

Physico-Chemical and Phytochemical Evaluation of *Dendrobium macraei* Lindl. (Whole Plant)

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ABSTRACT

In the present study, an attempt has been made to highlight the importance of the plant *Dendrobium macraei* Lindl. (family-Orchidaceae) in the field of traditional medicines. It is commonly known as Swarna Jivanti. The plant is one of the important Rasayana drugs in Ayurveda. It is mainly found in Sikkim Himalayas, Bengal and Khasia mountains at an altitude of 7000-8000 ft. The plant has been reported to be useful as cooling, alterative, astringent to the bowels, stimulant, nervine tonic, aphrodisiac, expectorant, in asthma, bronchitis, 'tridosha', throat troubles, fevers, burning sensations, biliousness, diseases of the eye and blood. Plant is stimulant and tonic. The plant is reported to contain alkaloids, carbohydrates, flavonoids, steroids, tannins and phenolic compounds. Jibantine, resinous principles α and β jibantic acid and diosgenin derivatives like denfigenin and defuscin as steroids are reported as chief constituents in *Dendrobium macraei*. The present study was undertaken for the development of physico- chemical parameters and phytochemical evaluation including development of chromatographic fingerprinting profile of various extracts prepared by thin layer chromatography (TLC) and fluorescence analysis of various parts of plant used. This study will help in authentication of plant material and will acts as standardization parameter for future study purpose.

Keywords: *Dendrobium macraei*, Physicochemical, Phytochemical, fluorescence analysis, TLC, Swarna Jivanti, Rasayana drug, standardization parameter.

INTRODUCTION

Dendrobium macraei is the important botanical source of Ayurvedic drug Swarna Jivanti belonging to family Orchidaceae. Various synonyms of *Dendrobium macraei* viz. *Desmotrichum fimbriatum*, *Flickingeria macraei* (Lindl.), *Flickingeria fimbriata*, *Ephemerantha macraei* (Lindl.), *Callista macraei* (Lindl.), *Flickingeria rabanii* (Lindl.), *Dendrobium fimbriatum* (Bl.), *Dendrobium nodosum* (Dalz.), *Flickingeria nodosa* (Dalz.) are also used as Swarna Jivanti. It is an epiphyte with creepy rhizome and pendulous stem¹. The plant is sweet with a flavour, cooling, alterative, astringent to the bowels, tonic, aphrodisiac, expectorant, useful in asthma, bronchitis, 'tridosha', throat troubles, fevers, burning sensations, biliousness, diseases of the eye and the blood. Plant is stimulant and tonic¹⁵. The plant is reported to contain alkaloids, carbohydrates, flavonoids, steroids, tannins and phenolic compounds. Jibantine, resinous principles α and β jibantic acid and diosgenin derivatives like denfigenin and defuscin as steroids are reported as chief constituents in *Dendrobium macraei*⁹. Jivanti is one of the important Rasayana drugs in Ayurveda and is used as an ingredient in formulations like *jivantadya ghrita*, *jivantayadi rasa*, *jivantadyadi taila*, *Ashwagandhadi ghrita*, *anuthaila*, *chandanadi thaila* which are used in treatment of tuberculosis, emaciation, fever, haemorrhage and various types of cardiac ailments^{19,23}. Therapeutically, Jivanti is

used as alterative, aphrodisiac, astringent, cooling, diuretic and as a tonic in various types of debilities¹⁶.

MATERIALS AND METHODS

Chemicals

All the chemicals which were used in study are of analytical grade and purchased from the Merck, Himedia Lab. Pvt. Ltd, Rankem.

Plant collection and identification

Plant material (*D. macraei* whole plant) was procured from Kankhal, near Haridwar, Uttarakhand, India. The plant was identified and authenticated at the Herbarium of Council of Scientific and Industrial Research –National Institute of Science Communication and Information Resources (CSIR-NISCAIR), Delhi vide reference no. NISCAIR/ RHMD/ Consult/ 2015/ 2565-144.

Physicochemical evaluation

Foreign organic matter

Foreign organic matter in plant was determined by spreading 100 g of crude drug on clear smooth surface background by using magnifying lenses (10X). The experiment was done in triplicates.

Moisture content

3 g of shade-dried drug was taken in a tared porcelain dish. The crude drug was heated at 105°C in an oven till a constant weight. Percentage moisture content of the

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sample was calculated with reference to the shade-dried drug material⁶.

Calculate the Percentage of moisture content of air dried material as

$$\% \text{ Moisture content} = \frac{\text{Loss in weight of the sample on heading}}{\text{Weight of total amount of drug taken}} \times 100$$

Extractive value^{6,21}

Various methods used for determination of extractive value are as follows

Method 1. Hot extraction

4.0 g of coarsely powdered, accurately weighed air-dried material was placed in a glass-stoppered conical flask. 100 ml of water was added and weighed to obtain the total weight including the flask. Shake well and allowed to stand for 1 h. A reflux condenser was attached to the flask and gently boil for 1h; cool and weighed. Readjusted to the original total weight with the solvent specified in the test procedure for the plant material concerned. Shake well and filter rapidly through a dry filter. 25 ml of the filtrate was transferred to a tared flat-bottomed dish and evaporated to dryness on a water-bath. Dried at 105°C for 6 h, cooled in a dessicator for 30 min, then weigh without delay. The content of extractable matter was calculated in mg per g of air-dried material.

Calculate the percentage of extractive value of air dried material as

$$\% \text{ Extractive value} = \frac{[\text{final wt.} - \text{initial wt.}] \times 4}{\text{Wt. of drug}} \times 100$$

Method 2. Cold maceration

4 g of coarsely powdered air-dried material, accurately weighed, was placed in a glass-stoppered conical flask. Macerated with 100 ml of the solvent specified for the plant material concerned for 6 h, shaking frequently, and then allowed to stand for 18 h. Filtered rapidly taking care not to lose any solvent, 25 ml of the filtrate was transferred to a tared flat-bottomed dish and evaporated to dryness on a water-bath. Dried at 105°C for 6 h, cooled in a dessicator for 30 min and weighed without delay. The content of extractable matter was calculated in mg per g of air-dried material.

Calculate the percentage of extractive value of air dried material as

$$\% \text{ Extractive value} = \frac{[\text{final wt.} - \text{initial wt.}] \times 4}{\text{Wt. of drug}} \times 100$$

By following both the methods described above various types of extractive values were calculated which are as follows.

