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Pharmacognostical and Phytochemical Evaluation of *Melissa parviflora*andHPTLC finger printing of its extracts

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ABSTRACT

Melissa parviflora is an aromatic perennial herbaceous plant of Lamiaceae family; it is a brightly colored shrub or sub-shrub that ranges from 60-100cm in height. Leaves are ovate-obtuse. During summer the plant produces bunches of pink blue and more rarely white fragrant flowers. Flowers are bisexual, zygomorphic, rarely sub actinomorphic, bracteolate. The different extracts were subjected to preliminary phytochemical screening for the presence of carbohydrate, flavonoids, phenolics etc. The objective of the present investigation was the development of quality standards and phytochemical analysis of Melissa parviflora. This included organoleptic properties, pH of aqueous solution, ash values, extractive values, successive extractive values, and loss on drying, HPTLC finger printing profile and preliminary phytochemical screening. The findings of this study might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario lacking regulatory laws to control quality of herbal drugs.

Keywords: Melissa parviflora, Pharmacognostical, Phytochemical, HPTLC

INTRODUCTION

Herbal medicine is a triumph of popular therapeutic diversity. Almost in all the traditional medicine, the medicinal plants play a major role and constitute the backbone for the same .[1]. In order to make sure the safe use of these medicines, a necessary first step is the establishment of standards of quality, safety and efficacy. Keeping this fact in the consideration, the attempts were made to establish physiochemical standards of the plant. *Melissa parviflora*(MP) is pubescent or glabrate herb.60-100 cm high ,found in temperate Himalaya from Garhwal to Sikkim ,Darjeeling and Khasi .Aka and Mishmi hills ,at altitudes of 1200-3000m .leaves ovate –or ovate lance late ,flowers white or pale pink ,rarely yellow .It has stomachic,anti-tubercular,anti-pyretic properties , it is used to strength the gums, and to remove bad taste from mouth ,and is also considered as tonic for brain [2-4].The Persian physician Avicenna recommended it for heart problems.[5] Its main action is as a tranquillizer. It is a soothing and calming

agent for stressed nerves. The herb is a common constituent of relaxants, nervine and sleeping aids throughout the world[6]. It is primarily indicated where there is dyspepsia associated with anxiety or depression. It relieves tension and stress reactions, is widely valued for its calming properties and has a tonic effect on the heart and circulatory system causing mild vasodilation of peripheral vessels, thus lowering blood pressure[6, 7]. Preliminary phytochemical analysis performedshowed that the tannin, saponins and flavonoid are themajor components of the extract[8]. The flavonoids show various biologicalactivities including antioxidant, anti-inflammatoryactivity, activity on coronary heart diseases andcytotoxic-antitumor activity [9, 10]. The plants containingsaponins or flavonoids exhibit anticonvulsant activity[11-13]

Keeping in view the importance of the *Melissa parviflora*, the present investigationwas carried out to establish pharmacognostical and physicochemical standards which would help in authentication as well as in checking adulteration, if any; further the study will greatly help in quality assurance of finished products containing this herbal drug as component.

MATERIALS AND METHODS

Plant material

The flower of *Melissa parviflora*was purchased from ShamsiDawakhana,Ballimaran,Delhi-110006,India.The authenticity and identity was confirmed on the basis of classical description in unani literature at department of IlmulAdvia F/O Medicine(u),Jamia Hamdard, New Delhi and modern Botanical information was established by matching with the specimens available at the national institute of Science Comminications,The wealth of Indian division,Dr K .Krishnan Marg, New Delhi,100012. Reference no. of drug sample NISCAIR/RHMD…1656/254.Voucher deposited in D/O IlmulAdvia F/O Medicine,Jamia Hamdard,New Delhi-110062

Macroscopical and microscopical study

Macroscopical and microscopical characters of the drugs were studied according to the WHO and pharmacopoeial guidelines[14-16]

Physico-chemical studies

Different physicochemical values such as extractive values (cold & hot extracts), ash values (total ash, acid-insoluble ash & water soluble ash), loss on drying, and pH of 1% and 10% solution of *Melissa parviflora*were determined according to the standard methods .[16]

Preliminary Phytochemical Analysis

The preliminary phytochemical screening was carried out using the extracts for different types of chemical constituents as per method described by Evans et al. [17] The extracts were subjected to preliminary phytochemical investigation for detection of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins & amino acids, and lipids/fats.

