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Downstream Processing of Secondary Metabolite Extraction from Medicinal Plants (Tinospora cordifolia, Ocimum tenuiflorum, Asparagus racemosus, Bacopa monnieri): Optimization and Equipment Utilization

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ABSTRACT

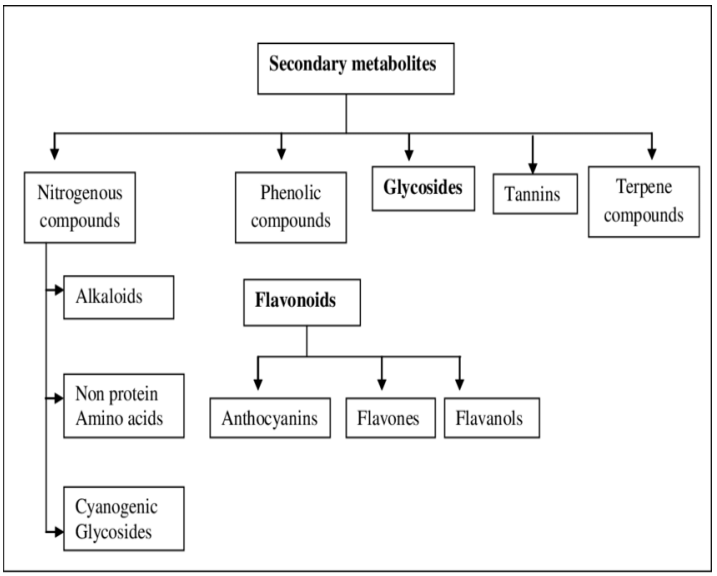
Tinosoporacordifolia, Ocimum tenuiflorum,Asparagus racemosus and Bacopa monnieri are all important and plants with significant medicinal properties from these plants secondary metabolites extracts were derived and TLC characterization was done.
This paper delves into the intricate downstream processing steps and the utilization of specialized equipment in the extraction and purification of secondary metabolites from four medicinal plants: Tinospora cordifolia, Ocimum tenuiflorum, Asparagus racemosus, and Bacopa monnieri. The study emphasizes the optimization of extraction methods, purification techniques, and the role of specific equipment in enhancing the efficiency of the downstream processing workflow.

INTRODUCTION

Tinospora cordifolia commonly named as "Guduchi" in Sanskrit belonging to family Menispermaceae is a genetically diverse, large, deciduous climbing shrub with greenish yellow typical flowers, found at higher altitude. In racemes or racemose panicles, the male flowers are clustered and female are solitary. The flowering season expands over summers and winters. A variety of active components derived from the plant like alkaloids, steroids, diterpenoid lactones, aliphatics, and glycosides have been isolated from the different parts of the plant body, including root, stem, and whole plant.

Medicinal plants have been a prolific source of bioactive compounds with therapeutic potential. This study focuses on the downstream processing involved in harnessing the secondary metabolites from selected medicinal plants, highlighting the significance of optimized extraction and purification methods.

Secondary metabolites are compounds that are produced by plants and are not directly involved in their growth or reproduction . They are often produced in response to environmental stresses, such as infection, herbivory, or environmental pollutants. Secondary metabolites can have a variety of functions, including defense against predators, attracting pollinators, or providing medicinal properties.The extraction of secondary metabolites from plants is a complex process that can vary depending on the type of compound being extracted and the plant material being used. The above mentioned properties of the plants was the reason why it was choosen.For secondary metabolites extraction,initially using methanol or any such reagent is used then purification is done



Getting a methanol extricate from clears out is to disconnect and concentrate the bioactive compounds show within the plant fabric for different logical, therapeutic, or industrial applications. Methanol extraction may be a common strategy utilized in phytochemical inquire about to think about and harness the potential benefits of these bioactive compounds.

TLC(Thin layer chromatography) is a widely used analytical technique in chemistry and biochemistry for separating and characterising complex mixtures of compounds;here it separate to methanolic extract from plant leaves because it can solve the following purposes such as compound identification and compound separation along with purity assessment .It can also aid in phytochemical profiling where their chemical constituents of plant extracts are profiled to help understand the quantities of compounds present in an extract along with quality control,it helps in measuring the consistency and quality of metallic extracts from batches of plant leaves and it also helps to detect active compounds which can be taken by the spots on the TLC sheet

Fig 1: Tinospora corifolia

Fig 2: Ocimum tenuiflorum

Fig3: Asparagus racemosus

METHODOLOGY

1. Firstly the leaves were taken and the leaf samples were washed.Then weighed the sample, using up mortar and pestle the leaves were grinded thoroughly and after this methanol was added in the ratio 1:1 to maintain a consistency that was neither too dilute nor too concentrated
2. This was then kept at room temperature for a period of 24 hours in an effendorf tube
3. After 24 hours the effendorf tube was taken and centrifugation was carried out.
4. After centrifugation the supernatant was removed and placed in a separate effendorf tube
5. After this TLC was carried out for TLC process firstly with TLC plate was to be prepared that was prepared by taking the TLC plate and marking the horizontal line about one centimetre above the bottom edge of the plate using a pencil.
6. The sample was sent spotted using a small capillary tube and small amounts were taken from the sample
7. Then the TLC plate was developed using the solvent n-butanol, acetic acid and water(8:2:1) in a 250mL beaker.The solvent was prepared
8. for 20mL(n- butanol:16mL,acetic
9. acid:2mL and water:2mL)
10. After the preparation of the solvent,the TLC plate with samples spotted was places in the solvent and it was sealed carefully using an aluminium foil.
11. After around 30 minutes the TLC plate was taken out and it was air dried and kept in the oven for a few minutes.
12. After the TLC plate was thoroughly dried a suitable spray was sprayed onto the surface of the plate.
13. The TLC plate was then visualised under UV light since the leaves contains a lot of secondary metabolites each metabolite has a different colour which can be clearly seen after visualisation of the plate.
14. Rf values were also calculated.
15. Phenolics and flavonoids assay was from the calibration curve also done and the procedure for that was as follows:
16. Flavanoids assay