Water soluble extractive value

Ethanol soluble extractive value

Acetone soluble extractive value

Chloroform soluble extractive value

Petroleum ether soluble extractive value

Ash value^{23,21}

Total ash

2 g of powdered *D. macraei*, was incinerated in a crucible at a temperature 500- 600°C in a muffle furnace till carbon free ash was obtained. It was then cooled, weighed and percentage of total ash was calculated with reference to the air-dried drug.

Calculate the percentage of Total ash value of air dried material as

$$\% \text{ Total ash value} = \frac{\text{weight of total ash}}{\text{Weight of crude drug taken}} \times 100$$

Determination of acid insoluble ash

Ash, above obtained, was boiled for 5 min with 25 ml of 70 g / L hydrochloric acid and filtered using an ashless filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid-insoluble ash was calculated with reference to the (40 #) air-dried powdered drug.

Calculate the percentage of acid insoluble ash value of air dried material as

$$\% \text{ Acid insoluble ash value} = \frac{\text{weight of acid insoluble ash}}{\text{weight of crude drug taken}} \times 100$$

Determination of water soluble ash

Total ash was boiled for 5 min with 25 ml water and insoluble matter which was collected on an ash-less filter paper was washed with hot water and ignited for 15 min at a temperature not exceeding 450°C in a muffle furnace. Difference in weight of ash and weight of water insoluble matter gave the weight of water-soluble ash. The percentage of water-soluble ash was calculated with reference to the (40 #) air-dried powdered drug.

Calculate the percentage of water soluble ash value of air dried material as

$$\% \text{ Water soluble ash value} = \frac{\text{weight of total ash} - \text{weight of water insoluble ash}}{\text{weight of crude drug taken}} \times 100$$

Determination of sulphated ash

A silica or platinum crucible was heated to redness for 10 min, allowed to cool in a dessicator and weighed. Unless otherwise specified in the individual monograph, 1 g of the substance was transferred to the crucible under examination and the crucible and the contents were weighed accurately. Gently, ignited at first until the substance was thoroughly charred. The residue was cooled and moistened with 1 ml of sulphuric acid, gently heated until the white fumes was no longer evolved and ignited at 800 ± 25°C until all black particles was disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool and few drops of sulphuric acid was added and heated. Ignited again as

Figure 1: Whole plant *D. macraei*Table 1: Moisture content of *D. macraei*.

S. No.	Quality parameter	Results (% w/w)
1.	Moisture content	9.6%

Table 2: Extractive values of *D. macraei*.

S. No.	Extractive values	Results (% w/w)	
		Hot extraction	Cold maceration
1.	Water soluble	12%	11%
2.	Ethanol soluble	11%	9%
3.	Acetone soluble	7%	5%
4.	Chloroform soluble	7%	5%
5.	Petroleum ether soluble	3%	1%

Table 3: Ash values of *D. macraei*.

S. No.	Ash values	Results (% w/w)
1.	Total ash	5.3%
2.	Acid- insoluble ash	1.3%
3.	Water soluble ash	3%
4.	Sulphated ash	7%

before, allowed to cool and weighed. The operation was repeated until two successive weighings do not differ by more than 0.5 mg²⁰.

Calculate the percentage of sulphated ash value of air dried material as

$$\% \text{ Sulphated ash value} = \frac{\text{weight of sulphated ash}}{\text{weight of crude drug taken}} \times 100$$

Preparation of extracts

Dried parts of whole plant were pulverized using a mechanical grinder. Powdered material was subjected to successive soxhlet extraction by solvents in increasing order of polarity viz. petroleum ether, chloroform, ethyl acetate, methanol and distilled water. Before each extraction the powdered material was dried in hot air-oven below 50°C. Finally, marc was boiled with distilled water for 4 h to obtain the aqueous extract. All extracts were concentrated in a rotary vacuum evaporator (40°C), freeze-dried and stored at 4°C until further use in the experiment.

Extracts was weighed, and percentage was calculated in terms of the air-dried weight of the plant material⁸.

Phytochemical evaluation

The preliminary phytochemical screening was carried out using the extracts prepared with different solvents for different types of chemical constituents by successive soxhlet extraction as per the methods described. The extracts were subjected to preliminary phytochemical investigation for detection of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins, amino acids, lipids/fats etc.

Tests for carbohydrates²

Molisch's test

1g powdered drug was extracted with 10 ml ethanol for 15 min on a boiling water bath and filtered. On addition of α -naphthol and concentrated H_2SO_4 to the filtrate a mild purple ring at the junction of two liquids indicate presence of carbohydrates.

Fehling's test

To the mixture of 1 ml Fehling A and 1 ml Fehling B solutions, (boiled for 1 min) add equal volume of test solution. After heating it on boiling water-bath for 5- 10 minutes' formation of red precipitates indicates presence of carbohydrates.

Keller Killiani test

To 2 ml extract add glacial acetic acid, one drop 5% FeCl_3 and conc. H_2SO_4 . Reddish brown colour appearing at junction of the two liquid layers and upper layer if appear bluish green, indicates presence of 2-deoxy sugars.

Tests for proteins and amino acids³

100 mg of methanol extract of *D. macraei* was dissolved in 10 ml of water and filtered. Filtrate was used to test the presence of proteins and amino acids.

Millon's test

To 2 ml of filtrate add 2 ml of Millon's reagent in a test tube and heat in a water bath for 5 minutes. After cooling add few drops of NaNO_2 solution. Formation of white precipitates turning to red upon heating indicates presence of proteins and amino acids.

Ninhydrin test

To 2 ml of filtrate add 2-3 drops of Ninhydrin reagent in a test tube and boil for 2 minutes. Formation of distinct blue colour indicates presence of amino acids.

Biuret test

Treat 2 ml of filtrate with 2 ml of 10% sodium hydroxide solution in a test tube and heat for 10 minutes. Then add a drop of 7% of copper sulphate in the above solution. Distinct violet colouration indicates presence of proteins.

Tests for glycosides¹⁷

The aqueous extract of *D. macraei* was prepared by cold maceration with 3% methanol-water for 7 days with occasional shaking.

Legal test

Take 1 ml filtrate in a test tube. To it add 3 ml sodium nitroprusside in pyridine and KOH in methanol. If the alkaline layer turns to blue, it indicates presence of cardiac glycosides.

