

ISSN 2278-4136
ISSN 2349-8234
JPP 2014; 2 (6): 29-33
Received: 21-01-2014
Accepted: 25-01-2014

Rehana Banu, H
Department of Botany, Nirmala
College for Women, Coimbatore,
Tamil Nadu, India.
Email: bundybanu@gmail.com

N. Nagarajan
Department of Botany, Kongunadu
Arts and Science College, Coimbatore,
Tamil Nadu, India.
Email: nnagaraj7@yahoo.com

TLC and HPTLC fingerprinting of leaf extracts of *Wedelia chinensis* (Osbeck) Merrill.

Rehana Banu, H., N. Nagarajan

ABSTRACT

Objective: To establish the fingerprint profile of *Wedelia chinensis* using thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC) technique. **Methods:** TLC and HPTLC studies were carried out in two different solvent systems, which showed different R_f value. **Results:** TLC profiling of the extract confirm about the presence of various phytochemicals. HPTLC finger printing of methanol extract of leaf revealed various peaks with R_f values in the range of 0.01 to 0.97. **Conclusion:** It can be concluded that different R_f value of various phytochemicals provide valuable clue regarding their polarity and selection of solvents for separation of phytochemicals. The study will help in future for identifying this plant for further research.

Keywords: *Wedelia chinensis*, profile, TLC, HPTLC Fingerprinting.

1. Introduction

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. This knowledge is accessible from thousands of medical texts and manuscripts. The substances having medical value have been extensively used for treating various disease conditions. Herbs being easily available to human beings have been explored to the maximum for their medicinal properties. Products of primary metabolism such as aminoacids, carbohydrates and proteins are vital for the maintenance of life processes, while others like alkaloids, phenolics, steroids, terpenoids are products of secondary metabolism and have toxicological, pharmacological and ecological importance [1]. Many medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the Indian traditional health care system, (Ayurveda) and proposed for their interesting multilevel activities. Amongst the medicinal plants used in Ayurvedic preparations for their therapeutic action, some have been thoroughly investigated and some of are still to be explored [2].

There are many "families" of phytochemicals which help the human body in variety of ways. Phytochemicals may protect human from a host of disease. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemical to protect itself but recent research demonstrates that many phytochemicals can protect humans against disease. There are many phytochemicals in fruit and shrubs and each work differently [3]. The phytochemical evaluations of plants which have a suitable history of use in folklore have often resulted in the isolation of principles with remarkable bio-activities [4]. Hence, methodologies that can generate a fingerprint of each extract in large collections would be useful to detect stability of the same extract over time. Preferably, the method should be based on electronic storage, retrieval and analysis of the data [5]. A lot of extraction methods and analytical methods as spectrophotometry, high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS) and Fluorescence Transmission-Infrared Spectroscopy (FT-IR) are developed for the study about plant active compounds [6].

High-performance thin layer chromatography (HPTLC) based methods could be considered as a good alternative, as they are being explored as an important tool in routine drug analysis. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase.

Correspondence:
Rehana Banu, H
Department of Botany, Nirmala
College for Women, Coimbatore,
Tamil Nadu, India.
Email: bundybanu@gmail.com
Tel: +919629226949

This reduces time and cost of analysis. In addition, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environment pollution. HPTLC also facilitates repeated detection of chromatogram with same or different parameters [7,8].

Wedelia chinensis (Osbeck) Merrill is a perennial herb of family Asteraceae, commonly known as "Pilabhamgara" or "Bhringraj" in Hindi, *Wedelia* in Chinese and "Manjal karisalanganni" in Tamil [9]. Traditionally, the fruits, leaves and stem are used in child birth and in the treatment of bites and stings, fever and infection. The leaves are used in the treatment of kidney dysfunction, cold, wounds and amenorrhea [10]. The leaves are also for dyeing hair and for promoting their growth. The tonic of the leaves is used in cough and cephalgia. Decoction of the plant is used in menorrhagia and skin diseases [11, 12]. The main objective of this study was to evaluate and optimize the HPTLC fingerprint method in standardization of *Wedelia chinensis* to provide beneficial information in regarding the standardization according to WHO guidelines. These HPTLC fluorescence images coupled with scanning profiles provided adequate information and parameters for comprehensive identification, assessment and comparison of major active constituent fingerprints in the samples studied to serve as a basis for their use in medicinal preparations.

