Chromatographic Evaluation of Medicinal Plant

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Abstract-

Premise of the Research*: Medicinal plants contain different bioactive compounds which have great importance to the health of individuals and communities. These compounds produce definite physiological action on the human body.The present study was carried out to verify the Thin layer chromatographic profiling and phytochemical screening of chloroform and methanol extract of Dodonaea Viscosa.*
Methodolgy*: Phytochemical screening was performed by various qualitative methods and extraction was carried out using a various solvent like cyclohexane, chloroform, acetone, methanol system using Soxhelt extraction. This extracts are further used for the development of mobile phase.*

Result: *Qualitative phytochemical determination by chemical test reflects the presence of alkaloid, flavonoid, tannins and glycoside in different plants. Thin layer chromatographic profiling of these plants were carried out using different solvents of methanol and chloroform extracts and it showed different Rf value.*

Conclusion: *The results obtained in the present work can be used for the further plan investigation and determination of plants activity.*

Keywords- Thin layer chromatographic, Extraction, Dodonaea viscosa, Solvents, Phytochrmicals.

I. INTRODUCTION

Thin layer chromatography (TLC) is a commonly used laboratory technique and it is similar to paper chromatography. Thin layer chromatography was based on the principle of separation. The separation depends upon the relative affinity of the compound towards mobile and stationary phase(fig no. 1).

- **Experimental Techniques in TLC:**

A Preparation of thin layer on plates:

The plates used in TLC must be uniform and consistent throughout. Various methods are used to apply thin layers of powdered or their suspensions or their slurries to the carrier plates with a view to achieve an uniform layer throughout the length of the plates. These are namely:

1. Pouring of Layers:
2. Dipping
3. Spraying
4. Spreading

B Choice of Adsorbents:

The choice of proper adsorbent in TLC plays an important role in the separation of components from the mixture of components. In the actual practice the adsorbents used are of mainly two types i.e. Inorganic and Organic adsorbents.

- Inorganic Adsorbents: Examples: Aluminium oxide, Aluminium silicate, Bentonites, Calcium carbonates, Calcium hydroxide, Calcium oxalate, Calcium silicate, Calcium sulphate, Dicalcium phosphate, Magnesium silicate, Silica gel, Zinc carbonate.
- Organic Adsorbents: Examples: Cellulose and Acetylated Cellulose, Charcoal and Activated Carbon, Dextran Gels, Cellulose Ion-Exchange Powder, Ion-Exchange Resins.

C Choice of Solvent System:

The choice of solvent or mixture of solvents used is mainly depend upon the following factors:

- The nature of the constituent to be separated (Polar or non polar)
- The nature of the process involved (‘adsorption’ or ‘partition chromatography’)

D Activation of the Adsorbents:

In TLC it is important to eliminate the solvent imbedded into the thin layer of coated adsorbent. It is achieved conveniently first by air-drying the TLC plates for duration of 30 minutes and then in a hot-air oven maintained at 110 °C for another 30 minutes and subsequently cooling them in desiccators. This drying process helps in the activation of the adsorbent layer. In order to achieve very active layers, silica

gel and alumina coated plates may be heated up to 150 °C for duration of 4 hours and cooling them in desiccators.

E Purification of Silica Gel Layers:

The 'iron-free' layers may be achieved by providing the pre-coated and air-dried plates a preliminary development with a mixture of methanol and concentrated hydrochloric acid. By this process the entire iron gets migrated with the solvent front to the upper boundary of the TLC plate. Then the purified plates are again dried and activated at 110°C. The cleaning process usually washes out the CaSO4 originally present as binder. Therefore, the silica gel thus obtained by purification may be reused to prepare TLC plates with other appropriate binders like gypsum, starch etc.

F Spotting of the Mixture of components:

The following points should be taken into consideration while spotting the mixture of components on a TLC plate:

(I) The sample is normally applied as a solution in a 'non-polar solvent' as far as possible, since the use of a polar solvent may cause:

- Spreading out of the starting spot, and
- Affect directly the Rf value of components.

