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THIN LAYER CHROMATOGRAPHY & BIOAUTOGRAPHY OF MENTHA ARVENSIS (L) LEAF EXTRACT.

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ABSTRACT :

Thin layer chromatography (TLC) is one of the important separation technique. In TLC, the stationary phase is a thin layer of adsorbent particles (silica gel) attached to a solid plate. A small amount of sample is applied (spotted) near the bottom of the plate and the plate is placed in the mobile phase. This solvent is drawn up by capillary action. Separation occurs as each component, being different in chemical and physical composition, interacts with the stationary and mobile phases to a different degree creating the individual bands on the plate. The retardation factor, Rf value, is used to characterize and compare components of various samples.

$$\text{Rf value} = \frac{\text{Distance from origin to component spot}}{\text{Distance from origin to solvent front}}$$

The pigments & chemical components in medicinal plants, vegetables, flowers and leaves can be separated and identified by using thin-layer chromatography. Menthol (35-70%), Menthone (15-30%) Menthyl acetate (4-14%), Pulegone (1-4%). It also contains Isomenthone, Limonene, Pinene, Caryphyllene, Piperitone, Cineole, and Acetaldehyde in trace. The phenolic constituents Rosamarinic acid, Flavonoids,

Eriocitrin, Luteoline, Hesperidin are contained by *M. arvensis L.* which can be isolated and identified using chromatographic techniques.

Bioautography can be employed in the target directed isolation of active constituents. Thin layer chromatography – bioautography was introduced by R. Fisher and H. Lautner in 1961. There are three different approaches for Bioautography to localize antimicrobial activity on TLC, Agar diffusion or contact Bioautography, Direct bioautography Immersion or agar overlay bioautography. Those assays supply a quick screen for new antimicrobial compounds through bioassay – guided isolation. Hence bioautography was used as a tool to screen out antimicrobial compounds in *M. arvensis.*

Bioautography belongs to microbial screening methods commonly used to the detection of antimicrobial activity. The screening can be defined as the first procedure, which is applied to an analysed sample in order to establish the presence or absence of its activity. Basically it is a simple measurement providing a 'yes or no' response. Quite often screening methods give higher sensitivity than any other method. Moreover, they are simple cheap. Time saving and do not require sophisticated equipment.

MATERIALS AND METHODS:

Thin layer Chromatography-

Aluminium coated silica gel plates
Solvent system : 100% dichloromethane
Spray reagent : Anisaldehyde sulphuric acid reagent
Oven at 150^{0}C
Heating water bath
Capillary

Methods:

20 ml of dichlorobenzene were taken in chromatographic chamber and kept for saturation.

Extracts of *M. arvensis L.* were spotted on silica gel plates, allowed to dry and then kept in saturated solvent system that is dichloromethane. Solvent was allowed to run on stationary phase. The plates were allowed to remain undisturbed until the solvent reaches to within 1 cm of the top. Then plates were taken out and allowed to dry. Spray reagent anesaldehde sulphuric acid were sprayed on plates and kept in oven at 1500C. To remove the colour of reagent the plates were exposed to steam. Spots were appeared on silica gel plates.

Rf values were calculated and compared with standard Rf values.

Bioautpgraphy-

Material –

Organism culture suspension of –*Bacillus sp 1*
-Bacillus sp 2
TLC plates
Muller Hinton agar butt
Petri plates

Method –

Agar Overlay Method –

Muller hingtone base agar were poured in petri plates & allowed to set. Then the chromatograms were kept on settled Muller hingtone agar. Seed agar were poured on chromatogram and allowed to set. These petri plates were then kept in refrigerator at 4^{0}C for pre-diffusion for 2hr. Then these plates were kept for incubation at 37^{0}C for 24 hr. These plates were then observed for zone of inhibition.