Fluorescence Analysis

Chemical tests of powder drug with different reagents were performed to observe the colour reactions according to the reported method .[18]

Determination of total phenolic content.[19]

Preparation of standard curve

Standard Gallic acid (10 mg) was dissolved in 100 mL distilled water in a volumetric flask (100 µg/mL of stock solution). From the above stock solution 0.5 to 2.5 ml of aliquots were pipetted out into 25 mL volumetric flasks. Then 10 mL of distilled water and 1.5 mL of Folin-Ciocalteu reagent, diluted according to the label specification to each of the above volumetric flasks were added. After 5 min, 4 mL of 1M sodium carbonate was added and volume was made up to 25 mL with distilled water. After 30 min, absorbance at 765 nm was recorded and calibration curve of absorbance vs concentration was plotted.

Preparation of test sample

Plant material was dried at room temperature and grounded in a mortar. Powder (50 gm) was extracted with 500 mL of methanol by maceration (48 h). The solvent was removed under vacuum and the extract was freeze-dried. From

the above prepared test solutions, 1 mL of solution was pipetted out into a 25 mL volumetric flask and then the same steps were followed as given above (standard curve preparation) for color development. The amounts of total phenolicsusing the standard curve of Gallic acid were determined.[20] .Total phenol values are expressed in terms of gallic acid equivalent (mg/g).

Determination of total flavonoid content [21]
Preparation of standard curve
Aluminum chloride colorimetric method was used for flavonoids determination. Plant extract (0.5 mL of 1:10 mg/mL) in methanol were separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M sodium acetate and 2.8 mL of distilled water. It was kept at room temperature for 30 min and absorbance of the reaction mixture was measured at 415 nm with a double beam UV spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at concentrations 10, 20, 40, 50 to 100 µg/mLin methanol. The results are reported in table.

Preparation of test sample
Plant powder (50 gm) was extracted with 500 mL of methanol by maceration (48 h). The solvent was removed under the vacuum and the extract was freeze-dried. From the above prepared test solutions, 1 mL of solution was pipetted out into a 25 mL volumetric flask and then the same steps were followed as given above (preparation standard curve) for the colour development.

Development of chromatographic fingerprint profile of different extractsby HPTLC
Preparation of extract
The plant materialwas coarsely powered and extracted in Soxhlet apparatus for 6-24 h using solvents; chloroform, and methanol.The extracts were evaporated to dryness in a rota-vapour and the solvents were recovered. Gummy residues so obtained, were stored in deep freezer at -20 °C till further application. TLC and HPTLC samples were prepared by dissolving each extract in their respective solvent to get the concentration of 10µg/mL. These solutions were further passed through syringe filter to remove any impurities and applied on TLC plate for finger printing analysis.The extract was applied on TLC aluminum sheets silica gel 60 F 254 (Merck) 10 micro liter each with band length 6 mm using Linomat 5 sample applicator set at a speed of 100 nL/sec CAMAG, Switzerland.Different solvent systems were used for separation of constituents of different extracts. The chromatograms were developed in twin trough chamber for 20 min up to the distance of 80 mm and the spots were visible without derivatization at 254 and 366 nm wavelengths.[22]