2. Materials and Methods

2.1. Collection, identification and preparation of plant materials

The plant specimen *Wedelia chinensis* (Osbeck) Merrill leaves were collected from Attakatti Hills, Western Ghats, India and authenticated by Dr. V. Balasubramanian, Associate Professor, Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India and was deposited at the college herbarium (Voucher Specimen No: KASC435). The leaves were washed, shade dried and is made powder mechanically and the fine powder was used for extraction procedure.

2.2. Preparation of Extracts

50 g of the shade dried and powdered leaf material was taken for extraction. The crude drug powder was subjected for extraction (Soxhlet extraction) in round bottom flask, first with petroleum ether (60–80 °C) for 24 hours [13, 14] followed by successive extraction with different solvents ranging from non-polar to polar organic solvents namely chloroform, ethyl acetate and methanol separately. The extracts were filtered over Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure to pasty mass [15] for further studies.

2.3. Thin Layer Chromatography (TLC)

TLC studies were carried out following the methodology of Wagner [16] *et al.*

Procedure: Glass capillaries were used for spotting TLC plate. Silica gel plate having the size of 5 × 20 cm was taken and it was marked using pencil. Firstly, straight line was drawn parallel to the short dimension of the plate, 1.5 cm from one end of the plate. Secondly, two small marks were made perpendicularly to this line to divide the line. The distance between the spots was 1.5 cm while the distance of the spot from the margin of plant was 1.0 cm. These marks served as a guide for placing the extracts of the plant. Silica gel G was used as the adsorbent. Slurry of it was prepared with distilled water and was poured on the clean and dry glass plates and spread on the plate as a uniform coating using a glass rod. These

plates were allowed to air dry for 20-25 minutes. Spots (leaf extract) were applied on the activated plate at a distance of 2 cm from one end of the plate with the help of a wing micropipette. While developing the plate, the solvent front was allowed to rise to the second line. Then the solvent was allowed to completely evaporate from the spot. Second deposit was made on same spot. Again the solvent was allowed to completely evaporate. The spotted plates were put in a chromatographic solvent chamber which contained various solvent systems where they placed in such a way that the loaded spot should not touch the solvent. The chamber was covered with proper lid and then it was allowed to stand for sufficient time to reach the solvent phase at maximum. When the solvent front reached second line on plate, it was taken out and dried at room temperature [17, 18]. After the separation of phytochemicals, specific spray reagents were sprayed and allowed to dry. The coloured spots developed on the stationary phase were marked and identified by specific reagent and their distances were measured. The term retention factor R_f , is commonly used to describe the chromatographic behavior of sample solutes. The R_f value for each substance is the distance it has moved divided by the distance the solvent front has moved. Usually, the center of each spot is the point taken for measurement. Comparison of R_f values makes it possible to research complex mixtures qualitatively. The extent of the surface of the spot is a measure for the quantity of the material present [19]. R_f value was calculated for well [20, 21] using the following formula:

$$R_f = \text{Distance travelled by solute} / \text{Distance travelled by solvent}$$

2.4 HPTLC Profile (High Performance Thin Layer Chromatography)

HPTLC studies were carried out following the method of Reich and Schibli [22].

2.4.1 Sample Preparation

100 mg methanolic extract of *Wedelia chinensis* was dissolved in 1 ml of methanol.

2.4.2 Developing Solvent System

A number of solvent systems were tried, for extract. The satisfactory resolution obtained for the phytochemical constituent alkaloid was in the solvent Ethyl acetate-methanol-water (10:1.35:1); for flavonoid was in the solvent Ethyl acetate-butanone-formic acid-water (5:3:1:1); for phenols was in the solvent Toluene: Acetone: Formic acid (4.5:4.5:1) and for that of tannins was in the solvent Toluene-ethyl acetate-formic acid-methanol (3:3:0.8:0.2).

2.4.3 Chromatography

Chromatography was performed on silica gel 60 F₂₅₄ TLC precoated plates using Hamilton syringe and CAMAG LINOMAT 5 instrument. 2 µl of standard solution and 2 µl of the test solution (extract) were loaded as 5 mm band length in the 4×10 glass plates, with the help of a CAMAG LIWOMAT 5 sample applicator at the distance of 10 mm from the edge of the plates.