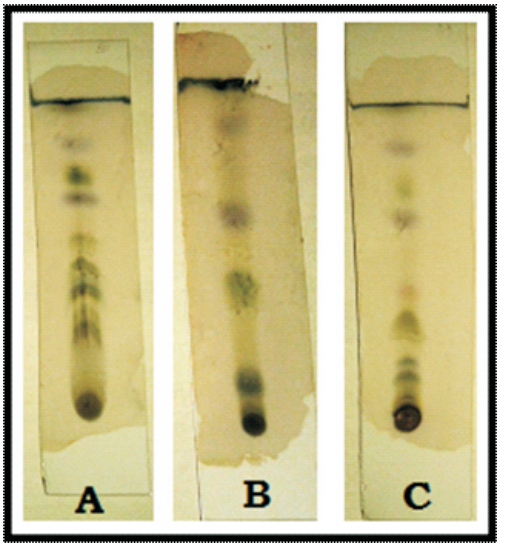
Table 1- Standard Rf values –

Component	Rf value
Menthol	0.30
Piperitone	0.35
Cineole	0.40
Pulegone	0.48
Isomenthone	0.55
Menthone	0.70
Methyl acetate	0.75

Results:

Table 2 - R_f values obtained

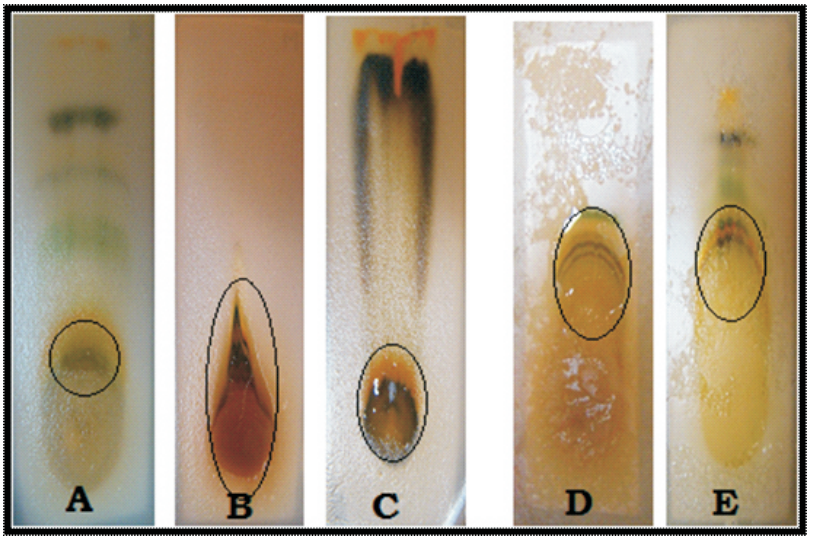
Extract in	R_f value – component	Standard R_f value
Ethanol	0.29 – Menthol 0.34 – Piperitone	0.30 0.35
Methanol	0.38 – Cineole	0.40
Ethyl acetate	0.28 – Menthol 0.37 – Piperitone 0.72 – Menthone 0.78 – Menthyl acetate	0.30 0.35 0.70 0.75



A – TLC of extract of *Mentha arvensis* in ethanol.
B - TLC of extract of *Mentha arvensis* in methanol.
C - TLC of extract of *Mentha arvensis* in ethyl acetate.

Fig 1- Thin layer chromatography

Bioautography –



A – Bioautography of ethanol extract for *Bacillus sp 1*
B - Bioautography of methanol extract for *Bacillus sp 1*
C - Bioautography of ethyl acetate extract for *Bacillus sp 1*
D - Bioautography of ethanol extract for *Bacillus sp 2*
E - Bioautography of methanol extract for *Bacillus sp 2*

Fig 4.5- zone of inhibitions observed on bioautography

Table 3 - The R_f value of zone of Inhibition

Extract in	*Bacillus sp 1* Rf value – component	*Bacillus sp 2* Rf value – component
Ethanol	0.29 – Menthol	0.82 – Menthyl Acetate
Methanol	No proper resolution	No proper zone
Ethyl acetate	0.37 – Piperitone	-

DISCUSSION:

In Thin layer chromatography followed by Bioautography the phytocompounds present in *M. arvensis* which show the antimicrobial activity are screened using dichloromethane as a solvent system and anesaldehyde sulphuric acid reagent as a developer. In this procedure we got the separation of compounds. By comparing the obtained R_f values with standard Rf values the compounds were identified.

Using bioautography the bioactivity of identified compounds was checked. In extract of M. arvensis in ethanol the Rf of zone of inhibition was obtained as0.29 that is Menthol. In methanolic extract no proper separation of components were observed. In ethyl acetate extract the Rf value of zone of inhibition observed was 0.37 that in Piperitone. So that it may be concluded that the compound menthol & Piperitone are showing antimicrobial activity.

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