RESULTS

Macroscopic: Leaves are 2.5 to 10 cm, ovate or ovate-lanceolate,base acute rotund orcordate; opposite, rarely whorled or alternate, simple to pinnately dissected or compound. Petioles are 6-25mm, slender. Whorls are numerous or few or many. The petiole varies in length from 1-1.2cm while the lamina is about 2.5x1-3.5x1.2cm
Microscopic: Transverse section of leave shows single layered upper and lower epidermis provided with glandular and non glandular hairs. The glandular hairs are characterized by the presence of unicellular circular head and uni to multi celled tail. The non glandular hairs are unbranched, uni seriate, multicellular, with the outer most cells tapering. These are ornamented with small bristles. Upper epidermal cells are mostly larger than lower ones, while both are covered with thick cuticles. The epidermis is followed by single layered palisade tissue continuous to lamina whereas it discontinues at the veins or midrib which is replaced by 3to 4 layered collenchymatous tissue on the upper side and three to six layered on the lower side of leave .The collenchyma is followed by circular parenchyma tissue with large intercellular space .the vascular bundle is kidney shaped and is collateral .The vessel members are mostly with spiral to reticulate thickenings with simple perforation plates and tracheid's are mostly pitted. The stomata are indistinct.

Powder: The powder under the microscope consists of fragments of parenchymatous tissue with intercellular spaces, collenchyma and strands of vascular bundles. The multicellular, uni seriate non-glandular trichromes together with glandular trichomeswithglobular unicellular head and uni-or multicellular uni seriate tail .Palisade tissue and spongy tissue are also met with .The color is dull green and taste slightly bitter.

Physico-chemical studies
Preliminary physicochemical studies of the test drug

Table 1:Extractive values of*Melissa parviflora*

S No.	Organic solvent	Mean %age of extracts cold	Hot Mean %age	Successive Mean %age
1	Petroleum ether	1.9	3.33	2.78
2	Chloroform	4.06	6.70	2.44
3	Alcohol (methanol)	5.2	6.44	2.88
4	Aqueous	4.7	15.08	16

Table 1 (b): Physicochemical observations of *Melissa parviflora*

Ash values (%)	
Total ash	: 20.8%
Water soluble ash	: 17%
Acid insoluble ash	: 19.6%
Moisture content	: 9.6%
Volatile content	: <1%
Specific gravity	: 1.015
Fixed oil	: Present
pH Values	
1% solution	: 8.85%
10% solution	: 8.37%
Loss on drying at 105^0	: 8.87%
Phenolic content	:3.84 (38.4%)

Table 2: Phytochemical screening of different extracts of *Melissa parviflora*

S. No	PHYTOCHEMICAL TESTS	Petroleum Extract	Chloroform Extract	Methanolic Extract	Aqueous Extract
1.	**Alkaloid**	-	+	+	+
2.	**Sterol**	+	+	+	+
3.	**Carbohydrates**	-	-	+	+
4.	**Phenolic compound**	-	-	+	+
5.	**Flavonoids**	+	+	+	+
6.	**Proteins &amino acids**	-	-	+	+
7.	**Acidic compounds**	-	+	+	-
8.	**Mucilage**	-	-	-	_
9.	**Resin**	-	--	-	-
10.	**Glycosides**	-	+	+	+
11.	**lipids**	-	-	-	-

Table 3: Fluorescent analysis *Melissa parviflora*

S. No.	**Solvent used**	**Ordinary Light**	**UV Light (254 nm)**	**UV Light (366 nm)**
1.	Pet Ether	Transparent	Colourless	Light brown
2.	Benzene	Green	Violet	White
3.	Acetone	Light Green	Light Brown	Green
4.	Ethyl Acetate	Light Green	Green	Violet
5.	Chloroform	Greenish yellow	Light Brown	Light Black
6.	Methanol	Dark Green	Orange	Light Yellow
7.	Water	Straw coloured	Colourless	Transparent
8.	Dil. HCL	Brown	Colourless	Light Green
9.	Dil. HNO_3	Reddish	Light Green	Green
10.	Dil. H_2SO_4	Black	Light Brown	Light black

Preliminary phytochemical studies of the test drug (*Melissa parviflora)*
The preliminary phytochemical screening was carried out using the extracts for different types of chemical constituents as per method described by Trease and Evans, 1985. The extracts were subjected to preliminary phytochemical investigation for detection of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids,

proteins and amino acids, proteins & amino acids, and lipids/fats. Presence and absence of different phyto-constituents are presented in Table 2.