(ii) The solvent employed for dissolving the sample must be easily volatile-in-nature so that it should be removed from the TLC plate before development occurs.
(iii) The 'area of application' should be smallest as far as possible so as to achieve a sharper resolution.

G Development of Thin Layers:

The spotted TLC plates, after evaporation of the sample solvent, are placed in a closed chamber saturated with vapors of the developing solvent (mobile phase). One end of the plate is then wetted with the developer by means of either 'ascending technique or the 'descending-technique'. After the developer has reached one-half to two-thirds the total length of the TLC plate, the latter is removed from the chamber, air-dried and the positions of the components are located by any of several methods (fig no. 2).

H Visualization of Spots:

The visualization of spot is done by two methods i.e. first by Short length UV light and second by the Iodine chamber. In short length UV the light must be held close to the plate to see the spots. Some spots may be very faint. The observed spots should be outlined with the pencil. 15 In second method the plate is kept in the iodine chamber for few minutes. The iodine chamber should be kept in fume hood. The observed spots should be circled with pencil.

The compound examination of Dodonaea viscosa (Sapindaceae), uncovered that the plant contained alkaloids, flavonoids, fixed oil and fat, steroids, phenolics, saponins,tannins, gums, mucilage's, starches, decreasing sugar, glycosides and follow components. The pharmacological examinations indicated that Dodonaea viscosa had antidiabetic, antimicrobial, insecticidal, cell reinforcement, cytotoxic, antifertility, injury, mitigating, pain relieving, against ulcer, antispasmodic, hostile to diarrheal and detoxification effects (fig no. 3).

Botanical Name	Senna uniflora (Mill.) H.S.Irwin & Barneby
Synonyms	Dodonaea angustifolia
Common name	Hopbush
Plant form	Herb
Class	Manoliopsida
Order	Sapindales
Family	Sapindaceae
Genus	Dodonaea
Species	Viscosa

II. OBJECTIVES

In recent years the use of herbal medicines is increased worldwide. As there is increase in use, there is increasing the identification of plants for their new activities. But the standardization of natural products or plants is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof. To analyze the extract of various plants parts the Thin Layer Chromatography is an essential tool. TLC is a strategy for investigating mixture by isolating the components in the mixture. TLC can be utilized to help decide the quantity of components in a mixture, the identity of mixes, and the purity of a compound. TLC used to help the identity of a compound in a mixture when the Rf of a compound is contrasted and the Rf of a known compound. The mobile phase development in the TLC is an important part. The proper development of mobile phase gives the maximum numbers of components present in the extract. Also the developed mobile phase can be used for the further research work on the plant in future.

III. METHODOLOGY

1. COLLECTION OF PLANT AND AUTHENTICATION:
The plant roots of Dodonaea Viscosa were collected from the Hatkanangle in Kuntugiri region. The plant sample was

authenticated by Department of Botany, Smt. K. W. College, Sangli and voucher specimen number is smkc/dbot-11.

2. DRUG AND CHEMICALS:

The chemicals used such as Chloroform, Methanol, Cyclohexane, Acetone were produced from Loba Chem. Silica gel and other chemicals like ethyl acetate, petroleum ether, ethanol used are with analytical grade and water used was double distilled.

3. PREPARATION OF EXTRACT (fig no. 4):

♦ Cyclohexane extract:

The roots were cleaned by using water. These cleaned roots were dried in sunlight. The reduction of size of the dried roots is done by using the cutter mill or mixer. These roots then passed through the sieve to obtain the desired particle size. The 300 gm powder of root which is to be extracted has been taken into the thimble, which is placed inside the Soxhelt extractor. The Soxhelt extractor is equipped with the condenser. The Cyclohexane was heated using the heating mantle and it began to evaporate, moving through the apparatus to the condenser. The condenser condenses the vapors of cyclohexane and condensate it. When the Soxhelt gets filled with cyclohexane, the siphon tube pours back it into the flask and the cycle begins again. The process was run continuously until the clear extract was not obtained. The yield obtained after the drying of extract was 0.41 gm (fig no. 5).

