (5.6.2.1) was boiled for 5 minutes with 25 ml of 2 M HCL. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weighed. The percentage of Acid insoluble ash was calculated with reference to the air dried drug.

Acid insoluble ash = Initial weight – Final weight/ Initial weight×100

Water Soluble Ash: Water-soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.

Procedure: The ash obtained in (5.6.2.2) was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weighed. Weight of insoluble matter was subtracted from the weight of the total ash; the difference in weight represents the water soluble ash.

Water Soluble ash = Initial weight – Final weight/ Initial weight×100

Determination of Extractive Values: Extractive value plays an important role in evaluation of crude drug. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulation.

Water Soluble Extractives:

Procedure: 5 g of air dried plant material was macerated with 100 ml of water in a closed flask, shaking frequently during the first 6 hour and allowed to stand for 18 hour. There after it was filtered rapidly taking precaution against loss of water. 25 ml of filtrate was evaporated to dryness in a tarred flat bottom shallow dish dried at 105ºC weighed and the percentage was calculated.

Alcohol Soluble Extractives:

Procedure: 5g of air dried plant material was macerated with 100 ml of ethanol in a closed flask, shaking frequently during the first 6 hour and allowed to stand for 18 hour. Thereafter it was filtered rapidly taking precaution against loss of water. 25 ml of filtrate was evaporated to dryness in a tarred flat bottom shallow dish dried at 105ºC weighed and the percentage was calculated.

Extractive value =Initial weight – Final weight/ Initial weight×100

Determination of moisture content by loss on drying:

Procedure: About 5 g of accurately weighed leaf powder was taken in different Petri dishes and kept in a hot air over at 110ºC for four hours. After cooling, the losses in weight were recorded in each case. This procedure was repeated till constant weight was obtained.

M. C. (%w/w) = (Initial weight of sample – Final weight of sample) / Initial weight of sample ×100

Determination of foaming index:

The Saponins are high molecular weight compounds containing phyto-constituents having detergent activity. Saponins are mostly characterized on basis of their frothing property. Many medicinal plants contain Saponins that can cause persistent foam when an aqueous solution is shaken. The foaming ability of plant materials and their extracts is measured in terms of foaming index.

Procedure: About 1 g of coarsely powdered plant material was taken and transferred to 500 ml conical flask containing 100 ml of boiling water and maintained at moderate boiling for 30 minutes. It was than cooled and filtered into a 100 ml volumetric flask and sufficient water was added through the filter to dilute to volume. This decoction was then poured into 10 stoppered test tubes (height 16 cm, diameter 16 mm) in successive portions in 1 ml, 2 ml, 3 ml and the volume of the liquid in each tube was adjusted with water to 10 ml. The test tube were stoppered and shaken them in a lengthwise motion for 15 seconds. Allowed to stand for 15 minutes and the height of foam were measured.

Foaming Index = 1000/A

Where, A = Volume (ml) of decoction used for preparing the dilution in the tube where foaming to height of 1 cm was observed.

Determination of swelling index: Many medicinal plant materials are of specific therapeutic or pharmaceutical utility because of their swelling properties, especially gums containing an appreciable amount of mucilage, pectin or hemicellulose.

Procedure: It was carried out simultaneously no fewer than three determinations for any given material. Introduce the specified quantity of the plant material concerned, previously reduced to the required fineness and accurately weighed 1g of plant material into a 25 ml glass-stopper measuring cylinder. The internal diameter of the cylinder was about 16 mm, the length of the graduated portion about 125mm, marked in 0.2 ml divisions from 0-25 ml in an upwards direction. 25 ml of water was added and the mixture was shaken thoroughly every 10 minutes for 1 h. It was the allowed to stand for 3 h at room temperature and the volume in ml occupied by the plant material, was measured including any sticky mucilage.

Determination of Total Flavonoids Content:

Aluminium Chloride Colorimetric Method:

Preparation of Standard solution (Quercetin) for calibration curve: In this method, Quercetin was used as standard.

Stock solution of standard: Stock solution of Quercetin (0.1 mg/ml) was prepared by using ethanol. From this stock solution, 1 to 10 ml of solutions was taken separately in clear test tubes. Then volume was made up to 10 ml with ethanol (10-100µg/ml).