Fluorescence Analysis

Chemical tests of powder drug with different reagents were studied in day light, U.V. 254 nm and U.V. 366 nm, the results are presented in Table 3.

Table 4: Powdered drug reaction with different reagents*Melissa parviflora*

Treatment	Observation
Conc. HCL	Dark Brown
Conc. HNO_3	Reddish
Conc. H_2SO_4	Black
Iodine Solution	Black Brown
Glacial Acetic Acid	Dark Brown
Powder as such	Light Brown

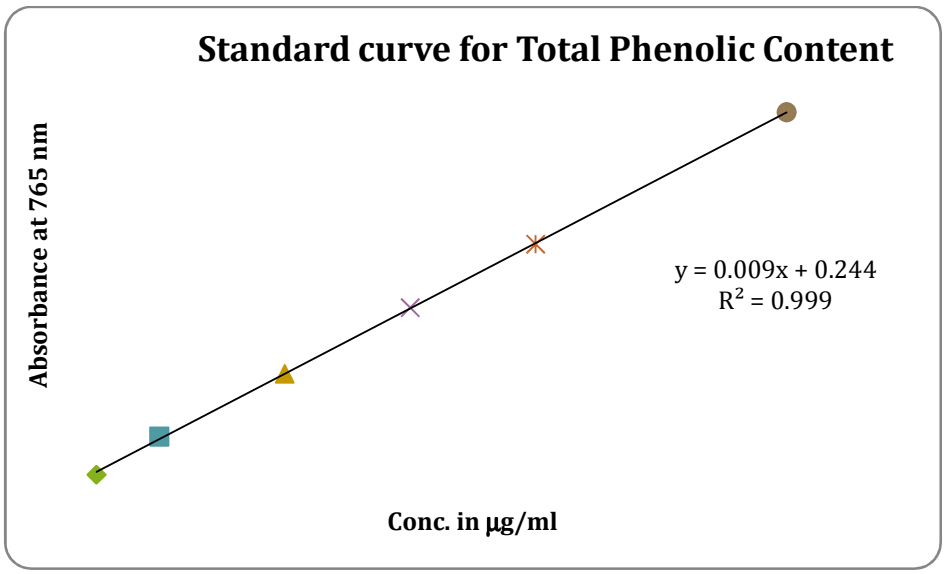
Total Phenolic content

Total phenolic content was measured by FolinCiocalteu method in term of Gallic acid equivalent in mg/g of the extract.The amount of phenolic content was calculated with the help of graph (figure 1) was found to be 68.41 µg/mL (0.68% w/w). Results are presented in Table 5

Table 5: Phenolic content of *Melissa parviflora*

S. No	Concentration of the standard solution (µg/mL)	Absorbance (765 nm)
1.	25	0.4533
2.	50	0.7166
3.	100	1.1523
4.	150	1.6103
5.	200	2.1526
6.	300	2.7666
7.	**Sample**	0.967

Figure 1: Standard calibration curve for determination of total phenolic content



Total Flavonoid content

The results of total flavonoids content of *Melissa parviflora*, determined by aluminum chloride colorimetric method, are showed in Table 6. The standard calibration curve is shown in the form of Figure 2. The total flavonoid content was found to be 28.41 µg/mL (0.28 % w/w).