♦ Chloroform extract:

After the completion of the cyclohexane extraction cycle the extract is removed and the marc is dried. This marc is used for the extraction of chloroform. The Soxhelt extractor is equipped with the condenser. The Chloroform was heated using the heating mantle and it began to evaporate, moving through the apparatus to the condenser. The condenser condenses the vapors of chloroform and condensate it. When the Soxhelt gets filled with chloroform, the siphon tube pours back it into the flask and the cycle begins again. The process was run continuously until the clear extract was not obtained. The yield obtained after the drying of extract was 0.65 gm (fig no. 6).

♦ Acetone Extract:

After the completion of the chloroform extraction cycle the extract is removed and the marc is dried. This marc is used for the extraction of Acetone. The Soxhelt extractor is equipped with the condenser. The Acetone was heated using the heating mantle and it began to evaporate, moving through the apparatus to the condenser. The condenser condenses the vapors of acetone and condensate it. When the Soxhelt gets filled with acetone, the siphon tube pours back it into the flask and the cycle begins again. The process was run continuously until the clear extract was not obtained. The yield obtained after the drying of extract was 0.58 gm (fig no 7).

♦ Methanol Extract:

After the completion of the acetone extraction cycle the extract is removed and the marc is dried. This marc is used for the extraction of methanol. The Soxhelt extractor is equipped with the condenser. The methanol was heated using the heating mantle and it began to evaporate, moving through the apparatus to the condenser. The condenser condenses the vapors of methanol and condensate it. When the Soxhelt gets filled with methanol, the siphon tube pours back it into the flask and the cycle begins again. The process was run continuously until the clear extract was not obtained. The yield obtained after the drying of extract was 0.93 gm (fig no. 8).

Table no. 1: The percentage yield of various extract of Dodonaea viscosa root.

Sr. No	Solvent	Color of Extract	Yield of extract (in gm)	Percentage Yeild (%w/w)
1	Cyclohexane	Light Yellow	0.41 gm	0.1367%
2	Chloroform	Light Yellow	0.65 gm	0.2167 %
3	Acetone	Light Yellow	0.58 gm	0.1933 %
4	Methanol	Light Yellow	0.93 gm	0.31

IV. PHYTOCHEMICAL ANALYSIS

The phytochemical analysis of prepared extract was performed for the detection of the phytoconstituents present in the extracts. The extracts of Cyclohexane, chloroform, acetone and methanol was performed for the following phytochemical tests :

A. Test for Alkaloid: In the 2-3 ml of extract the few drops of Dragendroff's reagent (potassium bismuth iodide) was added. After the addition the brown ppt may form.
B. Test for Flavonoids: In the extract a few drops of 66% or 80% Sulphuric acid was added and dissolved. It forms a Deep yellow solution.

C. Test for Phenolic Compounds: In the 1 ml of extract a Dilute Iodine solution was added. The transient red color was observed.

D. Test for Tannins: In the 1 ml of extract the 5% Ferric chloride solution was added. The deep blue black color was observed.

E. Test for Saponins:The extract was shaken vigorously, persistent stable foam was observed.

4. Development of mobile phase for Chloroform extract by thin layer chromatography:

The glass slides were cleaned with distilled water. For preparation of Stationary Phase, Silica gel G paste was prepared and applied over the slides. The slides were activated at 105 ^{0}C temperature by hot air oven. After activation of Stationary Phase, with the help of pencil two spots were marked one at the top and one at the bottom. The sample of Chloroform extract was applied at the centre of bottom spot which was previously marked. Poured the Mobile Phase into the TLC chamber and to maintain equal humidity, placed a moistened filter paper in the Mobile Phase. The TLC plate was placed into the TLC chamber and closed it with the lid. It was kept in such a way that the sample faces the Mobile Phase. Immersed the plate for development. The sample spot was placed in such a way that it lies above the level of mobile phase. After few min the development of spots had stated. Once the spots were obtained the plate was removed from the chamber and dried. The sample spots were observed under a UV light chamber at short range. The spots observed in the UV chamber are given below in fig no 9 :