2.4.4 Development of Chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10×10 cm saturated with previously equilibrated mobile phase for 30 minutes. The chromatographic conditions were previously optimized to active the best resolution and peak shape.

2.4.5 Detection of Spots

The air-dried plates were viewed in ultraviolet radiation to mid-day light. The chromatograms were scanned by densitometer at 420 nm after spraying with specific reagents like Dragendorff's reagent for alkaloids, Ethanolic aluminium chloride for flavonoids, Sodium carbonate solution and Folin- Ciocalteu reagent for Phenols and Ferric chloride reagent for Tannins respectively. Colchicine, apigenin, quercetin and gallic acid were used as the reference standards. The plates were kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254 nm and UV 366 nm.

2.4.6 Scanning

After derivatization, the plates were fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 366 nm, 254 nm and 500 nm. The Peak table, Peak display and Peak densitogram were recorded.

The retention factors (R_f) and % area were calculated by the WinCats software. The TLC was run under laboratory conditions. The spots were quantified using a Camag TLC scanner model 3 equipped with Camag WinCats Software.

3. Results and Discussion

3.1 TLC: The TLC studies showed that among the four solvents (petroleum ether, chloroform, ethyl acetate and methanol) used for extraction, the high polarity solvent methanol extracted higher quantity of secondary metabolites of medicinal importance viz., alkaloids, flavonoids, phenols and tannins from the leaves of *Wedelia chinensis*. Suitable mobile phases with appropriate proportion have been determined for the methanol extract. The corresponding R_f value of various secondary metabolites were recorded in Table 1. The corresponding TLC is presented in Figure

1. For the secondary metabolites phenols, two suitable mobile phases have been determined such as the combination of Chloroform: Methanol in the ratio of 27:0.3 and the combination of Ethyl acetate: Toluene: Formic acid in the ratio of 2.2:1.1:1.1.

Ethyl acetate: Butanol: Formic acid in the ratio of 2.5:1.5:0.5 was determined to be the suitable mobile phase for the secondary metabolite flavonoid. The suitable mobile phase for alkaloid was determined as the combination of Chloroform: Methanol in the ratio of 12:2. This combination is suitable for alkaloid separation as described by [23]. At UV-365 nm the spots were identified and the plate was sprayed with Dragendorff reagent [24]. Tannins were eluted in the mobile phase of Methanol: Water in the ratio of 6:4. R_f value 0.08 and confirmed the presence of Tannins [25].

3.2 HPTLC

The results from HPTLC finger print for methanolic extract of *Wedelia chinensis* leaves are given in Tables 2 to 5. Presence of alkaloid was confirmed with R_f values (0.01 to 0.93). R_f value range of 0.97 confirmed the presence of flavonoids in the extracts. Phenol was confirmed with R_f value range 0.01 to 0.97. Tannin was confirmed in the extract by the R_f value range 0.01 to 0.94. These phytoconstituents in the methanolic extract had several visible colour spots on the TLC plate (Plate II and III). The colored spots were visualized under the UV light between 254 and 366nm after the TLC plate was sprayed with specific spraying reagents, thus indicating the presence of phytoconstituents. The composition of the mobile phase for TLC was optimized by testing different solvent mixtures of varying polarity. The specificity of the method was ascertained by analyzing standards and sample. Spectral studies revealed that the peaks obtained from both the standard and test samples were identical (Figures 2 to 9).

Table 1: TLC finger printing of methanolic leaf extract of *Wedelia chinensis*

Phytoconstituents	Mobile phases	Spraying reagent	Spot colour	R_f value
Alkaloids	Chloroform: Methanol (12:2)	Dragendorff reagent	Orange	0.8
Flavonoids	Ethyl acetate: Butanol: Formic acid (2.5:1.5:0.5)	$AlCl_3$ reagent	Orange	0.87
Phenols	Chloroform : Methanol (27:0.3)	Folin Ciocalteu reagent	Blue	0.87
	Ethyl acetate : Toluene: Formic acid (2.2:1.1:1.1)	$FeCl_3$ reagent	Green	0.8
Tannins	Methanol : Water (6:4)	$FeCl_3$ reagent	Brownish Grey	0.83