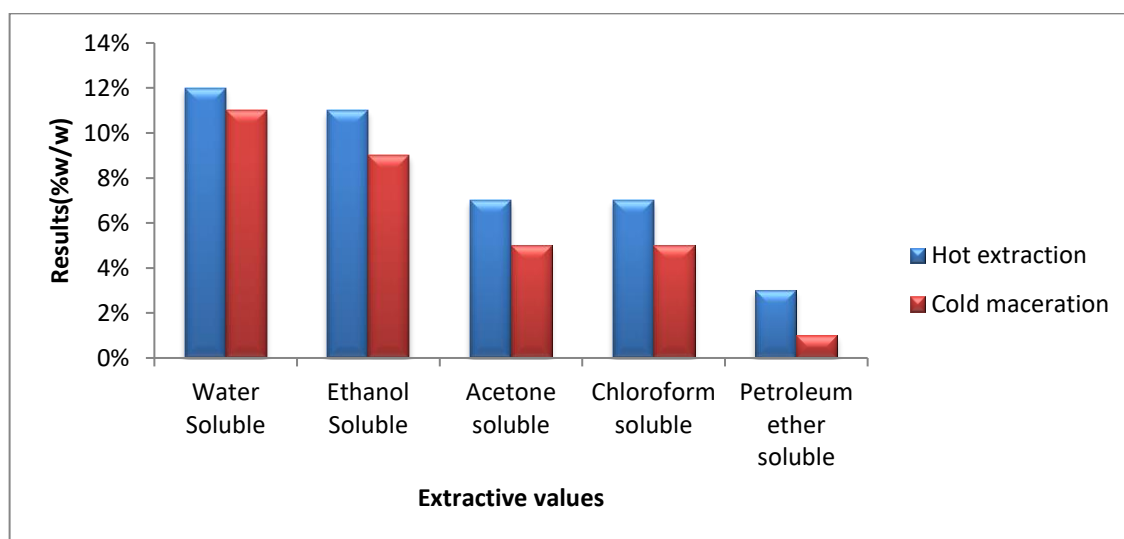
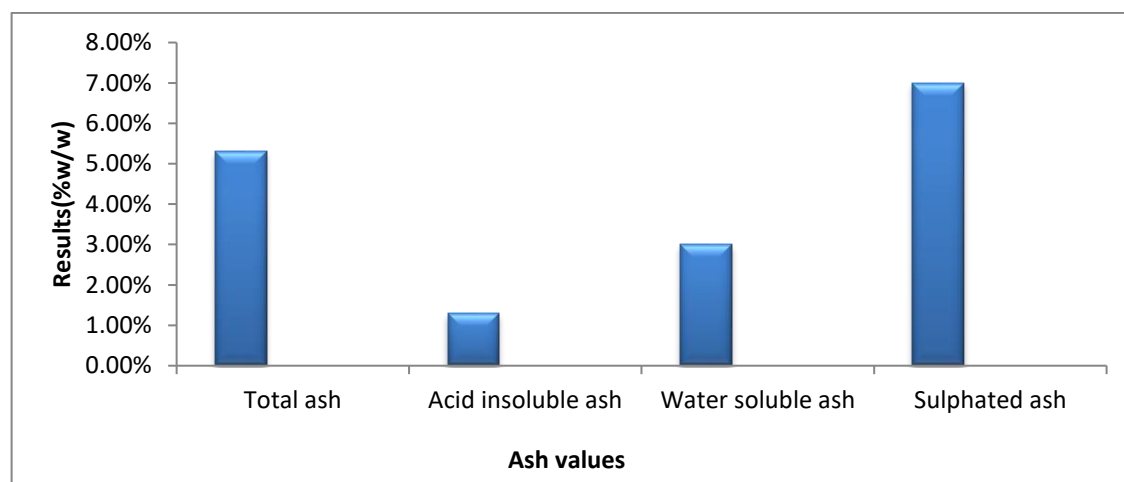
Keller-killiani test

Take 1 ml filtrate; shake with 1ml of glacial acetic acid containing traces of ferric chloride. Carefully add 1 ml of

Table 4: Phytochemical screening of different extracts of *D. macraei*.

S. No.	Phytochemical tests	Petroleum Ether Extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
1.	Carbohydrates	-	-	-	+	+
2.	Fats & Oils	+	-	-	-	-
3.	Proteins	-	-	-	-	-
4.	Amino acids	-	-	-	-	-
5.	Alkaloids	-	+	-	-	-
6.	Glycosides	-	-	-	-	-
7.	Flavonoids	-	-	++	+	+
8.	Tannins and Phenolic compounds	-	-	-	+	+

Note: (+) means positive, (-) means negative.

