Thin Layer Chromatographic study of three different extract of Calendula Officinalis leaves

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ABSTRACT

Calendula officinalis is a plant that possess many pharmacological actions like Stomach upset, Ulcer, Menstrual Period Problems, Eye Infections, Inflammation, Wound Healing, Anti-Septic etc. most widely it is used as wound healing purposes. If the leaf of that plant can be applied after crunching at the affected area, blood will be clot within a few time. But while this leaf can perform this action, it means it possess some active compound in it. To find out what the actual component it is, first we have to perform the Thin Layer Chromatographic study. In our present study we are going study the TLC profile of that Calendula officinalis leaf.

Keywords: Ulcer, Inflammation, Wound-Healing, Clot, TLC

INTRODUCTION

*Calendula officinalis*is a plant in the genus Calendula of the family Asteraceae. It is probably native to Southern Europe, through its long history of cultivation makes its precise origin. It is also widely naturalized further north in Europe & elsewhere in warm temperate regions of the world. It is commonly known as marigold, gold bloom & holligold [1]. The anti-inflammatory and anti-oedematous properties of *Calendula officinalis* have been linked to the pentacyclic mono-, di- and trihydroxytriterpenoid fatty acid esters, especially the faradiol esters, faradiol 3-O-laurate, faradiol 3-O-palmitate and faradiol3-O-myristate (Figure 1) [2-7]. The unesterified faradiol produced by hydrolysis, has been found to have the same effect as an equimolar dose of indomethacin which is a Non-Steroidal Anti-Inflammatory Drug (NSAID) [8]. However, the claimed benefits of these herbal formulations cannot be guaranteed in commercially available preparations unless standardised methods of regulation and testing are introduced. Thus, it has been suggested that the concentrations of the triterpenoid fatty acid esters in *Calendula officinalis* formulations may be an effective method to assess and monitor the quality of products on the market[5].

The main aim of this study is to differentiate & identify the different compounds present in *Calendula officinalis* leaf by using three different solvent extraction of this leaf.

EXPERIMENT

Extraction procedure:

Decoction procedure with the help of three different solvents that's are Chloroform, Ethanol & Benzene.

Instruments Used:

Beaker, Water Bath, Glass Rod, Funnel, Filter Paper, Conical Flask, China Dish, Solvent Chamber, TLC Plate, UV Chamber, Scales, Pencil.

Chemicals Used:

Chloroform, Ethanol, Benzene, Acetone, Dist. Water.

MATERIALS & METHOD

The *Calendula officinalis* leaves are collected & it was shade dried first for three days. As for because three solvent extract is needed for this study so three solvents are selected as per depends upon its polarity. The selected solvents are Chloroform, Ethanol & Benzene. Three 100 ml beaker was taken & took 30 ml of the each solvents in each beaker. Then added 10 gm. of the shade dried leafs in each beaker. For the extraction procedure heat was applied by the water bath. After 2hr of heating upon water bath the beakers were separated from the water bath, & allowed to cool. Then the misella was filtered out by the help of filter paper & funnel in a

conical flask & the marc was discarded out. Now the misella was transferred into three successive china dish, & again place on the water bath for the complete dryness. Once the solvents gets completely evaporated leaving behind the crude extract cut off the heat & allow the china dish for cooling. [Figure-1]

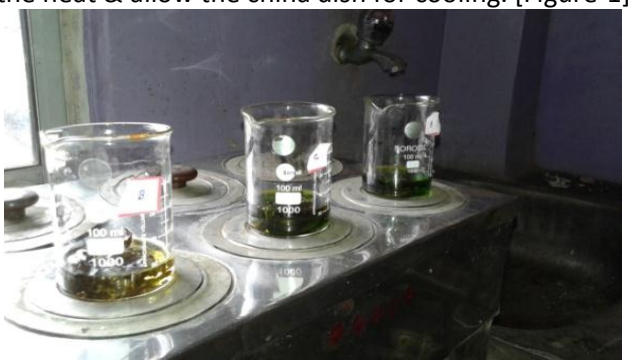

Figure-1

Now we took one pre-coated TLC plate & apply three samples on the plate & allow it to dry in normal environment. Now the solvent system was prepared using Chloroform:Ethanol: Benzene in 1:1:1 ratio. Now the TLC plate was moved into the solvent chamber & cover up the upper portion of the chamber & let the solvent run through the plate.[Figure 2, 3]

Figure-2

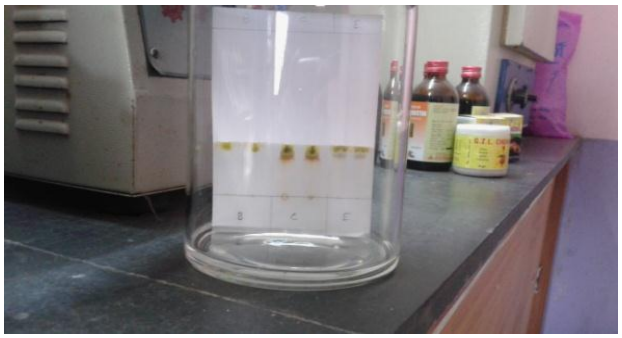

Figure-3

RESULTS & DISCUSSIONS

First the distance that the solvent runs was determined by using the scale.[Figure-4] Then the plate was placed in the UV cabinet for the detection of the sample peaks.