From each of the above test tubes 0.5 ml of solution was taken separately in different test tubes and to each, 1.5 ml of 95% ethanol, 0.1 ml of 10% aqueous aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml distilled water were added and incubated for 30 minutes at room temperature. The absorbance of each reaction mixture was measured at 415 nm with Shimadzu UV-1800 spectrophotometer.

Preparation of test solution: Stock solution of plant extract (1mg/ml) was prepared by using ethanol. From this stock solution, 1 to 10 ml of solutions was taken separately in clear test tubes. Then volume was made up to 10 ml with ethanol (10-1000 µg/ml).

From each test tube 0.5 ml of plant extract sample was taken and to each, 1.5 ml of 95% ethanol, 0.1 ml

10% aqueous aluminium chloride, 0.1 ml of 1m potassium acetate and 2.8 ml distilled water were added and incubated for 30 minutes at room temperature. The absorbance of each reaction mixture was measured at 415 nm with a Shimadzu UV-1800 spectrophotometer.

The blank solution was prepared by substituting 10% aluminium chloride with the same amount of distilled water.

The calibration curve graph of standard Quercetin was plotted using absorbance against concentration.

The total flavonoid content of plant extract was calculated using following formula:

$$C = c. V/m$$

Where: C = total content of flavonoid compound (mg/g) plant extract, In Quercetin equivalent (QE),

c = the concentration of Quercetin established from the calibration curve (mg/ml).

V = the volume of extract in ml,

m = the weight of plant extract fraction in g. **Determination of Total**

Tannin Content:

Tannins are the polyphenolic compounds which can be classified as hydrolysable and non-hydrolysable (condensed tannin). They have property to bind and precipitate proteins.

Preparation of Reagents:

Preparation of Folin – Denis Reagent:

10 g of sodium tungstate and 2 g of phosphotungstomolybdic acid were dissolved in 75 ml of distilled water along with 5 ml of phosphoric acid. The mixture was refluxed for 2 hours and the volume was made up with water up to 100 ml.

Preparation of Sodium carbonate solution Reagent:

35 g of sodium carbonate was dissolved in distilled water and volume was made up to 100 ml. It was allowed to stand overnight and filtered through filter paper.

Procedure:

Preparation of standard solution (Tannic acid) for calibration curve:

100 mg of accurately weighed tannic acid was dissolved in distilled water and volume was made up to 100 ml with distilled water in volumetric flask. 5 ml of this solution was diluted with water to 100 ml in another volumetric flask to give 50 µg/ml tannic acid solutions.

From this working stock solution, 1 ml to 9 ml were taken in clear separated test tubes and 0.5 ml of Folin–Denis Reagent and 1 ml of sodium carbonate solution were added to each test tube and volume was made up to 10 ml with distilled water. All the reagents in each test tube were mixed well and kept undisturbed for about 30 minutes. A blue colour solution was formed and absorbance was read at 700 nm against reagent blank using UV spectrophotometer.

Preparation of test solution (Aqueous extract):

Accurately weighed 0.1 g of the crude aqueous extract was dissolved in distilled water and volume was made to 100 ml in volumetric flask. 5 ml of this solution was diluted with water to 100 ml in another volumetric flask to get 50 µg/ml concentration..

From this stock solution, 1 to 9 ml of was taken separately in clear test tubes. Further same was followed as that of Standard solution.

The calibration curve graph of standard (Tannic acid) was plotted using absorbance against concentration.

The percentage of total tannin was calculated from using formula:

C = c. V/m

Where: C = total content of tannin (mg/g) test extract, In Folin- Denis Reagent equivalent;

c = the concentration of Folin- Denis Reagent established from the calibration curve (mg/ml)

V = the volume of extract in ml,

m = the weight of fraction in g.

HPTLC Analysis of Decoction[9]**:** HPTLC is a sophisticated form of TLC introducing shorter time of analysis with better resolution. It is more versatile and well established separation techniques; used for many applications in different areas. HPTLC is simplest of all the chromatographic techniques. It is based on the phenomenon on which the TLC works i.e separation may result due to adsorption or partition or by both phenomenon depending on the nature of adsorbents used on plates and solvent system used for development. Separation is based on migration of sample spotted on coated plate with one edge dipped in a mixture of solvent. The whole system is contained in enclosed tank. Detection techniques include fluorescence, UV and sprays for compounds that are not naturally coloured. The location of analyse on the TLC plate is described by the Rf value which is the ratio of the migration distance of the compound of interest to the mobile phase front. This technique widely used in the many fields for both qualitative and quantitative (identification and estimation) of constituent mixture.