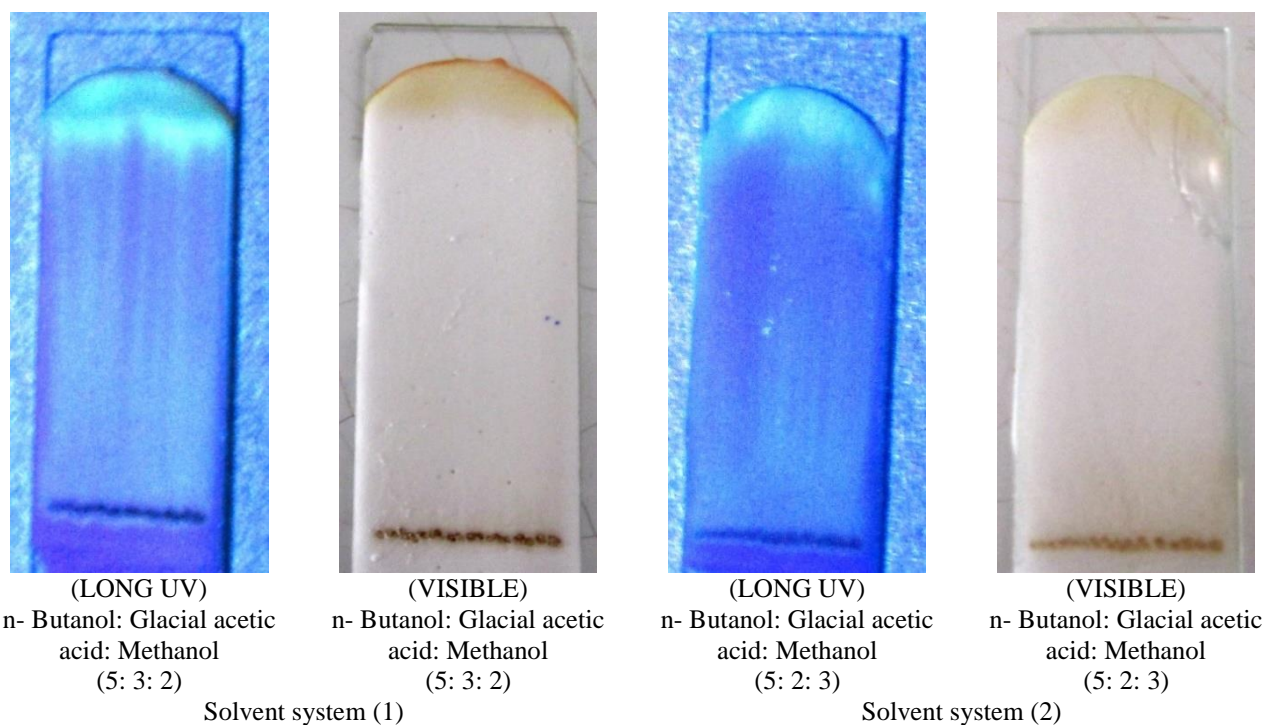
Figure 2: Histogram representing different extractive values of *D. macraei*.Figure 3: Histogram representing different ash values of *D. macraei*.Table 5.1: TLC fingerprinting profile of Aqueous extract of *D. macraei*.

S. No.	Solvent systems	Rf values
1.	n- Butanol : Glacial acetic acid: Methanol (5: 3: 2)	One spot (0.89)
2.	n- Butanol : Glacial acetic acid: Methanol (5: 2: 3)	One spot (0.84)

Detecting agent used: Conc. Sulphuric acid

concentrated sulphuric acid by the side of test tubes. Blue

colouration in acetic acid layer or red colour at the junction of the two liquids indicates presence of glycosides.

Figure 4: TLC Plates representing chemical constituents in aqueous extract of *D. macraei*.Table 5.2: TLC fingerprinting profile of Petroleum ether extract of *D. macraei*.

S. No.	Solvent systems	Rf values
1.	Pet.Ether: Toluene: Ethyl acetate (8:1:1)	Two spots (0.52, 0.72)
2.	Toluene: Ethyl acetate : Formic acid (8:1:1)	Three spots (0.291, 0.52, 0.68)
3.	Pet.Ether: Toluene: Ethyl acetate (7: 1: 2)	Four spots (0.8, 0.72, 0.65, 0.5)
4.	Toluene: Ethyl acetate : Formic acid (7: 2: 1)	Three spots (0.75, 0.64, 0.39)

Detecting agent used: Conc. Sulphuric acid

Table 5.3: TLC fingerprinting profile of Chloroform extract of *D. macraei*.

S. No.	Solvent systems	Rf values
1.	Chloroform: Ethyl acetate (4:6)	Six spots (0.18, 0.20, 0.53, 0.72, 0.84, 0.94)
2.	Chloroform: Ethyl acetate (5:5)	Five spots (0.109, 0.27, 0.45, 0.72, 0.89)
3.	Methanol: Chloroform (9:1)	Three spots (0.18, 0.84, 0.93)
4.	Toluene: Ethyl acetate : Glacial acetic acid (7: 2: 1)	Three spots (0.66, 0.78, 0.87)

Detecting agent used: Conc. Sulphuric acid and FeCl_3 Table 5.4: TLC fingerprinting profile of Ethyl acetate extract of *D. macraei*.

S. No.	Solvent systems	Rf values
1.	Toluene: Ethyl acetate (5:5)	Seven spots (0.089, 0.16, 0.23, 0.39, 0.51, 0.78, 0.87)
2.	Toluene: Ethyl acetate: Formic acid (4:5:1)	Two spots (0.810, 0.775)
3.	Toluene: Ethyl acetate (6:4)	Two spots (0.5, 0.571)
4.	Toluene: Ethyl acetate (4:6)	Three spots (0.53, 0.58, 0.625)

Detecting agent used: Conc. Sulphuric acid, Draggendroff's reagent and FeCl_3 Table 5.5: TLC fingerprinting profile of Methanol extract of *D. macraei*.

S. No.	Solvent systems	Rf values
1.	Toluene: Ethyl acetate : Glacial acetic acid (5: 4: 1)	Three spots (0.89, 0.82, 0.77)
2.	Toluene: Ethyl acetate : Glacial acetic acid (6: 3: 1)	Five spots (0.75, 0.78, 0.82, 0.85, 0.89)
3.	Toluene: Ethyl acetate : Glacial acetic acid (7: 2: 1)	Five spots (0.48, 0.64, 0.69, 0.75, 0.85)
4.	Chloroform: Ethyl acetate: Glacial acetic acid (7: 2: 1)	Four spots (0.77, 0.82, 0.87, 0.91)