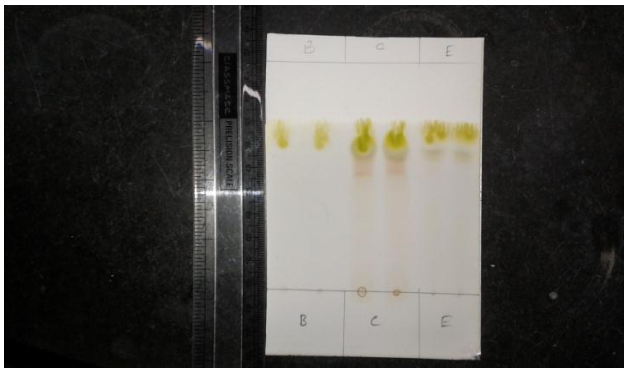

Figure-4

Now the plate was determined in 254nm & 366nm occasionally. [Figure- 5, 6]

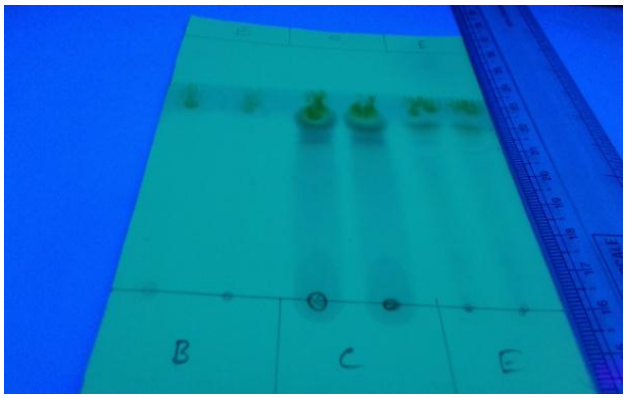

Figure-5

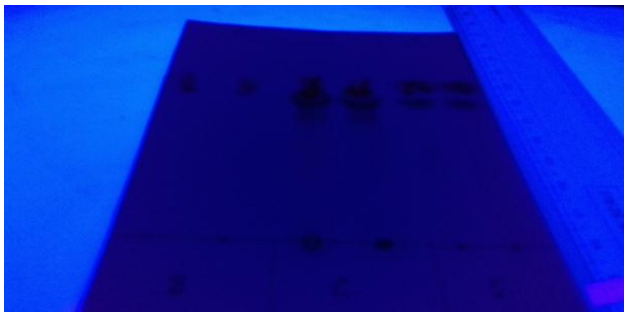

Figure-6

Calculation of R_f Value:

For the calculation of Rf Value the formula used is-

$$R_f = \frac{\text{Distance Travel by Solute}}{\text{Distance Travel by Solvent}}$$

Points Obtain: [Figure-7]

Solvent Runs- 7.5

Benzene Ext.-None.

$R_f$values for Chloroform Ext.-0.69, 0.86.

R_f Values for Ethanol Ext.- 0.58, 0.86.

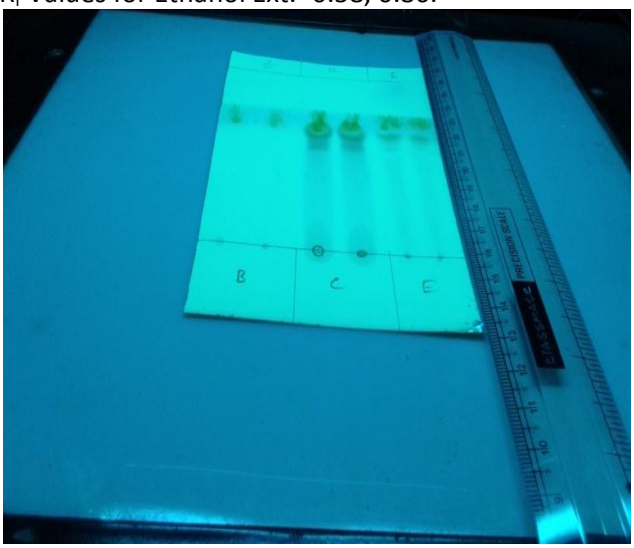

Figure-7

DISCUSSION

After performing the above test it has been clear that if this leaf is extracted in benzene no compound will appear in TLC plate, but in Chloroform & Ethanol extract it shows a many peaks, that indicates presence of many compounds that are extracted out by the solvent extraction procedure. It has been reviewed that the R_f value of Lutein & Zeaxanthin are 0.72 & 0.59 respectively[9]. In our current study we found that R_f value of Chloroform Extract was 0.69 & 0.86, &R_f value of Ethanol Extract was 0.58 & 0.86. So it is clear that the Chloroform Extract contains the Lutein & Ethanol Extract contains Zeaxanthin respectively. Further isolation & purification is needed to confirm the presence of Lutein & Zeaxanthin in the leaf.

CONCLUSION

This study shows the need to perform the standardization procedure of a herbal ingredient. This TLC procedure shows that some solvent can extract out the active ingredient present in the herbal material. Like here Chloroform & Ethanol can extract out many component present in that leaf while at the same time Benzene couldn't perform the same. But further study is needed to confirm its efficacy & isolation techniques.

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