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# High Performance Liquid Chromatography (HPLC) and Phytochemical Screening of Three Plant Malvastrum Coromandelianum, Medicago Lupulina and Parathenium Hysterophorus

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**Abstract**

*Malvastrumcoromandelianum*is a herbal medicinal plant and famous for medicinal properties and use of agricultureas a pesticide for many years. These plants are recognized for their antibacterial and antifungal behavior due to the presence of alkaloids, carbohydrate, saponins, terpenoids and steroids and *paratheniumhysterophorus* is and herbal medicinal plant and use of agriculture as bio-pesticides. This is very useful for agriculture because N, P, K, Fe, Mn, Cu and Zn minerals present. *Parthenin,*hysterin, hymenin, and ambrosin are present in *Paratheniumhysterophorus.*Due to the present those chemicals, the weed exerts strong allelopathic effects on different crops. The current study dependsupon the provide details information about *Malvastrumcoromandelianum*, *Medicagolupulina*and *paratheniumhysterophorus* including phytochemical analysis which is very benefits in tomato crops as a Biopesticides and HPLC of *Paratheniumhysterophorus*Plant extract because all phytochemical present in this plant which is very beneficial as a biopesticides.

**Keywords**: *Malvastrumcoromandelianum*, *Medicagolupulina*and *paratheniumhysterophorus*, Phytochemical analysis, HPLC.

## INTRODUCTION

The increase of anextensive variety of chemicals has resulted in the rapid growth in industrialization along with an increasing population. Apply new technologies to reduced or get rid of these contaminants from the environment has led to a remarkable effort the frequency and widespread use of man-made "xenobiotic" chemicals. Due to the advancement of toxic intermediates, there has been an adverse effect on the environment due to land-filling, recycling, pyrolysis, which is not good at all.(Debarati*et al*., 2005).But these methods are not always important as sometimes they are not cost effective or sometimes it's not possible to execute these methods especially in case of Pesticides (Jain *et al*., 2005). It has been extensively used in agriculture due to widespread use of pesticide for pest control. However, the arbitrary use of pesticides has imposed serious damage and problems to

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humans and as well as to the biodiversity. The pesticides that are extremely applied to the land surface travel a long distance and contaminates water table, reaching the aquatic environment and thereby gradually also the soil(Suman.,2010). Bio pesticides may provide an answer to this problem. There are many plants that grow at the nearby areas of crop fields and remain unaffected by various pests without any intensive care. These plants may act as repellant for various insects and pests. Many ways have been adopted for the control of pests by Conventional agrochemicals, by using bio-insecticides but still there are numerous life-threatening factors.

## METHODS AND MATERIALS

The Botanical identification of all the plant material *Medicago lupulina, Malvastrum Coromandelianum* and *Parthenium Hysterophorus were* done under guidance Mr. Prabhakar at Department of Botany, Punjab University, Chandigarh. All the plant collected from the field with the help of botany expert which was in Botany department, Punjab University, Chandigarh. Table 1.1 showed the summary of names of the plants that were used in this study and it also given the information of the part of the plant that has been used for present Research.

Table 1.1: Represent the common names and part of the plant used in this study.

| S.No. | Scientific Name | Common Name | Part Used |
|---|---|---|---|
| *1* | ***Medicago lupulina*** | **black medick** | **Leaves** |
| *2* | ***Malvastrumcoromandelianum*** | **Kharenti** | **Leaves** |
| *3* | ***Paratheniumhysterophorus*** | **Carrot Grass** | **Leaves** |

### Preparation of Plant Extract

All the plant samples were washed thoroughly and thereafter the leaves of *Medicago lupulina, Malvastrum Coromandelianum* and *Parthenium Hysterophorus* were dried at 40℃ for 4-5 days. After the drying process, leaves were ground to fine powder. This powdered material was further used to prepare three different types of extract; aqueous, ethanolic and aqueous-ethanolic in respective solvents, with slight modification in the method described by Mishra and Bhatia, 2010. The yield of extraction was calculated by the following formula:

$$\text{Yield of extraction} = \frac{\text{Weight of extract (gm)}}{\text{Weight of Plant powder used f or extraction (gm)}}$$

### Preparation of sample extracts

The preparation of aqueous extract of all the three samples; *Medicago lupulina, Malvastrum Coromandelianum* and *Parthenium Hysterophorus* were dried till 3 - 4 days. During the drying procedure, the mixture was subjected to intermittent shaking. After this the aqueous mixture was filtered through a Buchner Funnel using Whatman filter paper no. 1 with the