Scanning and Documentation: Now a day's HPTLC equipments are supplied with computer recording and storing devices. Development of HPTLC plates are scanned at selected UV region wavelength by instrument and detected spots seen on computer in the form of peaks. The scanner converts bands into peaks and peak height or area is related to the concentration of the substance on the spot. The peak height and area under the spot (areas) are measured by the instruments and are recorded as percent on the printer. Furthermore, the plates carry supplies name, batch no., chemical code etc. This helps in storing the data of individual plates for further use as well as for photo documentation and storage. The HPTLC analysis of the residue was carried out at Qualichem Laboratory Pvt. Ltd., Nagpur[10].

RESULT AND DISCUSSION:

Macroscopic Characterization:

Fig. 1: *L. biflora* leaf

Table No. 1. Macroscopic characters of *L. biflora* leaf

Sr. No.	Characters	Observations
1	Color	Green
2	Odour	Odourless
3	Taste	Slightly bitter
4	State	Dry
5	Texture	Rough and Hairy
6	Shape	Ovate
7	Size	9 cm Long, 4 cm broad
8	Apex	Sub-acuminate
9	Margin	Serrate

Table No. 2: Macroscopic Characteristics of Aqueous extract of *L. biflora* leaves

Sr. No.	Extract	Color	% Yield
1.	water	Yellowish Green	2.72

Physical Characterization:

Table No. 3: Characteristics of Aqueous extract of *L. biflora* leaves

Parameters	Characteristics
Color	Dark Brown
State	Solid
Consistency	Dry
pH	6
Yield (%w/w)	7.2

Table No. 4: Preliminary Phyto-chemical screening of *L. biflora* aqueous extract [7-8]

Sr. No.	Phyto-chemical	Test performed	Aqueous extract
1.	Carbohydrates Test	Molish test Fehling's test Benedict's test Iodine test	+ve +ve +ve -ve
2.	**Glycosides Test** Anthraquinone glycosides Cardiac glycosides Coumarin glycosides	Keller killani test	 +ve +ve -ve
3.	Saponin test	Foam test Haemolysis test	+ve +ve
4.	Alkaloids test	Dragendroff's test Hager's test Mayer's test Wagner's test	-ve -ve -ve -ve
5.	Phytosterol/Steroid test	Salkowski's test Liberman test	+ve +ve
6.	Flavonoid test	Shinoda test Lead acetate test Alkali test	+ve +ve +ve
7.	Tannins and phenol test	Ferric chloride Potassium dichromate Lead acetate test Gelatin test Phenazone test	+ve +ve +ve +ve -ve
8.	Protein and amino acid	Biuret test Millon's test Ninhydrin test	-ve -ve -ve
9.	Test for fixed oils and fats		-ve

-ve: Indicates absence; **+ve:** Indicates presence

Preliminary Photochemical Screening of Aqueous extact of *L. biflora* leaves revealed the presence of carbohydrates, glycosides, saponins, flavonoids and tannins.

Physico-chemical Parameters:

Table No. 5: Determination of Total Ash Content, Extractive value and Moisture content

Parameters	Value (%w/w)
Total Ash content	
Total Ash	13.75
Acid insoluble Ash	3.87
Water soluble Ash	4.50
Extractive Value	
Water soluble Extractive	33.65
Alcohol soluble Extractive	8.31
Moisture Content	
Moisture Content	2.00

Table No. 6 : Determination of Foaming and Swelling index

Powder of *L. biflora* Leaves	Observation
Foaming index	Very slightly foam less than 1 cm
Swelling index	No swelling were observed

Determination of Total Flavonoid Content:

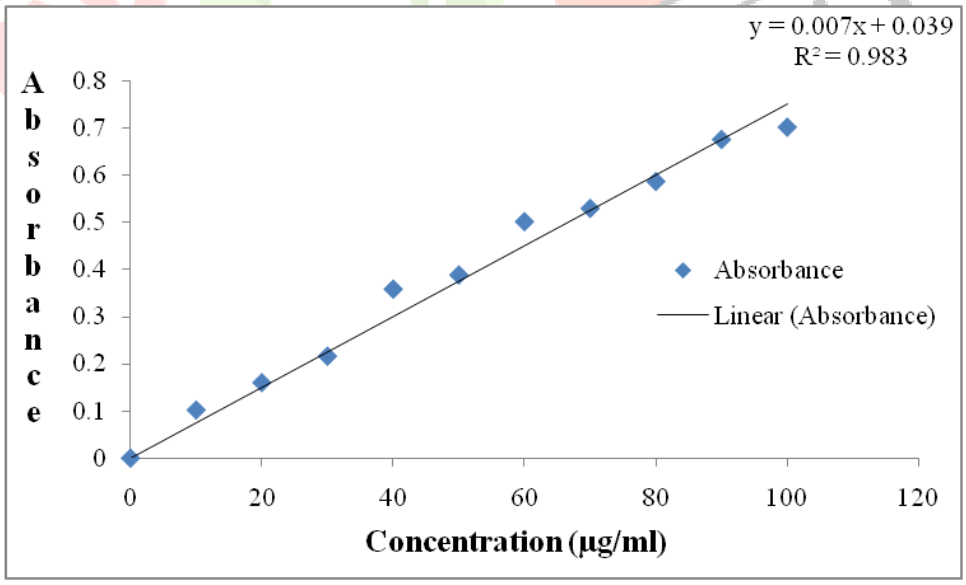

Fig. 2: Calibration curve of Quercetin

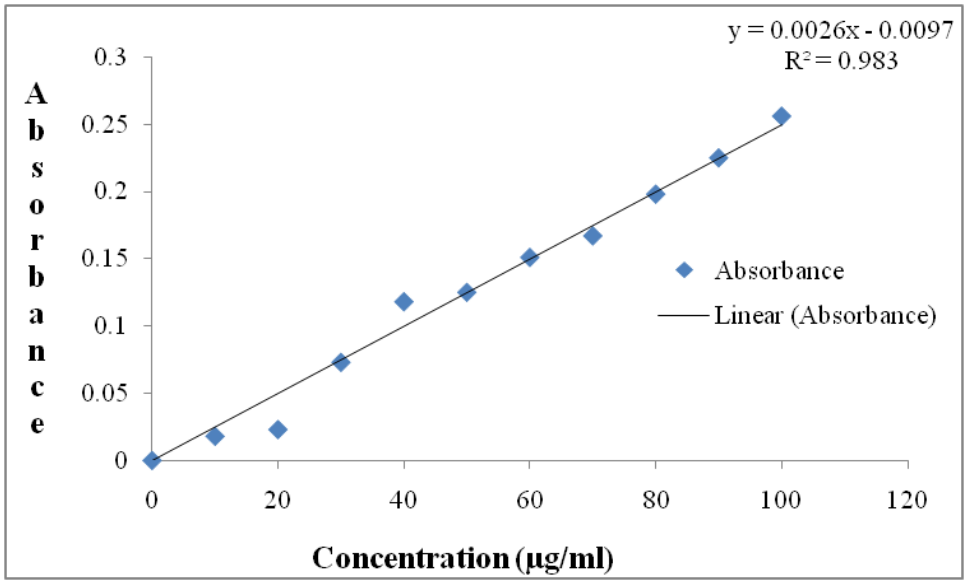

Fig. 3: Calibration curve of Extract

Table No. 07: Total Flavonoid Content of *L. biflora* aqueous extract

Sample solution (µg/ml)	Weight of dry extract per ml (g)	Absorbance	QE. Conc. (µg/ml)	QE. Conc. C (mg/ml)	TFC (µg/ml)
100	0.1	0.300	39.72	0.039	0.1986

Determination of Total Tannins Content:

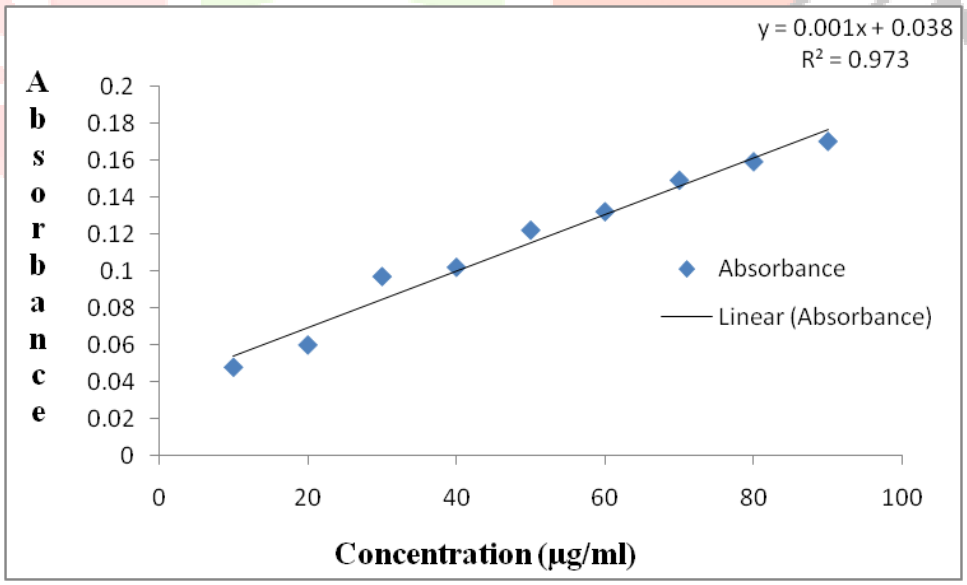

Fig. 4: Calibration curve of Tannic acid

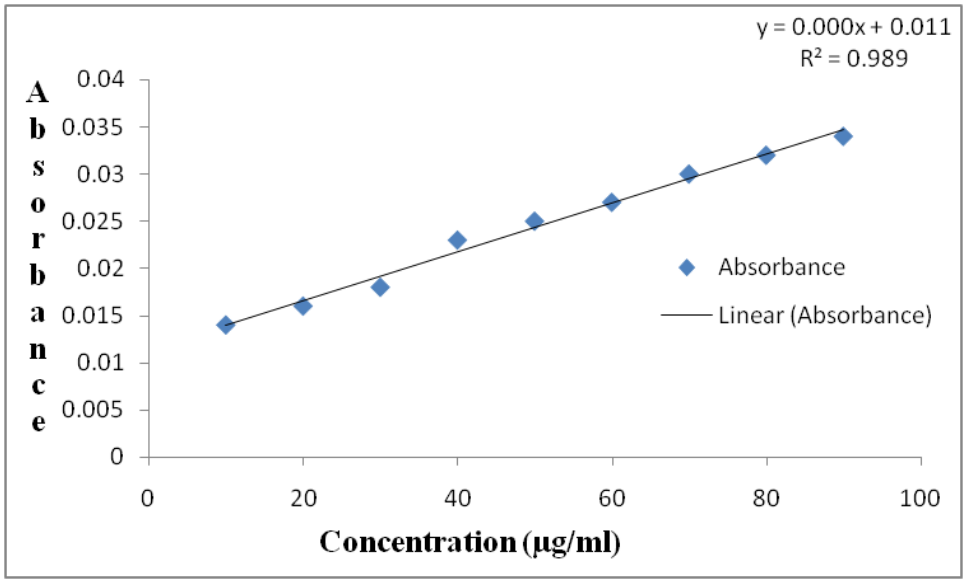

Fig. 5: Calibration curve of Test sample

Table 8: Total Tannin Content of *L. biflora* leaves

Sample solution (µg/ml)	Weight of dry extract per ml (g)	Absorbance	TA. Conc. (µg/ml)	TA. Conc. C (mg/ml)	TFC (µg/ml)
90	0.1	0.037	1.5181	0.1518	0.3062

Table 9: Thin Layer Chromatography of Aqueous extract:

Solvent system	Detection wavelength	Samples	No. of components	Rf values
Toluene: ethyl acetate: formic acid: water (3:3:0.8:0.3)	254 nm	Aqueous extract	9	0.02, 0.12, 0.19, 0.25, 0.39, 0.52, 0.64, 0.92, 1.02
	366 nm		7	0.03, 0.24, 0.31, 0.49, 0.53, 0.73, 1.02
n-butanol :Acetic acid: water (8: 1: 1)	254 nm		2	0.75 0.92 1.02
Petroleum ether: Ethyl acetate (7: 3)	254 nm		3	0.64 0.94 1.02

