

Chromatographic Study of Extracts/Fractions of Selected Plants *Mangifera indica*, *Cucumis sativus* and *Annona squamosa*

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ABSTRACT

Chromatographic fingerprinting of extracts/fractions of *Mangifera indica*, *Cucumis sativus* and *Annona squamosa* was done. Thin Layer and High Performance Liquid Chromatography were performed using different solvent systems. On the basis of trial and error for better resolution and separation of the phytoconstituents, following mobile phases gave best result. The presence of phytoconstituents was confirmed by reference standards. The solvent systems of different polarities were prepared for *Mangifera indica*, *Cucumis sativus* and *Annona squamosa*. For Co-optimization of extracts and their fractions, Chloroform: Glacial acetic acid: Cyclohexane (40:10:40) and Chloroform: Acetone: Formic acid (50:25:10) for CS, AS and Chloroform: Formic acid: Glacial acetic acid: Water (4:4:4:1) for MI were sorted out for better separation of phytoconstituents (Table 4.26, 4.27 and 4.28).

TLC study of MEMI gave 7 spots with R_f values 0.9, 0.85, 0.78, 0.6*, 0.5, 0.3, 0.16 while Ethyl acetate fraction gave 2 spots with R_f values 0.7, 0.6*. After matching with reference standard, study also revealed the presence of mangiferin in extract and fraction with R_f value 0.60*.

Key words:- Chromatography, TLC, fingerprinting, DM (Diabetes Mellitus)

INTRODUCTION

Most medications utilized in treatment of DM address the issue of hyperglycemia. In any case, the ongoing discoveries have demonstrated that, numerous elements other than carbohydrate metabolism play critical part in pathogenesis of DM. Components like chronic inflammation, activation of immune system, oxidative pressure, disturbance of protein and lipid digestion all play vital role in disease progression and recovery of diabetic complication. A few studies have proposed that hypoglycemic agents don't adequately give assurance against target organ harm caused by diabetes mellitus. As an outcome, diabetes mellitus in long haul comes full circle in micro and macro vascular intricacies like, neuropathy, nephropathy, retinopathy, cardiomyopathy and

coronary vein ailment.³⁻⁵

The term diabetes was coined by the Greek physician Aretaeus in the principal century A.D. In the seventeenth century, Willis saw that the pee of diabetics as wonderfully sweet as though imbued with nectar or sugar. The presence of sugar in the pee of diabetics was shown by Dobson in 1755. Novel drug delivery system is gainful in conveying the herbal constituent at ideal rate and delivery of medication at the site of activity which limits the toxicity and enhances bioavailability of the medications. In novel drug delivery system, distribution of medication is controlled by entrapping the medication in carrier or by altering the structure of the medication at atomic dimension. Herbal constituents are becoming more popular in the modern world for their application to fix assortment of maladies with less poisonous impacts and better restorative impacts.⁶⁻⁷ Anyway a few restrictions of herbal concentrates/plant actives like instability in exceptionally acidic pH, liver digestion and so forth prompted medication levels underneath restorative focus in the blood bringing about less or no remedial impact. Incorporation of novel drug delivery technology to herbal or plant actives minimizes the drug degradation or pre- systemic metabolism, and serious side effects by accumulation of drugs to the non-targeted areas and improves the ease of administration in the paediatric angieriatric patients. Different novel drug delivery systems, for example, liposomes, niosomes, microspheres and phytosomes have been accounted for the delivery of herbal medications. Incorporation of herbal medications in the delivery system likewise helps to increment in dissolvability, upgraded stability, protection from toxicity, enhanced pharmacological action, enhanced tissue macrophage distribution, sustained delivery and protection from physical and chemical degradation.

METHDOLOGY:-

Methods and procedures

Identification of Collected Plant Materials

The plants *Mangifera indica*, *Cucumis sativus* and *Annona squamosa* were collected from local farmers of different region of Uttar Pradesh, India. Plant materials were dried under shade and powdered coarsely before extraction.

Fractionation of Extracts and Their Chromatographic Studies

I. Fractionation of Extracts

Preliminary antidiabetic screening of different crude extract exhibited that, *Mangifera indica*, *Cucumis sativus* and *Annona squamosa* methanol extract have potent antidiabetic activity and hence they were selected for further fractionation to pin-point the activity. On the basis of polarity index (PI), fractionation of methanol extract was

done using benzene, ethyl acetate and ethanol by silica gel 60-80 mesh packed chromatographic column. Different fractions were collected. By using vacuum solvents were removed and fractions were dried with anhydrous Na₂SO₄, percentage yields (w/w) were calculated (**Table 4.22**).