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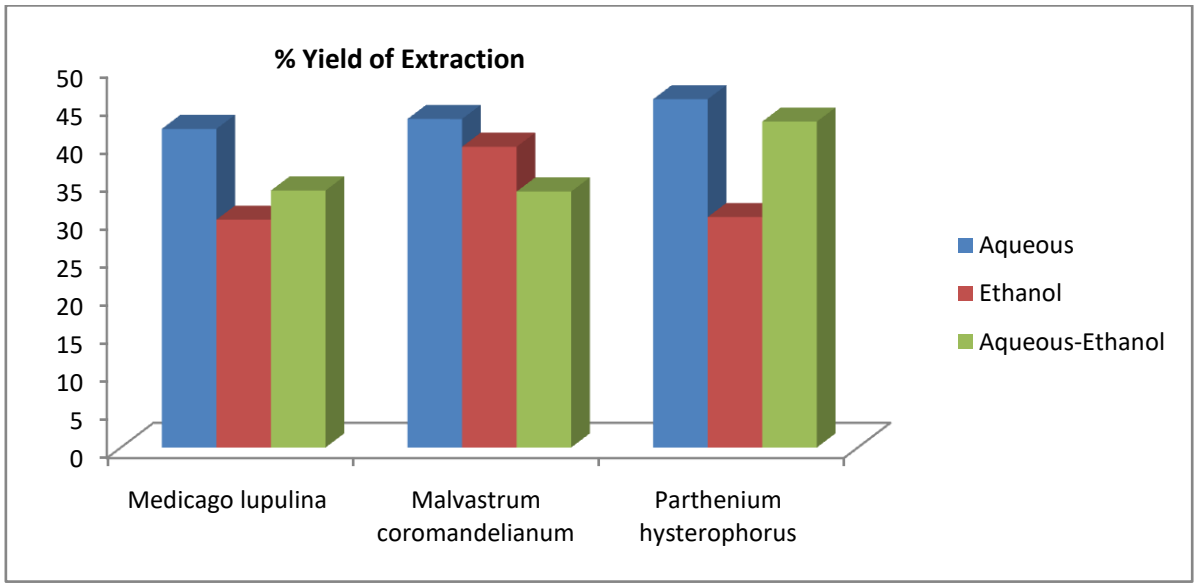
help of a suction system to remove the solid particles from it in order to get the filterate. Followed by this it was subjected to evaporation in the rotary evaporator at 40℃ under reduced pressure condition. Finally, the dried extracts were scrapped and collected from the evaporator flask, weighted and were stored at 4℃ for further use. Similarly, the preparations of ethanol extract of all three samples; *Medicago lupulina*, *Malvastrum Coromandelianum* and *Parthenium Hysterophorus* were dried and the preparations of aqueous-ethanolic extract in ethanol: water (1:1) (v/v). The remaining procedure is same as mentioned for the preparation of aqueous extract. In total, 9 different extracts were prepared from different plant samples that included three aqueous, three ethanolic and three aqueous-ethanolic extracts. All extract of the plant samples ready for present work.

Table:1.2 Extraction yields of Malvastrumcoromandelinum, Medicagolupulina and Paratheniumhysterophorus in three different Solvents.

| S.No. | Sample | Solvent Used | Weight of Extract (gm) | Yield (%) |
|---|---|---|---|---|
| **1** | ***Medicago lupulina*** | **Aqueous** | **4.19** | **41.9** |
| **2** | ***Malvastrumcoromandelinum*** | | **4.32** | **43.2** |
| **3** | ***Paratheniumhysterophorus*** | | **4.58** | **45.8** |
| **4** | ***Medicago lupulina*** | **Ethanol** | **2.996** | **29.96** |
| **5** | ***Malvastrumcoromandelinum*** | | **3.955** | **39.55** |
| **6** | ***Paratheniumhysterophorus*** | | **3.03** | **30.3** |
| **7** | ***Medicago lupulina*** | **Aqueous-Ethanol** | **3.38** | **33.8** |
| **8** | ***Malvastrumcoromandelinum*** | | **3.366** | **33.66** |
| **9** | ***Paratheniumhysterophorus*** | | **4.286** | **42.86** |



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**Phytochemical Analysis**

Phytochemical analysis is valuable to estimate the constituents present in the crude extract mixture as well as it also facilitates the analysis of specific components soluble in any specific solvent. Therefore, plants find their biopesticides values due to the presence of respective phytochemical constituents.Screening of the phytochemicals is an important tool in the analysis of bioactive compounds. Moreover, it is a convenient, fast and inexpensive assay that allows researchers to know about the presence of various phytochemical in a mixture. Phytochemical analysis was carried out for individual extract of Malvastrum Coromandelianum, Medicago lupulina and Parthenium hysterophorus and extract mixture of all the three plants in a defined concentration. All the extract combinations were subjected to the qualitative detection of various phytochemicals as per the methods described ahead.Trease and Evans, 1989.