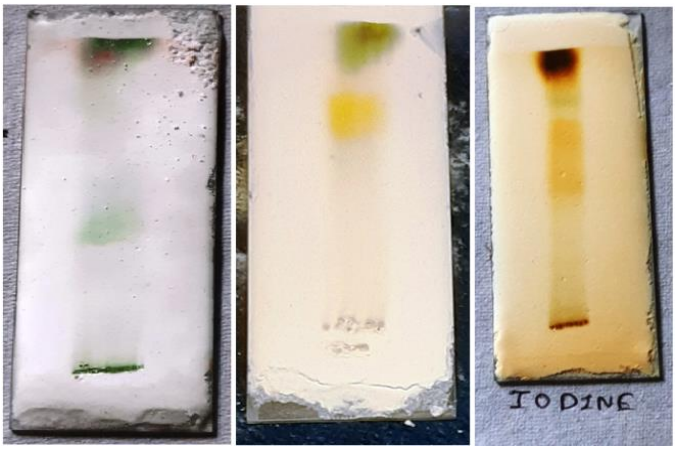

Fig. 6: Thin Layer Chromatography of Aqueous extract with different solvent system

HPTLC Analysis of Aqueous extract: HPTLC analysis of aqueous extract was carried out at Qualichem Laboratory, Pvt. Ltd. Nagpur.

The finger printing analysis for Aqueous Extract of *L. biflora* leaves.

1) Condition maintained during the sample analysis as follows:

1. Mobile phase: Toluene: ethyl acetate: formic acid: water (3:3:0.8:0.3)

Chamber type	Twin Trough Chamber 20x10cm
Solvent front position	70.0 mm
Volume	10.0 ml
Drying device	Oven
Temperature	60 °C
Time	5 Minutes

2. After Development:

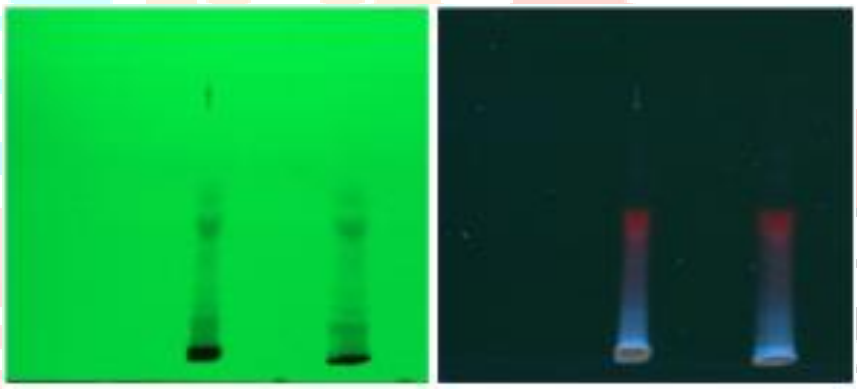

Fig. 7: HPLC At 254 nm (UV light) and AT, 366 nm (Fluorescence light)

It observed that two or three compounds are separated on ethyl acetate on 254 nm (UV-light). Two compounds are separated on 366 nm. (Fluorescence light)