Detecting agent used: Conc. Sulphuric acid and FeCl_3

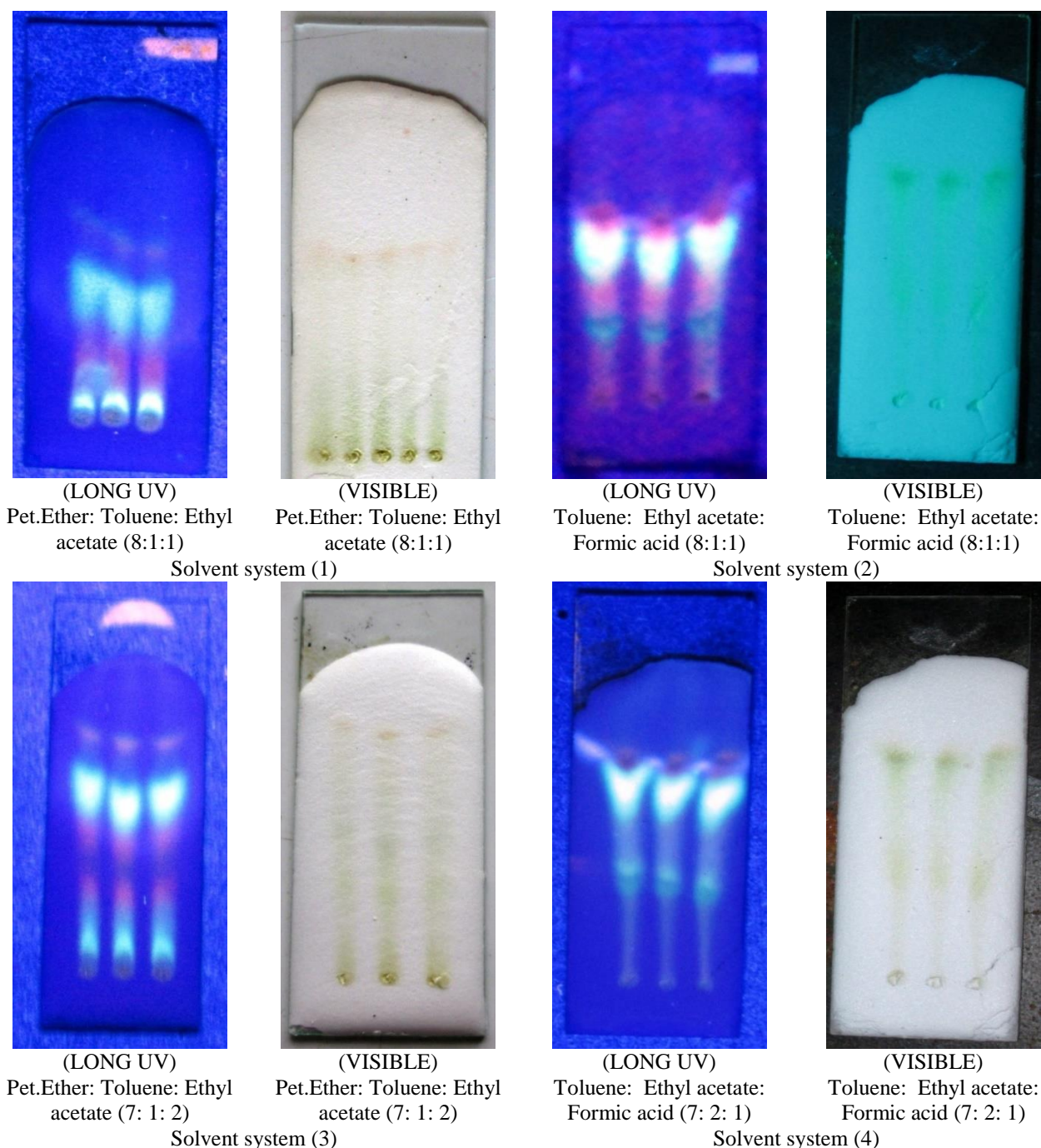


Figure 5: TLC Plates representing chemical constituents in pet.ether extract of *D. macraei*.

Tests for anthraquinone glycosides¹⁰

Borntrager's test

Prepare ether extract of whole plant powder of the drugs. Add ammonia to the filtered ethereal extract. If the aqueous layer shows pink red or violet colour after shaking then anthraquinone glycosides are present.

Modified Borntrager's test

Add ferric chloride and dilute HCl to the aqueous solution of drug and heat, cool and filter. Filtrate is shaken with ether or any other organic solvent. The ethereal extract is then shaken with dilute ammonia. If the aqueous layer

shows rose-pink to cherry red colour then anthraquinone glycosides are confirmed.

zone confirms the presence of saponins.

Tests for flavonoids⁵

Shinoda test

1g of powdered drug was extracted with 10 ml of ethanol (95 % v/v) for 15 min on a boiling water bath and filtered. To the filtrate was added a small piece of magnesium ribbon and 3 to 4 drops of concentrated sulphuric acid. Formation of red colour indicates presence of flavonoids.