5. Development of mobile phase for Methanol extract by thin layer chromatography:

The glass slides were cleaned with distilled water. For preparation of Stationary Phase, Silica gel G paste was prepared and applied over the slides. The slides were activated at 1050C temperature by hot air oven. After activation of Stationary Phase, with the help of pencil two spots were marked one at the top and one at the bottom. The sample of methanol extract was applied at the centre of bottom spot which was previously marked. Poured the Mobile Phase into the TLC chamber and to maintain equal humidity, placed a moistened filter paper in the Mobile Phase. The TLC plate was placed into the TLC chamber and closed it with the lid. It was kept in such a way that the sample faces the Mobile Phase. Immersed the plate for development. The sample spot was placed in such a way that it lies above the level of mobile phase. After few min the development of spots had stated. Once the spots were obtained the plate was removed from the chamber and dried. The sample spots were observed under a UV light chamber at short range. The spots observed in the UV chamber are given below in figure no 10:

V. RESULT

- PHYTOCHEMICAL ANALYSIS:

The phytochemical analysis root extracts of plant Dodonaea Viscosa Linn. was performed. The methanol extract shows the presence of alkaloids, Flavonoids, phenolic compounds and Saponins. The chloroform and acetone extract shows the presence of Flavonoids, tannins and saponins, while the Cyclohexane extract shows the presence of flvonoids and saponins.

Table no.2: Phytochemical analysis of root extracts:

Sr. No	Extract	Alkaloids	Flavonoids	Phenolic compunds	Tannins	Saponins
1.	Methanol	+	+	+	+	+
2.	Chloroform	-	+	-	+	+
3.	Acetone	-	+	-	+	+
4.	Cyclohexane	-	+	-	-	+

Key: + = Present, - = Absent

- DEVELOPMENT OF MOBILE PHASE:

The mobile phase is developed by using various solvents for both chloroform and methanolic extract and hence the results are shown below:

a. CHLOROFORM EXTRACT: The chloroform extract of roots of the plant Dodonaea viscosa Linn. shows the better separation in the mobile phase Ethyl acetate : chloroform in the ratio 2:3. The spots observed are clear and well identified as shown in figure no 11.

✓ Rf value:

The Rf values of the detected spots of the chloroform extract was calculated by the following formula: Rf value= Distance travelled by the solute / Distance travelled by the solvent The three spots i.e. spot no.1, spot no.2, spot no.3 are observed in the TLC of the chloroform extract. This three spots shows the Rf values 0.42, 0.62 and 0.82 respectively.

b. METHANOLIC EXTRACT: The Methanol extract of roots of the plat Dodonaea viscosa shows the better

separation in the mobile phase Ethyl acetate : Pet ether : Ethanol in the ratio 2.5:1.5:1. The spots observed are clear and well identified as shown in figure no 12.

- ✓ Rf Value:

The Rf values of the detected spots of the Methanol extract was calculated by the following formula: Rf value= Distance travelled by the solute / Distance travelled by the solvent The three spots i.e. spot no.1, spot no.2, spot no.3 were observed in the TLC of the chloroform extract. This three spots shows the Rf values 0.29, 0.48 and 0.87 respectively.

Table no. 3: Rf values of TLC solvent system for various extract of Dodonaea Viscosa root.

Sr. No	Extract	No of spots detected	Rf value
1	Chloroform extract	Spot no. 1	0.42
		Spot no. 2	0.62
		Spot no. 3	0.82
2	Methanolic extract	Spot no. 1	0.29
		Spot no. 2	0.48
		Spot no. 3	0.87

VI. SUGGESTIONS

Phytochemical Studies:

The existences of phytochemicals are engaged with the different sickness protection and curing. Functions of alkaloid are for the most part identified with the protection.Significant pain relieving and anticonvulsant exercises were observed.It is additionally utilized as a muscle relaxant, cough relief. Fundamental phytopharmacological examinations of Dodonaea viscosa leaves uncovered the nearness of flavonoid promising antiulcer movement which might be credited to cytoprotective and healing action of flavonoids present in plants. The antinociceptive adequacy of the concentrates might be ascribed because of the nearness of flavonoids. It additionally indicated cancer prevention agent movement. The presence of Phenolic compunds and tannins were also attributed to show antiulcer activity. The presence of saponins were responsible for curing of various diseases or disorders such as Antinociceptive , neurological disorders such as Analgesic, depressant and anti-convulsant and also show Antiulcer activity.