**Test for Alkaloid**

**Mayer and Wagner's Test**

Added 2 mL of sample in a test tube and 0.5Ml of 1% HCL, followed by addition of 1mL of Wagner's reagent (2.5 grams iodine is dissolve in 2.5 grams of potassium iodide ($KI_2$); added 250 mL of water to prepare solution). Appearance of Reddish- brown color indicates positive test.

**Test for saponins**

**Foam Test**

To 1mLof extract of each solvent was added the 1-2 drops of distilled water and shake dynamically until foam was observed. The presence of Saponins indicates formation of foam.

**Test for Terpenoids**

**Salkowski Test**

2Ml of chloroform was added to 1Ml of sample extract, followed by addition of 3 Ml of conc. $H_2SO_4$. Red brown color at upper phase will indicate a positive result.

**Test for carbohydrate**

1 ml of Barfoed's reagent (CuSO4) +plant extract added in the clean test tube. This solution was heated in a boiling water bath for 2-3 minutes. TheRedcolor change or precipitate formation showed presence of carbohydrate.

**Test for Proteins**

**Ninhydrin test**

1 ml of extract + 2ml of ninhydrin added and boiled. Violet color observed which indicates the presence of amino acids.

**Test for Phytosterols**

**LibermannBurchard's Test**

2 ml extract taken in a test tube and added 2 ml Chloroform+ 2 ml Acetic Anhydride + 2 ml conc.Sulphuricacid. After thattranslucent greencolor observed which is give the confirmtest.

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**Test forSteroid**

1 mlplant extract was taken in a test tube and dissolved with chloroform (10 mL), and added equal volume of concentrated sulphuric acid. The upper layer in the test tube was turns into red and sulphuric acid layer which showed yellow with green fluorescence.

**High Performance Liquid Chromatography (HPLC) of Extract**

The chromatographic equipment of Shimadzu HPLC incorporated with LC-solution software, RP-HPLC instrument equipped with PDA detector, Nucleodur, C 18 (4.6 × 250 mm i.d., 5 µm particle size) controlled at 35 $^{o}$C was employed for the chromatographic analysis of ethanolic extract mixture. Data acquisition and peak integration was performed using Shimadzu class LC solution software and results were obtained by comparison with standards at 254nm UV detection.

**Qualitative Chromatographic Analysis of extract**

The Chromatographic conditions described by Sawant *et al.*,2010 were followed with slight modifications. The gradient system of stationary phase was applied for the chromatographic analysis. Weighed amount of Parthenium (plant extract) was taken in 10 ml volumetric flask and dissolved in 10 ml of diluent and sonicated using bath sonicator for 10 minutes and filtered through 0.22 µ Millipore membrane filters and injected in HPLC system.

**RESULT**

The results confirm the presence of constituents which are knows to show photochemical and physiological activities.The resultsof Phytochemical analysis of the extract are summarized in Table 1 and 2 and 3. The results revealed the presence of medicinally active constituents in Table 1 such as proteins, alkaloid, saponins, carbohydrate, phytosterol and steroids. While terpenoids were found to be absent, Table 2 such as proteins, alkaloid, saponins, carbohydrate, phytosterol, terpenoids and steroids are present and Table 3 such as proteins, alkaloid, saponins, terpenoids, phytosterol and steroids. While carbohydrate was found to be absent. Then, I did HPLC technique of *Parthenium hysterophorus* plant extract which is defined all value of RT. It has been observed that the retention Time of *Parthenium hysterophorus* was 2.16 and 2.56 respectively using RP-HPLC. In this present study, four peaks were obtained in chromatogram of *Parthenium hysterophorus* plant extract, represented figure 1, with 2.16, 2.56, 3.31 and 5.9. due to present study Alkaloids compounds present in *Parthenium hysterophorus* plant extract. The alkaloids contained in plants may be responsible for central nervous system activity. In the results of this study, it has been found that phytochemical compounds can be bioactive constituents which is responsible for the plants due to these compounds. In which there has also been confirmation of pharmacological activities. Quality control parameters and phytochemical standards have also been established in this study.According to the particular research we can definitely say that there is the bright future and advantage of the photochemical activity. These particular properties of the plants will be widely used to predict and detect the survival of the general plants which will be helpful to the mankind in near future.