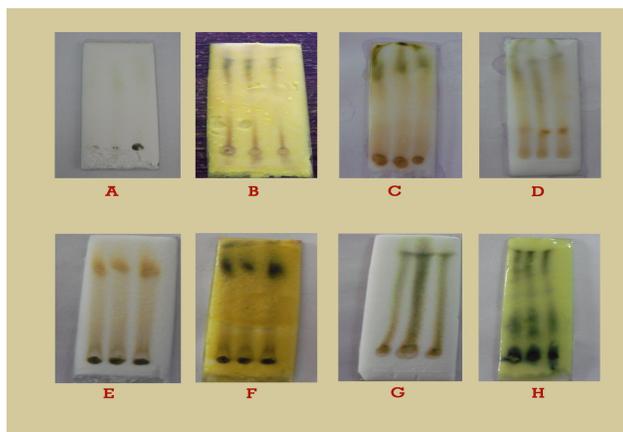


Fig 1: TLC profile of leaf extract of *Wedelia chinensis*

A- Alkaloids - Before spraying; B – Alkaloids - After spraying; C- Flavonoids - Before spraying; D – Flavonoids - After spraying; E- Tannins - Before spraying; F – Tannins - After spraying; G- Phenols - Before spraying; H – Phenols - After spraying

Table 2: HPTLC finger printing spectral values for the presence of alkaloids in *W. chinensis*

Peak	R _f	Height	Area	Assigned substance
1	0.01	169.3	1121.4	Unknown
2	0.03	24.5	206.6	Alkaloid 1
3	0.05	32.3	517.1	Unknown
4	0.14	97.6	2652.5	Alkaloid 2
5	0.21	61.3	1926.5	Alkaloid 3
6	0.46	13.4	204.6	Unknown
7	0.56	13.4	494.4	Unknown
8	0.93	173.2	11181.7	Unknown
1	0.39	570.0	15539.1	Colchicine standard

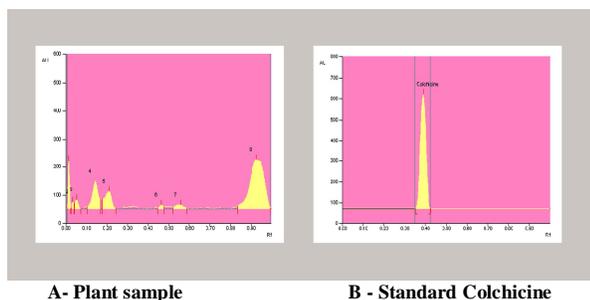


Fig 2: HPTLC Chromatogram of leaf extract of *Wedelia chinensis* confirm the phytoconstituent alkaloid

Table 3: HPTLC finger printing spectral values for the presence of flavonoids in *W. chinensis*

Peak	R _f	Height	Area	Assigned substance
1	0.01	41.3	667.9	Unknown
2	0.76	31.5	1209.3	Flavonoid 1
3	0.92	26.1	447.4	Unknown
4	0.97	86.4	1770.0	Unknown
1	0.87	47.5	1237.8	Apigenin standard

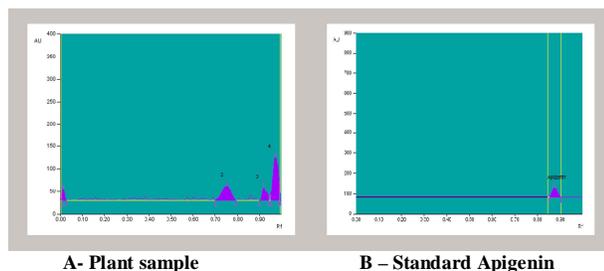


Fig 3: HPTLC Chromatogram of leaf extract of *Wedelia chinensis* confirm the phytoconstituent flavonoid

Table 4: HPTLC finger printing spectral values for the presence of phenols in *W. chinensis*