Fluorescence test

Extract 1g powder of *D. macraei* with 15 ml methanol for

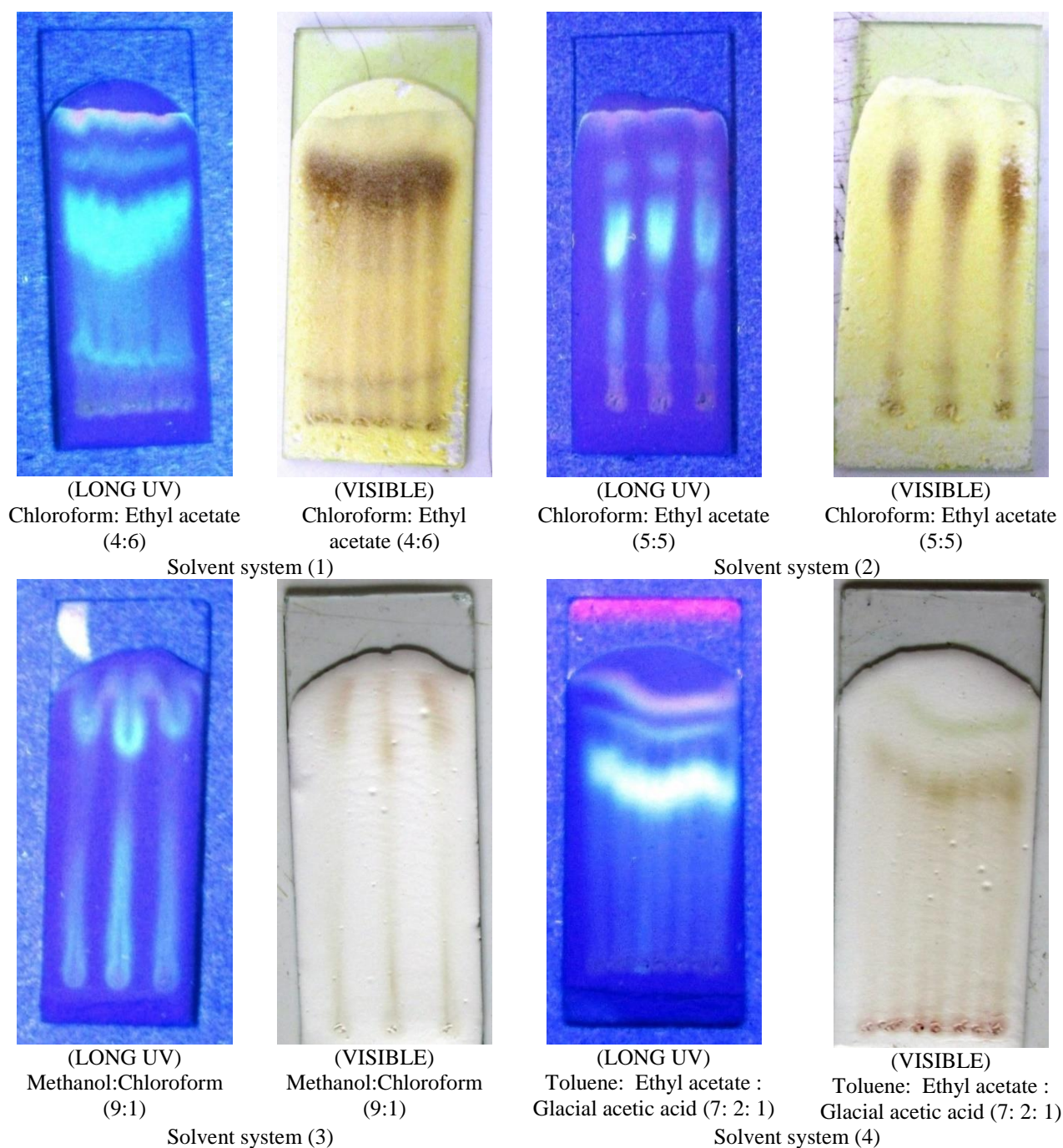


Figure 6: TLC Plates representing chemical constituents in chloroform extract of *D. macraei*.

2 min. on a boiling water bath. Filter while hot and evaporate to dryness. To the residue add 0.3 ml boric acid solution (3 % w/v) and 1 ml oxalic acid solution (10 % w/v). Evaporate the mixture to dryness and dissolve the residue in 10 ml ether. The ethereal layer showing greenish fluorescence under UV light indicates presence of flavonoids.

Tests for tannins^{22,4,14}

For following tests, aqueous extract of *D. Macraei* powder (10 g) was prepared by refluxing with 50 ml water for about 1h on water bath.

Test with gelatin

Add 2-3 ml of aqueous extract to 1% w/w

Gelatin solution containing NaCl. Formation of heavy white precipitates indicates presence of tannins.

Reaction with lead acetate

To the aqueous extract of drug add 2 ml of 10% w/w solution of lead acetate. Precipitation if obtained indicates presence of tannins.

Tests for phenolic compounds^{1,7}

Test with FeCl₃

To methanolic extracts of powdered drug add a drop of freshly prepared FeCl₃ solution. Brownish green colour indicates presence of phenolics.

Test with Folin ciocalteu reagent

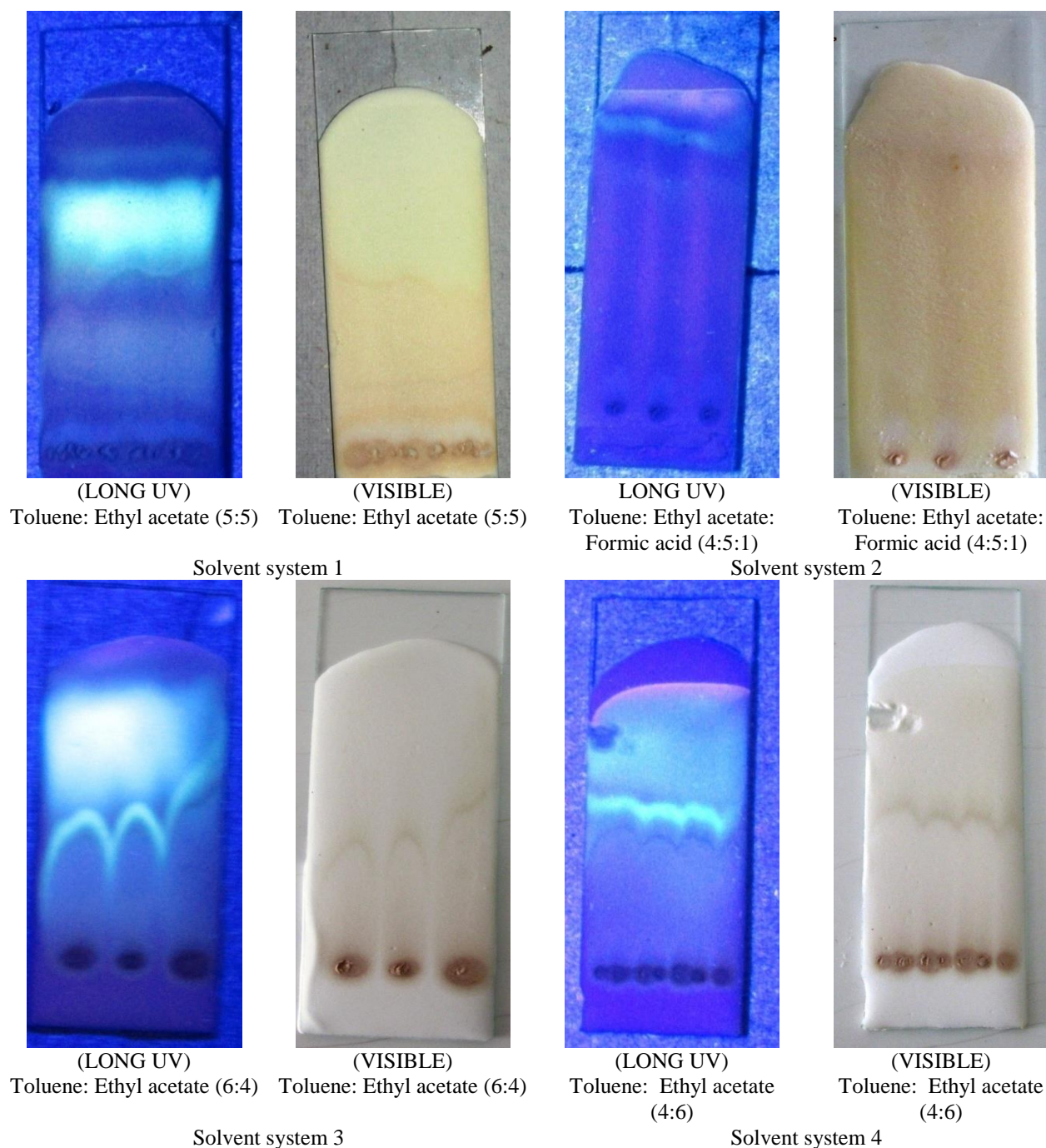


Figure 7: TLC Plates representing chemical constituents in ethyl acetate extract of *D. macraei*.