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Table 1 Phytochemicals present in*Malvastrumcoromandelianum* extract

| PHYTOCHEMICALS | Present(+)/Absent(-) |
|---|---|
| Proteins | + |
| Saponins | + |
| Terpenoids | - |
| Carbohydrate | + |
| Alkaloids | + |
| Steroids & Phytosterols | + |

Table 2 Phytochemicals present in*Partheniumhysterophorus*extract

| PHYTOCHEMICALS | Present(+)/Absent(-) |
|---|---|
| Proteins | + |
| Saponins | + |
| Terpenoids | + |
| Carbohydrate | + |
| Alkaloids | + |
| Steroids & Phytosterols | + |

Table 3 Phytochemicals present in *Medicago Lupulina* extract

| PHYTOCHEMICALS | Present(+)/Absent(-) |
|---|---|
| Proteins | + |
| Saponins | + |
| Terpenoids | + |
| Carbohydrate | – |
| Alkaloids | + |
| Steroids &Phytosterols | + |

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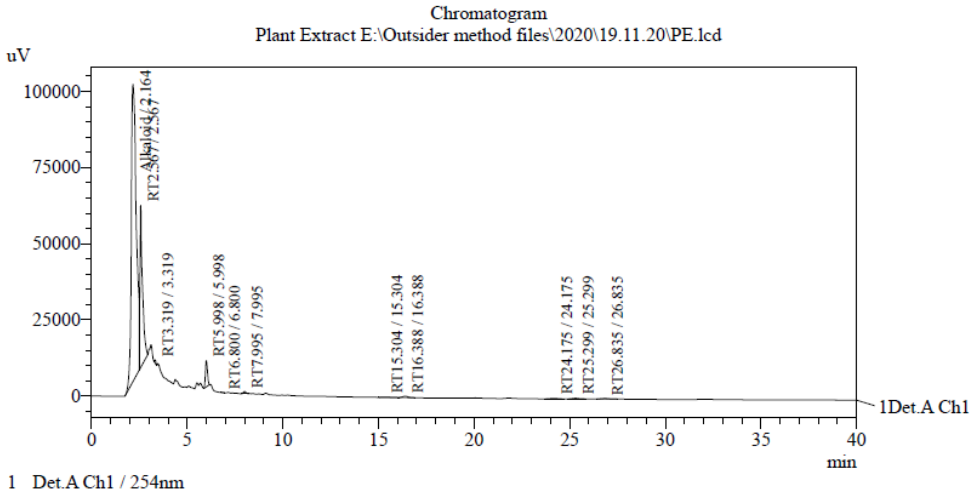


Figure 1. Chromatogram of *Parthenium hysterophorus* (Plant Extract)

## DISCUSSION

Three plants*Malvastrumcoromandelianum*and*Partheniumhysterophorus*were screened for their phytochemical constituent. It was found that all the plants have considerable proportion of important phytochemicals that are easily detected by qualitative tests. In our analysis it was cleared that the *Malvastrumcoromandelianum*and*Partheniumhysterophorus*is rich in proteins, alkaloid, saponins, carbohydrate, phytosterol, terpenoids and steroids etc. due to present study of HPLC, Alkaloids compounds present in *Parthenium hysterophorus* plant extract. The alkaloids contained in plants may be responsible for central nervous system activity.

## CONCLUSION

Current study involves quality control characterization of plant Malvastrumcoromandelianum and Partheniumhysterophorus. The plant extract evaluated for phytochemical analysis and has containd proteins, alkaloid, saponins, carbohydrate, phytosterol, terpenoids and steroids and the solvent extract of Malvastrumcoromandelianum and Partheniumhysterophorus. Alkaloids compound present in Parthenium hysterophorus plant extract which is good for human because it is responsible for CNS Activity also good for farmers because presence of high content of phytochemical compounds presents Because of which it also acts like biopesticides. Contains medicinally important bioactive compounds and this justify the use of plant, medicine as a biopesticides for treatment of various diseases of crops. The effectiveness of the bio-pesticide is obvious from the results. Considering the eco-friendly nature of the biopesticides, their easy availability and effectiveness, the biopesticides should be considered an alternative to the conventional synthetic pesticides.

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