Table No. 3.5 Extract fractions and their nomenclature

Drug	Fraction	nomenclature
<i>Mangifera indica</i> (MI)	Benzene fraction of Methanol extract MI	Benzene fraction of MEMI
	Ethyl acetate fraction of Methanol extract MI	Ethyl acetate fraction of MEMI
	Ethanol fraction of Methanol extract MI	Ethanol fraction of MEMI
<i>Cucumis sativus</i> (CS)	Benzene fraction of Methanol extract CS	Benzene fraction of MECS
	Ethyl acetate fraction of Methanol extract CS	Ethyl acetate fraction of MECS
	Ethanol fraction of Methanol extract CS	Ethanol fraction of MECS
<i>Annona squamosa</i> (AS)	Benzene fraction of Methanol extract AS	Benzene fraction of MEAS
	Ethyl acetate fraction of Methanol extract AS	Ethyl acetate fraction of MEAS
	Ethanol fraction of Methanol extract AS	Ethanol fraction of MEAS

II. Qualitative Phytochemical Evaluation of Fractions ²⁷⁴

Preliminary phytochemical screenings of fractions were done as per Kokate, 2002. Small quantities of investigating fractions were dissolved in their parent solvent and were subjected for analysis using specific reagents and the observations were tabulated (Table 4.23, 4.24 and 4.25).

(A) Tests for carbohydrates:

Molisch's test (For reducing sugars): In the solution of fraction Molisch's reagent was added

Purple ring at the junction confirm the presence carbohydrates.

Keller-Kiliani test (For de-oxy-sugars): With ferric chloride and sulphuric acid two layers were formed lower as reddish brown and upper as bluish green.

Borntrager's test (For anthraquinone glycoside): Small quantity of alcoholic potassiumhydroxide added in test solution, dilute with 4ml of water and filter, acidify with HCl, cool and shake well with 5ml of ether. Ether is separated and shakes with 2ml of dilute solution of ammonium hydroxide. Rose red to intense red color is

produced in the aqueous layer is indicative of presence of anthraquinone glycosides.

Liebermann - Burchard test: In test solution acetic anhydride and conc. H₂SO₄ were added, shaken and allowed to stand, bluish green lower layer confirm the presence of sterols.

(B) Tests for alkaloids:

A small portion of fractions are shaken in 5 ml of hydrochloric acid and filtered. The filtrates were separately tested with following reagents.

Dragendorff's test: With potassium bismuth iodide orange brown precipitate indicate the presence of alkaloid.

Mayer's test: Cream precipitate with potassium mercuric iodide confirms the presence of alkaloid.

Hager's reagent: Alkaloid gives yellow precipitate with saturated picric acid solution

(C) Test for terpenes / sterols:

Salkowaski test: On shaking with Conc. H₂SO₄ gives golden yellow lower layer.

Liebermann-Burchard test: With acetic anhydride and 1 ml of Conc. H₂SO₄ production of red color was confirm the presence of terpenes.

(D) Tests for saponins:

Foam test: Stable Foam produce on shaking, the solution shows the presence of saponins.

(E) Test for flavonoids:

Sinoda test: Pink color is obtained on adding 5 ml of 95% ethanol, to dry powdered fractions along with few drops Conc. HCl and 0.5 g of magnesium, in presence of flavonoids.

Zn/HCl reducing test:

Magenta red color obtained with, zinc dust and few drops of HCl in presence of flavonoid.

(F) Tests for tannins: With 2-3 ml of test solution, following color reaction is observable **Ferric chloride test:** Gives dark color with ferric chloride solution show the presence of tannins **Gelatin test:** White precipitate obtained on treating the solution with gelatin if tannins are present.

(G) Tests for Proteins

Biuret test: Blue color produce when treated with NaOH and dilute CuSO₄ indicate presence of protein.

RESULT & DISCUSSION

4.1.1 Chromatographic Study of Extracts/Fractions of Selected Plants

Table No. 4.26 Co-optimization of mobile phase for TLC profile of *Mangifera indica*

Mobile phase	Separation status	
	methanol extract	Ethyl acetate fraction
Chloroforme:Acetone:Water (4:4:2)	No separation	No separation
Chloroforme:Acetone:Water (5:4:1)	No separation	No separation
Chloroforme: Formic acid:Water (4:4:2)	Tailing	Tailing
Chloroforme: Formic acid:Water (4:4:4)	Poor separation	Poor separation
Chloroforme: Formic acid:Glacialacetic acid: Water (4:4:1:1)	Good separation	Good separation
Chloroforme: Formic acid: Glacialacetic acid: Water (4:4:4:1)	Better separation	Better separation

Table No. 4.27 Optimization of mobile phase for TLC profile of *Cucumis sativus*

Mobile phase	Separation status	
	Methanol extract	Ethyl acetate fraction
Toluene: Ethylacetate: diethylamine (70:20:14)	No separation	No separation
Toluene: Ethylacetate: diethylamine (60:30:20)	No separation	No separation
Chloroform: Ethylacetate: diethylamine (50:25:25)	No separation	No separation
Chloroform: Glacialacetic acid:Ethylacetate (50:30:20)	Tailing	Tailing
Chloroform:Glacialacetic acid:Cyclohexane(50:10:50)	Good separation	Good separation
Chloroform:Glacialacetic acid:Cyclohexane(40:10:40)	Better separation	Better separation