Peak	R _f	Height	Area	Assigned substance
1	0.01	82.6	1224.6	Unknown
2	0.10	10.4	216.6	Unknown
3	0.14	16.8	481.9	Phenolic 1
4	0.27	55.3	2154.1	Phenolic 2
5	0.38	76.0	3932.1	Phenolic 3
6	0.43	99.9	4124.0	Phenolic 4
7	0.56	25.1	676.7	Unknown
8	0.73	93.0	3856.6	Phenolic 5
9	0.79	82.1	3624.2	Phenolic 6
10	0.87	14.2	298.4	Unknown
11	0.97	23.1	606.3	Unknown
1	0.73	477.0	14623.4	Quercetin standard

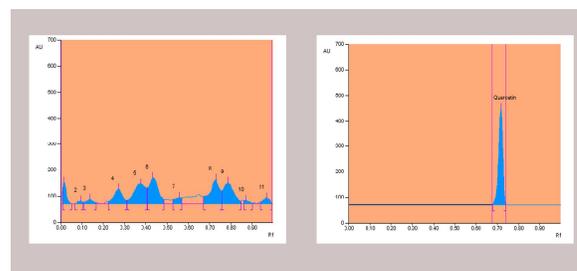


Fig 4: HPTLC Chromatogram of leaf extract of *Wedelia chinensis* confirm the phytoconstituent phenol

Table 5: HPTLC finger printing spectral values for the presence of tannins in *W. chinensis*

Peak	R _f	Height	Area	Assigned substance
1	0.01	357.8	5267.4	Unknown
2	0.11	84.3	2473.0	Unknown
3	0.19	44.3	1071.9	Tannin 1
4	0.22	62.4	1964.7	Unknown
5	0.28	85.3	2603.8	Tannin 2
6	0.82	146.7	15622.6	Unknown
7	0.94	151.4	5983.8	Unknown
1	0.46	299.9	10430.9	Gallic acid standard

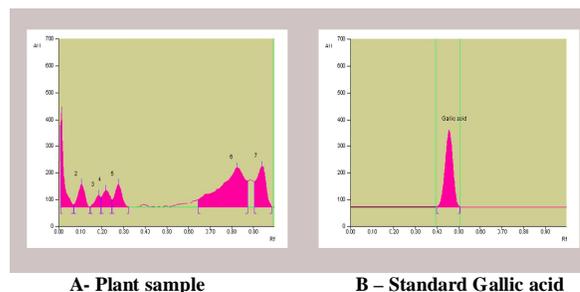


Fig 5: HPTLC Chromatogram of leaf extract of *Wedelia chinensis* confirm the phytoconstituent tannin