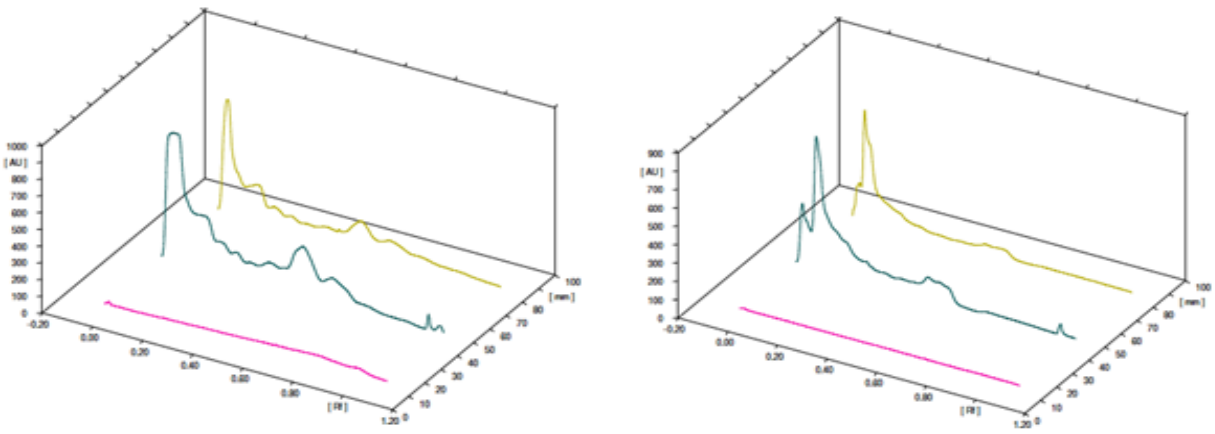

Fig. 8: 3D plot of HPTLC analysis of LBLD- (a) At 254nm (b) At 366nm

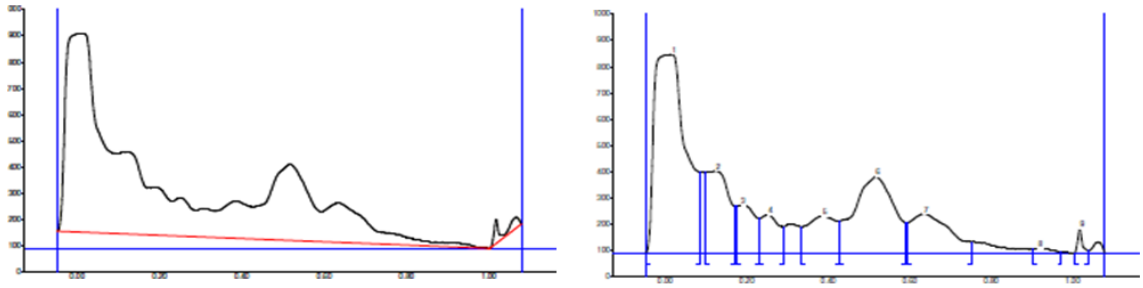

Fig. 9: At 254 nm, Tract 2

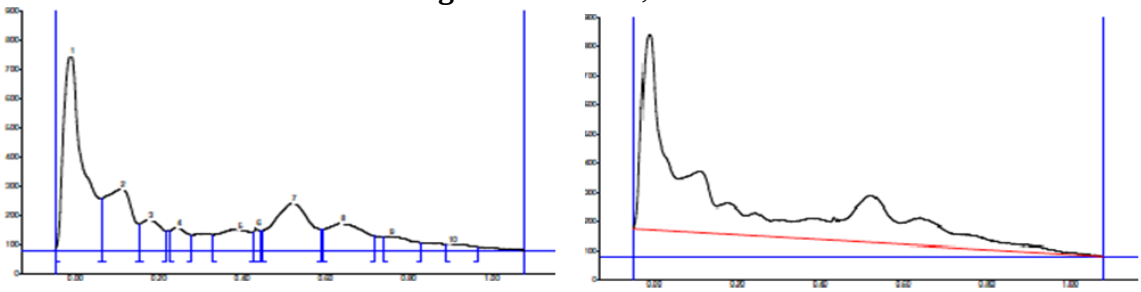

Fig. 10: At 254 nm, Tract 3

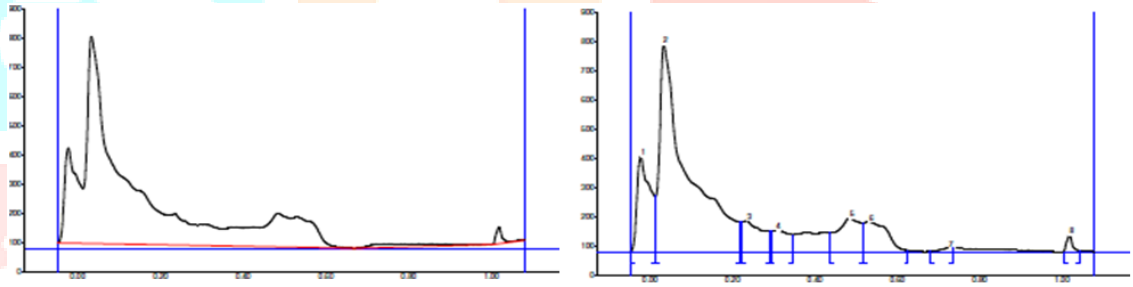

Fig. 11: At 366 nm: Tract 2

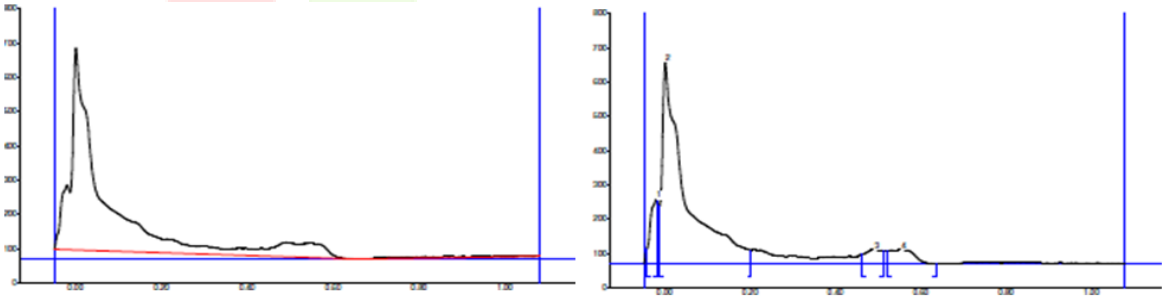

Fig. 12: At 366 nm: Tract 3

Table 12: HPTLC analysis of aqueous extract

Solvent system	Detection wavelength	Samples	No. of components	Rf values	Area	Area %
Toluene: ethyl acetate: formic acid: water (3:3:0.8:0.3)	254 nm	Tract 2	9	0.02, 0.12, 0.19, 0.25, 0.39, 0.52, 0.64, 0.92, 1.02	42573.9, 12767.0, 5965.5, 4702.6, 7255.3, 20403.0, 10367.9, 540.5, 739.2	40.43 12.12 5.66 4.47 6.89 19.37 9.84 0.51 0.70
		Tract 3	9	0.11, 0.18, 0.25, 0.39, 0.44, 0.52, 0.64, 0.76, 0.90	9795.2 3684.4 2245.6 3946.7 843.7 10213.8 6071.3 2283.8 955.6	15.57 5.86 3.57 6.27 1.34 16.24 9.65 3.63 1.52
	366 nm	Tract 2	7	0.03, 0.24, 0.31, 0.49, 0.53, 0.73, 1.02	34398.3 3880.9 2279.0 4525.3 4246.3 267.3 566.1	59.62 6.73 3.95 7.84 7.36 0.46 0.98
		Tract 3	2	0.00 0.49, 0.56	22913.5 1224.8 1760.2	81.16 4.34 6.23

CONCLUSION: From the research it was concluded that:-

1. *Leucas biflora* leaves show significant antimicrobial, antioxidant, antibacterial activity.
2. Aqueous extract of *L. biflora* leaves was subjected to preliminary phytochemical screening for detection of chemical constituents present in it and indicates the presence of carbohydrate, glycosides, flavonoids, tannins, Saponins.