To a drop of methanolic extract of whole plant add a drop of Folin ciocalteu reagent. Bluish green colour indicates presence of phenolics.

Test for alkaloids^{8,13}

Dragendroff's test

Extract 1g powdered drug with 20ml alcohol by refluxing for 15 min and filter; evaporate the filtrate to dryness. Dissolve the residue in 15 ml 2N H₂SO₄ and filter. After making it alkaline, extract the filtrate with chloroform. Treat the residue with Dragendroff's reagent. Development of orange precipitates indicates presence of alkaloids.

Hager's test

Dissolve 100 mg of methanol extract in 10 ml of dilute hydrochloric acid (0.1 N) and filter. Treat two ml of the filtrate with Hager's reagent; formation of yellow coloured precipitates indicates presence of alkaloids.

Development of chromatographic fingerprinting profile of various extracts prepared by thin layer chromatography

The TLC plates were washed and dried in oven. TLC plates were prepared by the pouring method. Silica gel G was taken in a beaker and the slurry was made with distilled water. The plates were then tipped back and to spread the slurry uniformly over the surface. These plates were dried at room temperature and then put in the oven at 110°C for 30 min activation of TLC plates. Various solvents were

Table 6.1: Fluorescence analysis of powdered *D. macraei* treated with various reagents

S. No.	Reagents	Visible light	Long UV	Short UV
1.	Powder as such	Light brown colour	Walnut brown colour	Henna green colour
2.	50% H ₂ SO ₄	Light brown colour	Dark brown colour	Henna green colour
3.	50% HNO ₃	Chocolate brown colour	Dark brown colour	Cascade green colour
4.	5% KOH	Light brown colour	Dark green colour	Cascade green colour
5.	Methanol	Light brown colour	Chartreuse green colour	Emerald green colour
6.	1 N HCl	Light brown colour	Walnut brown colour	Henna green colour
7.	1N Methanolic NaOH	Henna green colour	Light green colour	Cascade green colour
8.	Ethanol	Dark brown colour	Light green colour	Dark green colour
9.	1 N Ethanolic NaOH	Yellowish brown colour	Pale brown colour	Cascade green colour
10.	5% NaOH	Pale brown colour	Dark green colour	Cascade green colour
11.	5% FeCl ₃	Yellowish brown colour	Blackish violet colour	Henna green colour
12.	Conc. HCl	Light brown colour	Dark brown colour	Cascade green colour
13.	Conc. H ₂ SO ₄	Dark brown colour	Blackish violet colour	Dark green colour
14.	NH ₃	Light brown colour	Dark green colour	Cascade green colour

Table 6.2: Fluorescence analysis of extracts of *D. macraei*.

S. No.	Extracts	Fluorescence analysis			Consistency
		Visible light	Long UV	Short UV	
1.	Petroleum ether extract	Dark green colour	Black colour	Dark green colour	Semi-solid mass
2.	Chloroform extract	Henna green colour	Black colour	Henna green colour	Solid mass
3.	Ethyl acetate extract	Brownish green colour	Black colour	Light green colour	Soft and sticky mass
4.	Methanol extract	Dark brown colour	Dark violet colour	Blackish green colour	Soft and sticky mass
5.	Aqueous extract	Dark brown colour	Blackish violet colour	Violet colour	Semi-solid mass

used for TLC for the investigation of different compounds which were present in extracts¹¹.

Fluorescence Analysis

Fluorescence analysis is the one of the most important parameter for the evaluation of the quality, strength and purity of the selected plant material. The powdered material (whole plant) was analyzed under the three regions of light like visible, short U.V region and long U.V region after the treatment with various inorganic/organic reagents.

Statistical analysis

The values were represented as mean \pm S.D. (n=3) statistically significant differences between groups were calculated by using ANOVA, followed by Post hoc Tukey's multiple range test where $P < 0.05$ was considered statistically significant.

RESULTS

D. macraei was subjected to systematic physiochemical and phyto chemical screening includes TLC and fluorescence analysis by extracting with various organic solvents to determine the amount of soluble constituents in a given amount of medicinal plant material. The data generated is helpful in determining the quality and purity of a crude drug, especially in powdered form.

Physicochemical evaluation

In this study the parameters used for the evaluation of *D. macraei* were Foreign organic matter, moisture content, extractive values by different solvents (includes water, ethanol, acetone, chloroform and petroleum ether) and ash

values (total ash, water soluble and acid insoluble ash). The objective of reducing the vegetable drug to its ash is to remove all traces of organic matter, which may otherwise interfere in an analytical determination. On incineration, crude drugs leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The determination of ash is useful for detecting low-grade products, exhausted drugs and excess of sandy or earthy matter; it is more especially applicable to powdered drugs¹⁹. Results were clearly shown in Table No. 1, 2 and 3 respectively.

Phytochemical evaluation

Phytochemical analysis was performed on the whole plant powder and it was found to contain carbohydrates, steroids, coumarins, flavonoids, phenolics and alkaloids. Results of qualitative tests revealed the presence of carbohydrates, alkaloids, flavonoids, phenolics, fats and oils etc. Phytochemical screening of different extracts of *D. macraei* are presented in Table No.4. In the following table the (+) sign means positive and (-) sign means negative.

Thin layer Chromatography

Various mobile phases were employed to develop the TLC of different extracts. Results of TLC fingerprinting profile of different extracts of *D. macraei* are presented in Table No. 5.1- 5.5

Fluorescence analysis

Fluorescence analysis of powdered *D. macraei* treated with various reagents and analysis of extracts were clearly depicted in Table No. 6.1-6.2.