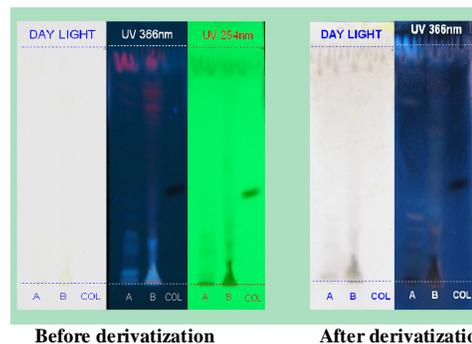


Fig 6: HPTLC profile of leaf extract of *Wedelia chinensis* for alkaloid

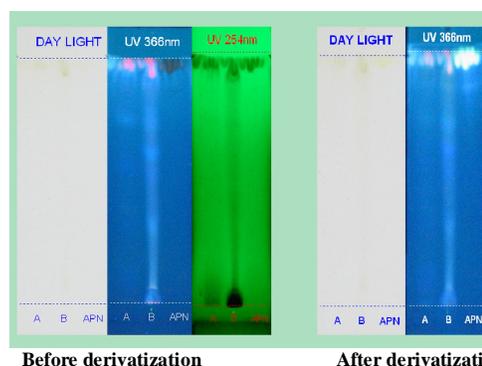


Fig 7: HPTLC profile of leaf extract of *Wedelia chinensis* for flavonoid

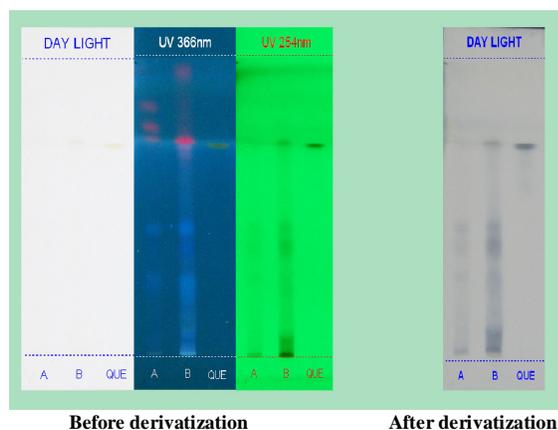


Fig 8: HPTLC profile of leaf extract of *Wedelia chinensis* for phenol

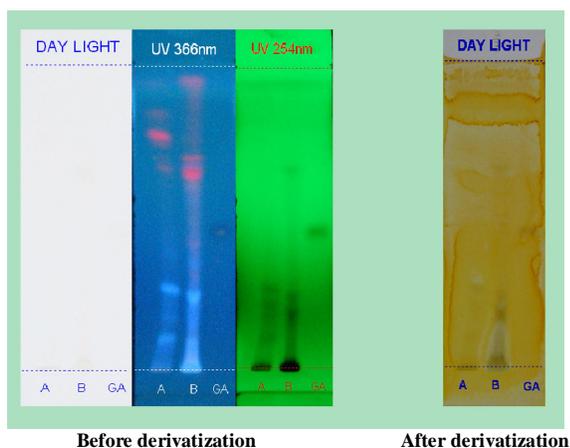


Fig 9: HPTLC profile of leaf extract of *Wedelia chinensis* for tannin

4. Conclusion

Sophisticated modern techniques of standardization such as TLC and HPTLC provide quantitative and semi quantitative information about the main active constituents or marker compounds present in the crude drug or herbal products. TLC serves as one of the many methods in providing a chromatographic plant extract fingerprint. HPTLC fingerprint analysis can be used as a diagnostic tool for the correct identification of the plant. Though further work to characterize the other chemical constituents and perform

quantitative estimation with marker compounds is also necessary these data can also be considered along with the other values for fixing standards to this plant.

5. Acknowledgement

I wish to express my sincere gratitude to my supervisor Dr. N. Nagarajan, Assistant Professor, Department of Botany, Kongunadu Arts and Science College, Coimbatore for his continuous guidance and support. I also thank Dr. V. Balasubramanian, Associate Professor, Department of Botany, Kongunadu Arts and Science College, Coimbatore for identification of the plant material and Dalmia Research Centre, Coimbatore for helping in the analytical results.

6. References

1. Sharma RK, Bhagwan D. Charak Samhita. Edn 4, Vol. 2, Chowkhamba Sanskrit Series, Varanasi, 1996, 17-101.
2. Warriar PK, Nambiar VPK. Ramankutt. Indian Medicinal Plants. Vol. 4, Universities Press, Hyderabad, 304-307.
3. Acharya PS. Dravyaguna Vignyan. Vol. 2, Chaukhamba Bharati Academy, Varanas, 2003, 284-286.
4. Kaviraj AG. Astang Sangrah. Krishnadas Academy Orientalia Publishers and Distributors, Varanasi, 1993, 4-32.
5. Pandey G. Dravyaguna Vignyan. Vol. 2, Chaukhamba Krishnadas Academy, Varanasi, 2004, 158-162.
6. Kirtikar KR, Basu BD. Indian Medicinal Plants. Edn 2, Vol. 1, International Book Distributers, Deharadun, 2005, 649-651.
7. Namjoshi AM. Studies in the Pharmacognosy of Ayurvedic Drug. Board of Research in Ayurveda, Bombay.
8. Khandelwal KR. Practical Pharmacognosy. Edn 11, Nirali Prakashan Pune, 2002, 7-10.
9. Uniyal MR. Prayogatmak Abhinav Dravyaguna Vignyan. Edn 1, Chaukhamba Orientalia, Varanasi, 2009, 514-517.
10. Dwivedi JN, Singh RB. Essentials of Plant Technique. Scientific Publishers Jodhpur, 1985.
11. Nadkarni KM, Nadkarni AK. Indian Materia Medica. Edn 2, Vol. 1, Popular Prakashan, Mumbai, 1062-1063.
12. Jaques HE. How to Know to Insects. Iowa academy of science, Iowa, 1936, 33.