Table 6: Total flavonoid content of *Melissa parviflora*

S. No.	Concentration of the Standard solution (µg/mL)	Absorbance (415 nm)
1.	10	0.365
2.	20	0.535
3.	30	0.617
4.	40	0.574
5.	50	0.640
6.	60	0.701
7.	70	0.673
8.	80	0.847
9.	100	0.927
10.	**Sample**	0.398

Figure 2: Standard calibration curve for determination of total Flavonoid content

Where x is conc. to be found and y is absorbance and c is interpretation

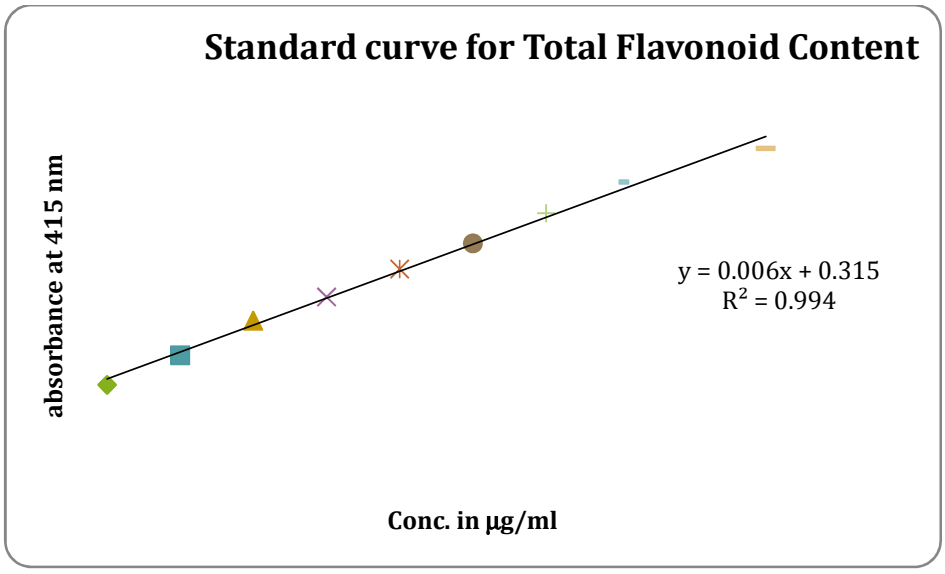


Table 7. Observation of Rf values and %area of the chromatogram of chloroform and methanol extracts of *Melissa parviflora*

Extract	Sample solution	Peak	Rf	% Area	Figure
Chloroform	Toluene:Ethylacetate: Formic acid (9:0.5:0.5)	8	0.03, 0.07, 0.12, 0.17, 0.31, 0.36, 0.49, 0.76, 0.05, 0.11, 0.13, 0.19, 0.33, 0.36 0.51, 0.77, 0.06, 0.12, 0.17, 0.21, 0.35, 0.44, 0.54, 0.79	3.74,16.72,57.39,8.56,2.81,5.01,3.10,2.67.	
Methanol	Toluene:Ethylacetate: Formic acid(9:0.5:0.5)	10	0.03, 0.06, 0.12, 0.15, 0.19, 0.29, 0.33, 0.40, 0.85, 0.90,0.02, 0.04, 0.11, 0.14, 0.17, 0.26, 0.31, 0.37, 0.78, 0.89 0.01, 0.03, 0.08, 0.12, 0.16, 0.24,0.29, 0.33, 0.74, 0.88,	23.78,8.11,11.39,7.58,3.92,9.55,5.34,20.63,8.40,1.29.	

HPTLC finger printing of *Melissa parviflora*

The chloroform and methanolic extracts of *Melissa parviflora* were subjected to generate HPTLC finger printing profile represented as chromatogram. The solvent system used in the investigation was found to give compact spots for extracts at different Rf values and there was no overlap with any other component in the analyzed sample at 254 and366nm, the results and observations are presented in table 7 and fig 3,4and 5.