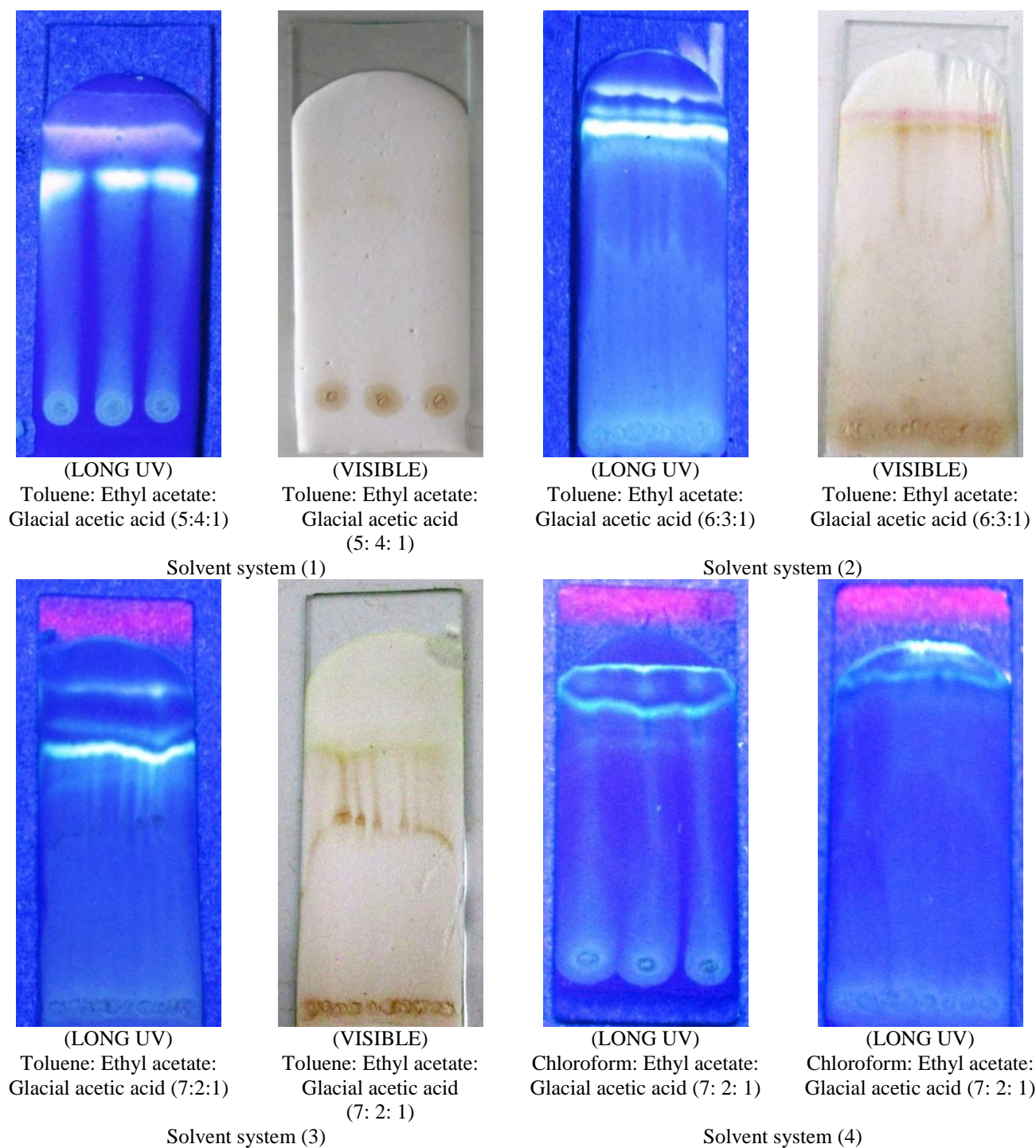


Figure 8: TLC Plates representing chemical constituents in methanol extract of *D. macraei*.

DISCUSSION

Physico-chemical parameters help to identify the authenticated plant material and to check the status of adulteration present in crude drug material. In the present study moisture content in the coarse powdered drug was estimated as 9.6% which is shown in Table No.1 above. Extractive value determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. In the present study, ethanol, chloroform, acetone, petroleum ether and water were used to evaluate the extractable constituents in the whole plant

of *D. macraei* by cold maceration and hot extraction method. Ethanol, chloroform, acetone, petroleum ether and water-soluble extractive values by cold maceration method were estimated as 9%, 5%, 5%, 1% and 11% respectively in which aqueous extract shows high amount of soluble extractable constituents, and by hot extraction method were estimated as 11%, 7%, 7%, 3% and 12% respectively in which aqueous extract shows high amount of soluble extractable constituents which is shown in Table No.2 above. The ash values were also estimated in the present study such as total ash, acid insoluble ash, water soluble ash and sulphated ash values to determine the extraneous

matter adhering to the plant surface and total amount of material remains after ignition. The estimated ash values such as total ash, acid –insoluble ash, water soluble ash and sulphated ash found were 5.3%, 1.3%, 3% and 7% respectively which is shown in Table No. 3 above. Phytochemical screening helps to investigate the phytoconstituents which are present in plant. It showed presence of mainly carbohydrates, flavonoids, phenolics, alkaloids in different extracts of *D. macraei* which is shown in Table No.4 above. Further, the plant was subjected to determine TLC finger printing profile in various prepared extracts which shows the presence of various types of phyto-constituents present in various extracts when treated against various types of solvent system. This method was particularly valuable for the qualitative determination of small amounts of impurities present in plants. Then, the retardation factor (Rf) was noted by taking the measurements of distance travelled by solute and solvent in the specific solvent system. Spots and phyto-constituents are identified by spraying of various types of detecting agents which is shown in Table No. 5.1-5.5 above. Fluorescence analysis shows the emission of light by a substance against absorbed light. Fluorescence occurs when the absorbed radiation was in the ultraviolet region of the spectrum, and thus invisible to the human eye, while the emitted light was in the visible region, which gives the fluorescent substance a distinct colour that can only be seen when exposed to UV light. Fluorescence analysis of powdered *D. macraei* when treated with various reagents shows different fluorescence after absorbing light in visible, long and short U-V was also reported in Table No.6.1 above. Fluorescence analysis along with the consistency of various prepared extracts of *D. macraei* in visible, long and short U-V was evaluated and reported in Table No. 6.2 above.

CONCLUSION

Despite a long history of use of *Dendrobium macraei* as a traditional medicine for the treatment of various ailments. The present study establishes the various pharmacognostic standards like physico- chemical parameters; phytochemical groups present in plant, TLC fingerprinting profile and Fluorescence analysis of the prepared extracts. Future prospects of the current investigations acts as a standardization parameter.

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