TLC Analysis:

During the TLC analysis, while preparing the extract various hurdles came across such as the roots of plant were very strong which delayes the drying process as well as create difficulties in producing powder. Along with that the preparation of stationary phase i.e silica gel also created problems while placing on the slide and also delays the derivatitaion/activation time.These Rf values acquired from the phytochemicals give the significant data about their polarity and significant intimations for the seperation of these phytochemical in the seperation procedure. Distinctive Rf values of the compound likewise reflects an thought regarding their polarity by the utilization of the different solvents frameworks system for TLC studies could be significant for the determination of the suitable dissolvable framework system. This data will help in choice of proper solvent framework system for additional seperation of compound from these plant extracts.

VII. CONCLUSION

The present study shows the presence of medicinally important bioactive compounds in the Dodonaea Viscosa plant which may be potential for novel drug discovery. TLC investigation of the phytochemicals demonstrated the great affectability and separation. These findings may also lead to the further isolation, purification, characterization of the active compounds from the root extract of the plant Dodonaea Viscosa Linn. for chromatographic and spectroscopic techniques. The rest of the extract i.e. Cyclohexane and acetone extract can used for the further development of TLC and mobile phase.

VIII. ACKNOWLEDGEMENTS

We pay our acknowledgement to Ms. Yasmin Momin for guiding us and also Annasaheb Dange College of B.Pharmacy, Ashta for providing all the necessary help to carry out the research.

Abbreviations:

Thin Layer Chromatography – TLC
Ultraviolet – UV
Retention Factor – Rf

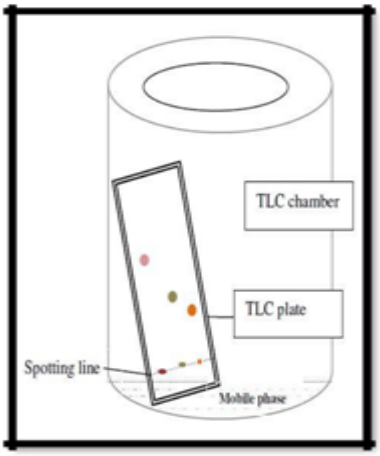

Fig. no.1. Thin Layer Chromatography

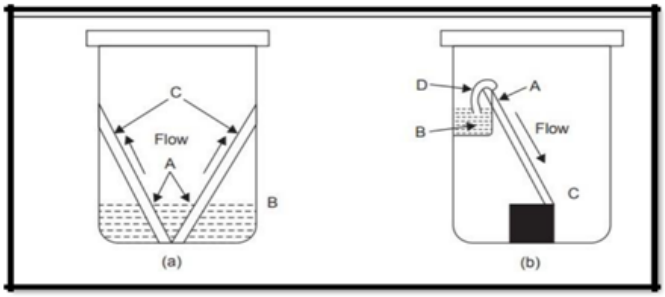

Fig no.2: (a) Ascending flow (b) Descending flow

Fig.no.3. Dodonaea Viscosa Linn

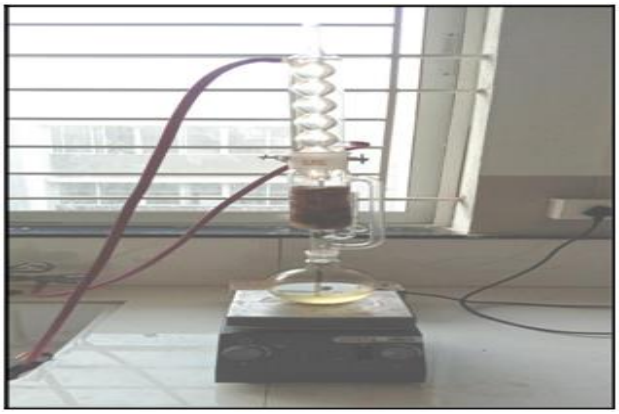

Fig. no.4: Soxhlet Extractor

Fig.no.5 Cyclohexane extract

Fig.no.6: Chloroform extract

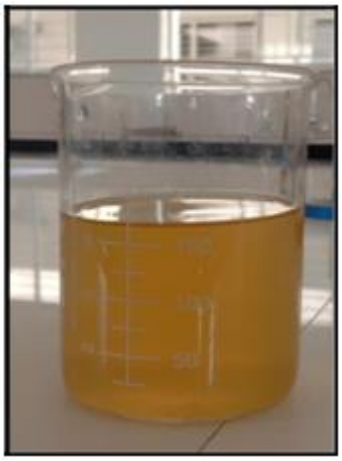

Fig.no.7: Acetone Extract

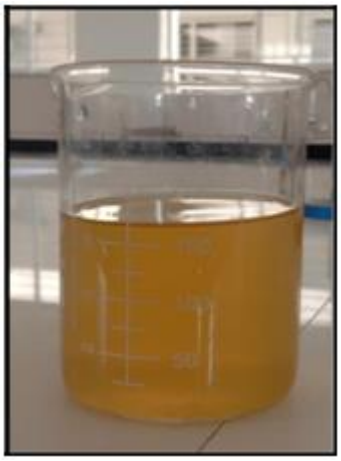

Fig.no.8: Methanol extract

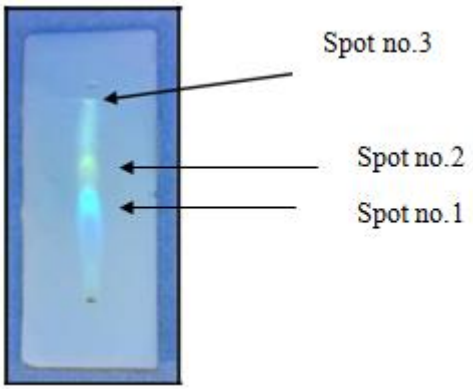

Fig.no.9: Detection of spots of the Chloroform extract

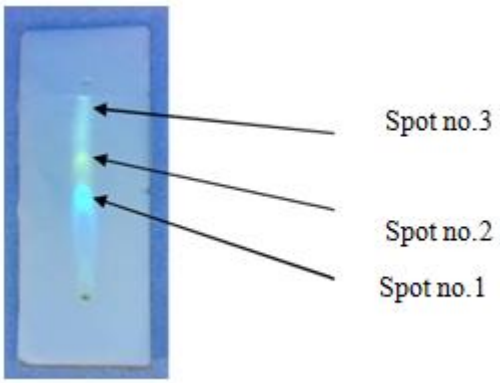

Fig.no.10: Detection of spots of the Methanol extract

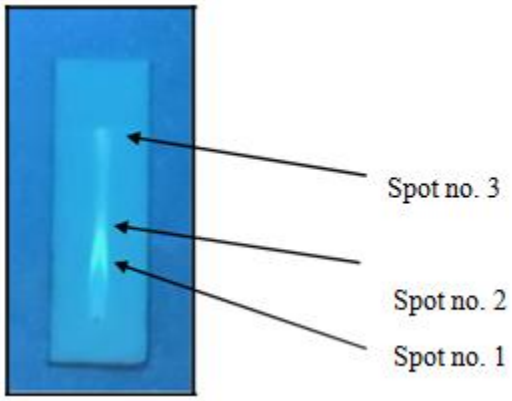

Fig no.11: TLC plate of chloroform extract

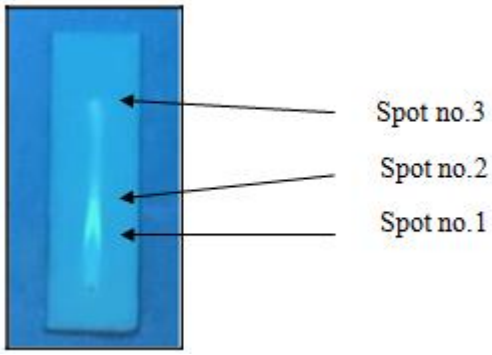

Fig no.12: TLC plate of Methanolic extract

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