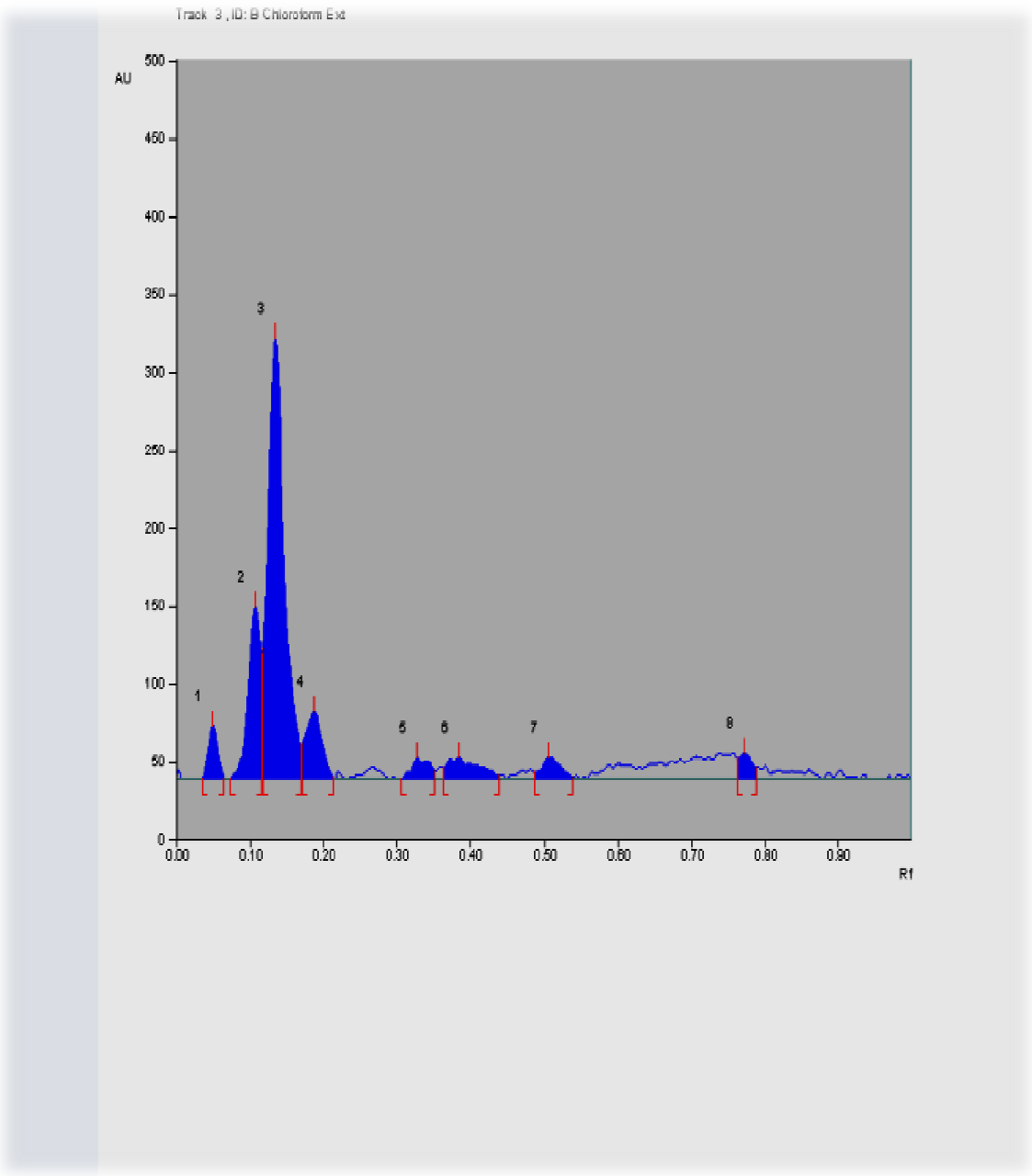


FIG3.HPTLC Chromotogram of the choloroform extract of Melissa parviflora

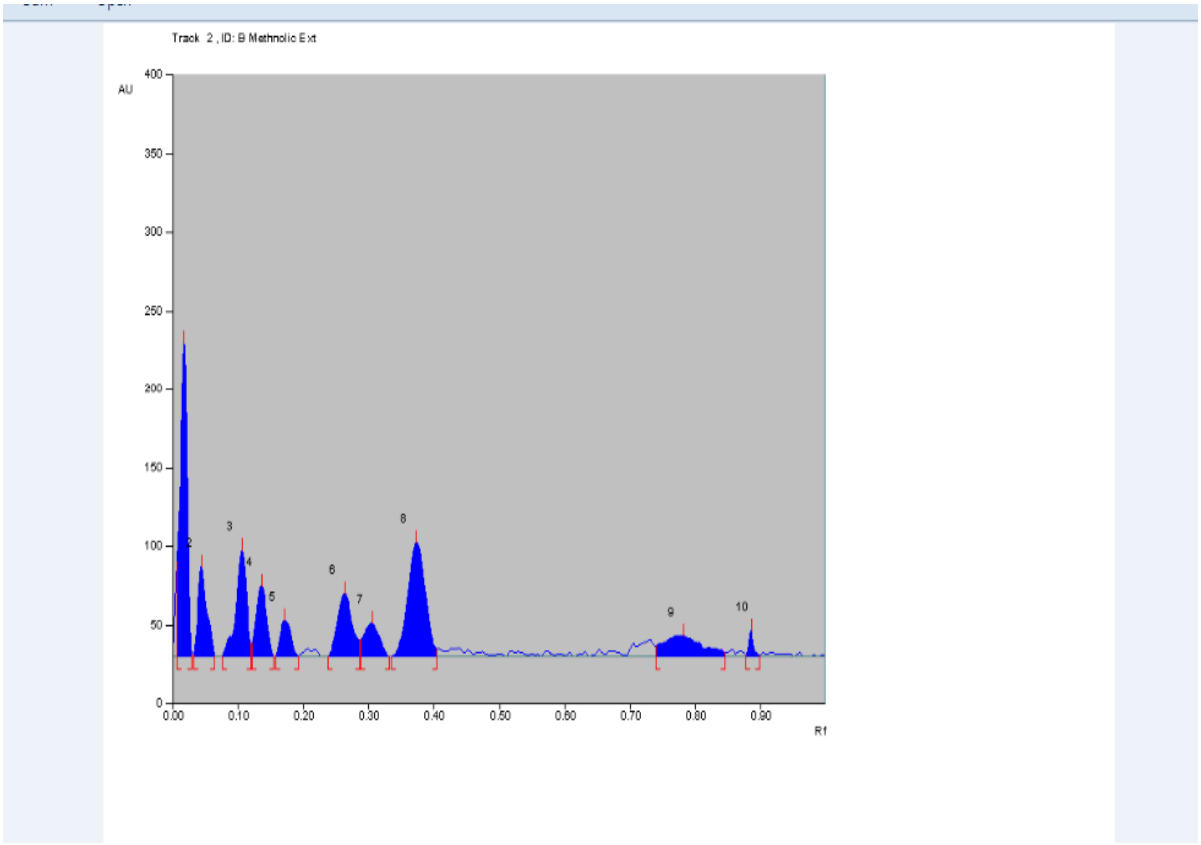


FIG4.HPTLC Chromotogram of the Methanolicextract of Melissa parviflora

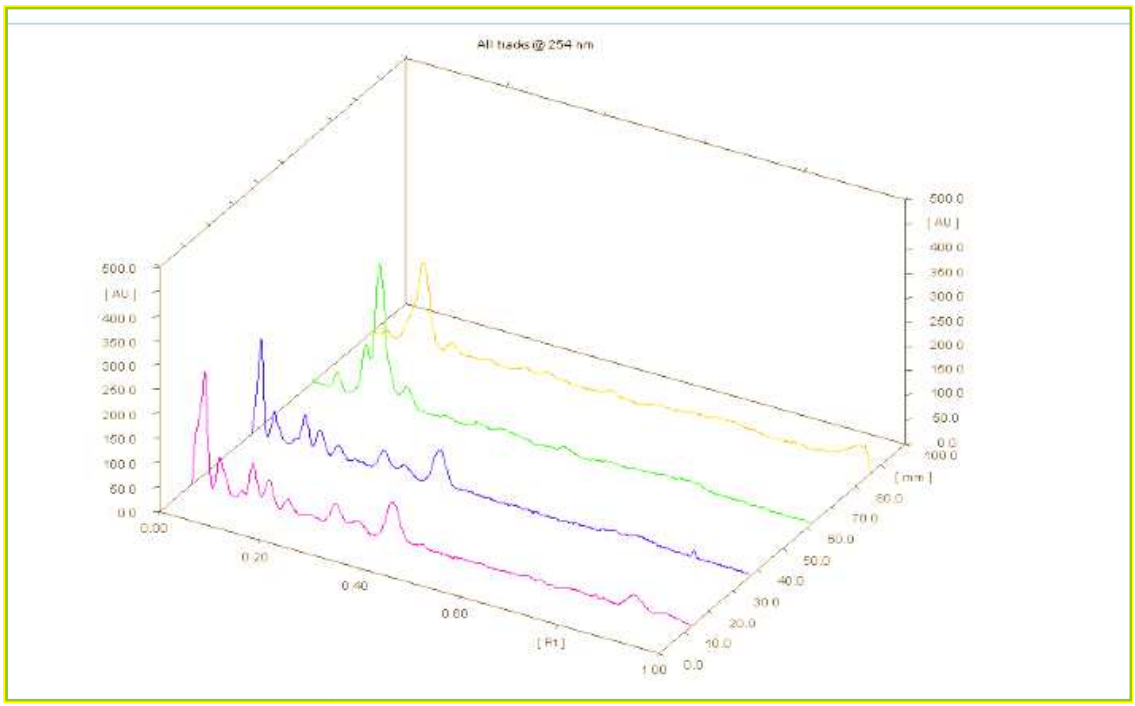


FIG5.3D Diagram of finger printing of Melissa parviflora

DISCUSSION

This study on the pharmacognostical and phytochemical analysis of*Melissa parviflora,* revealed a set of parameters which may enable to those who handle this plant to maintain its quality control. Adulteration and substitution have become a major problem due to the absence of standards relating to genuineness of drug. Skill hand and cost factors for pharmaceuticals purposes, the quality of medicine must be as high as that of other medicinal preparations. Quality refers to intrinsic value of the drug, the amount of medicinal principles or active constituents present.