3. In future these compounds can be isolated in pure form by various separation techniques such as column chromatography; preparative TLC or counter current extraction and their structure can be elucidated using the modern analytical technique such as IR, NMR and mass spectroscopy for the extract identification of the compounds.
4. As the plant is widely grown and abundantly available it can prove as a cheap and effective remedy in the treatment of various diseases.

REFERENCE:

1. Prajapati N.D. and Purohit S.S. Agro's colour atlas of medicinal plants. Agobias India-2003:1-5.
2. Handa S.S. Herbal raw materials and traditional remedies. The eastern pharmacist-1995:23-27.
3. Majumdar. Koushik. Datta B.K. *Leucas biflora* (Vahl) R. Br. (Lamiaceae): A new distributional record and its less known ethno-medicinal usage from tripura. Indian journal of traditional knowledge-2011:10(3):575-577.
4. Wagner and Bladt, 1996, Plant Drug Analysis: A Thin Layer Chromatography Atlas, 2nd Edition, Springer Publication: 281-288.
5. Stahl, 1964, Thin Layer Chromatography, Academic Press, New York 52-105: 133-151.
6. Khadbadi S.S. Deore S.I. Baviskar B.A. Experimental phyto-pharmacognosy – A comprehensive guide, 2011, Nirali Prakashan: 100.
7. Khandelwal K.R. "Practical Pharmacognosy-technique & experiments", Nirali Prakashan.
8. Korlam S. Papani S. Mylisetti V. Mittoori H. Chitoor M. Preliminary phytochemical profile of leaf extracts in different solvents of Cassia roxburghii. IJSEAS–2016:2(9):61-68.
9. Seasotiya L. Siwach P. Malik A. Bai S. Dalal S. Phytochemical evaluation and HPTLC fingerprint profile of Cassia fistula. IJAPBC– 2014:3(3):604-611.
10. Rangari V.D. Pharmacognosy & Phytochemistry. Career publication- 2012:ed.3:1:108.