Table No. 4.28 Optimization of mobile phase for TLC profile of *Annona squamosa*

Mobile phase	Separation status	
	Methanol extract	Ethanol fraction
Chloroform: Ethylacetate (50:50)	No separation	No separation
Chloroform: Ethylacetate (80:20)	No separation	No separation
Chloroform: Ethylacetate: Water (80:20)	No separation	No separation
Chloroform:Acetone (50:50)	Tailing	Tailing
Chloroform:Acetone (75:25)	Tailing	Tailing
Chloroform:Acetone: : Formic acid (50:25:25)	Poor separation	Poor separation
Chloroform:Acetone: : Formic acid (50:25:10)	Better separation	Better separation

Table No. 4.29 TLC profile of *Mangifera indica* methanol extracts and its ethyl acetate fraction

S.No.	Extract/ Fraction	Spot	R _f value
1.	Methanol extract	7	0.9, 0.85, 0.78, 0.6*, 0.5, 0.3, 0.16
2.	Ethyl acetate fraction	2	0.7, 0.6*

*R_f value matched with reference standard;

Table No. 4.30 TLC profile of *Cucumis sativus* methanol extract and its ethanol fraction

S.No.	Extract/ Fraction	Spot	R _f value
1.	Methanol extract	4	0.79, 0.73, 0.68, 0.59*
2.	Ethanol fraction	2	0.81, 0.65*

* R_f Value matched with reference standard;

Table No. 4.31 TLC profile of *Annona squamosa* methanol extract and its Ethanol fraction

S.No.	Extract/ fraction	Spot	R _f value
1.	Methanol extract	9	0.89, 0.78, 0.74, 0.61, 0.52*, 0.43, 0.29, 0.19, 0.07*
2.	Ethanol fraction	6	0.90, 0.75, 0.52*, 0.31, 0.20, 0.06*

* R_f Value matched with reference standard;

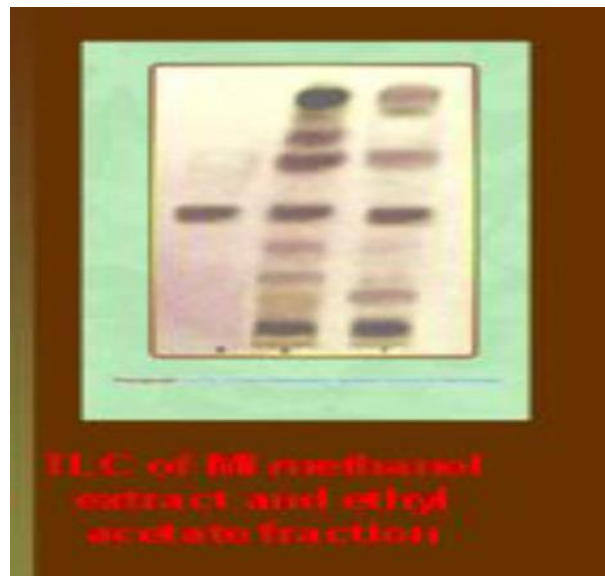


Figure No. 4.18 photograph of TLC of *Mangifera indica* methanol extract and ethyl acetate fraction



Figure No. 4.19 photograph of TLC of *Cucumis sativus* methanol extract and ethyl acetate fraction



Figure No. 4.20 photograph of TLC of *Annona squamosa* methanol extract and ethyl acetate fraction

CONCLUSION:-

Chromatographic fingerprinting of extracts/fractions of *Mangifera indica*, *Cucumis sativus* and *Annona squamosa* was done. Thin Layer and High Performance Liquid Chromatography were performed using different solvent systems. On the basis of trial and error for better resolution and separation of the phytoconstituents, following mobile phases gave best result. The presence of phytoconstituents was confirmed by reference standards. The solvent systems of different polarities were prepared for *Mangifera indica*, *Cucumis sativus* and *Annona squamosa*. For Co-optimization of extracts and their fractions, Chloroform: Glacialacetic acid: Cyclohexane (40:10:40) and Chloroform: Acetone: Formic acid (50:25:10) for CS, AS and Chloroform: Formic acid: Glacialacetic acid: Water (4:4:4:1) for MI were sorted out for better separation of phytoconstituents (Table 4.26, 4.27 and 4.28).

TLC study of MEMI gave 7 spots with R_f values 0.9, 0.85, 0.78, 0.6*, 0.5, 0.3, 0.16 while Ethyl acetate fraction gave 2 spots with R_f values 0.7, 0.6* After matching with reference standard, study also revealed the presence of mangiferin in extract and fraction with R_f value 0.60*. (TLC study of MECS gave 4 spots with R_f values 0.79, 0.73, 0.68, 0.59* while Ethanol fraction of methanol extract gave 2 spots with R_f values 0.81, 0.65* After matching with reference standard, it revealed the presence of Curcubitacine in extract and fraction with R_f value 0.44*.

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