The pharmacognostical parameters including HPTLC are helpful for the future identification and authentication of this plant in the herbal industry. The physical parameters, such as loss on drying, ash values and extractive values will be helpful to identify the authenticity of the drug even from the crushed or powdered plant materials. It will serve as a standard data for the quality control of the preparations containing this plant in future. The information obtained from the ash values and extractive values are useful during the time of collection and also during extraction process. Using these standards, the plant can be differentiated from other related species. The plant may be considered as biosynthetic laboratory for a variety of compounds (secondary metabolites) like alkaloids, glycosides, flavonoids, volatile oils, and saponins that exert physiological effects. The curative properties of medicinal plants are due to the presence of various secondary metabolites. Thus the preliminary screening tests may be useful in the detection of bioactive principles. HPTLC results indicate the number of constituents and further facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds. Phytochemical study was also useful to isolate the pharmacologically active principles present in the drug. More phytochemical research work is required for isolation, purification and characterization of biologically compounds.

CONCLUSION

Standardization of herbal drugs is a topic of great concern. They are subject to variability as derived from heterogeneous sources. This variability can have both merits and demerits. The main demerits are that the activity of the material may vary and that inferior material may be produced*Melissa parviflora*is an important plant and has been found to have various biological properties. So efforts have been made to provide scientific data to standardize the plant material for further studies. Microscopic, macroscopic data and other physical values including HPTLC will help to identify the correct species of the plant. The research out comings of the standardization parameters can also be used for evaluating the quality and purity of the drug and its formulation.

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