i. Prepare a series of quercetin standard solutions with known concentrations (e.g., 0, 10, 20, 30, 40, and 50 µg/mL)
using methanol as the solvent.

ii. Extract total flavonoids from plant samples using an appropriate solvent (e.g., methanol).

iii. Concentrate the extract if necessary and dissolve in methanol to obtain a suitable concentration

iv. Take 100 µL of each quercetin standard solution and plant extract solution in separate test tubes or microcentrifuge tubes.

v. Add 10 µL of 10% aluminum chloride solution to each tube.

vi. Add 10 µL of 1M sodium acetate solution to each tube.

vii. Mix well and allow the reaction to proceed for 30 minutes at room temperature.

viii. After 30 minutes, measure the absorbance of each solution at a specific wavelength (usually around 415 nm) using a UV-Visible spectrophotometer.

ix. Express the results as milligrams (mg) of quercetin equivalents per gram (g) or milligram (mg) of plant material.

x. Phenolics estimation:

xi. Set a blank containing only the solvent (methanol) and reagents (aluminum chloride and sodium acetate) and measure its absorbance.

xii. Plot a calibration curve using the absorbance values of the quercetin standard solutions on the y-axis and the corresponding concentrations on the x- axis.Determine the equation of the line (y = mx + b), where "m" is the slope and "b" is the intercept.

xiii. Calculate the concentration of total flavonoids in the plant extract using the equation derived

Preparation of the standard:

1. Pipette out different aliquots of standard Gallic acid stock solution (30µg/ml) ranging from 0.0 to 1.0 ml into different test tubes. Make up the volume to 1.0ml in each test tube with distilled water.

2. Add 0.5ml of FC reagent, mix by inversion and incubate in dark for 5 min at room temp.

3. Add 1ml 5%NaCO3 solution, mix well and incubate at room temperature for 5 min.

4. Measure the absorbance of the solution at 725nm.

5. The graph was plotted with concentration of gallic acid on X-axis and optical density on Y- axis and calibration graph was drawn.

Downstream Processing Steps:

Extraction:

- Maceration: Plant material was soaked in a solvent for an optimized duration to facilitate the dissolution of bioactive compounds.
- Soxhlet Extraction: Continuous extraction was achieved by using a Soxhlet apparatus, ensuring higher efficiency and yield
- Ultrasound-Assisted Extraction (UAE): Sonication was employed to enhance mass transfer, breaking down cell walls and facilitating compound release.

Filtration and Separation:

- Filtration: Solid impurities were removed using various filtration techniques, such as gravity filtration or vacuum filtration.

Liquid-Liquid Extraction: Separation of compounds was accomplished using selective solvents to partition the desired constituents.

Purification

Preparative HPLC:High Performance Liquid Chromatography with a preparative column enhanced the sepration of compound based on their chemical properties

Utilized Equipment:

Soxhlet Apparatus:

- The Soxhlet extraction method was executed using a Soxhlet apparatus to ensure a continuous and efficient extraction process.

Ultrasonic Processor:

- A pivotal role was played by the ultrasonic processor in ultrasound- assisted extraction, facilitating the disruption of plant cell structures and promoting enhanced mass transfer.

Rotary Evaporator:

- Following extraction, solvents were eliminated using a rotary evaporator, concentrating the crude extract in preparation for subsequent processing.

Filtration Equipment:

- Filtration equipment, including filter papers and vacuum filtration setups, played a crucial role in the separation process by effectively removing solid impurities.

Column Chromatography Setup:

- The purification of compounds was accomplished using a column chromatography setup, featuring a glass column packed with a stationary phase. This method facilitated the separation of components based on their differential interactions with the stationary phase.

Preparative HPLC System:

- Isolation of pure compounds was achieved through the use of a Preparative High-Performance Liquid Chromatography (HPLC) system. This system, equipped with a preparative column, enabled the separation and collection of compounds based on their elution profiles, ensuring the extraction of pure substances for further analysis or application.

Fig 5:TLC Plate with the samples spotted

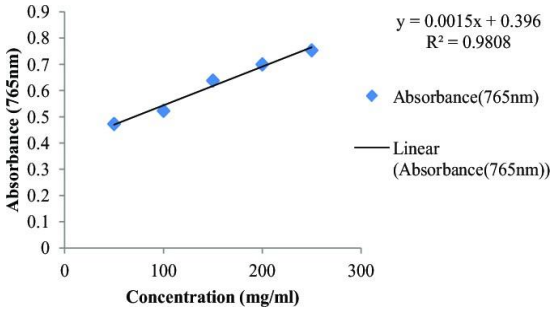


Fig 8:Standard gallic acid curve

Fig 6:Supernatant stored at -20C

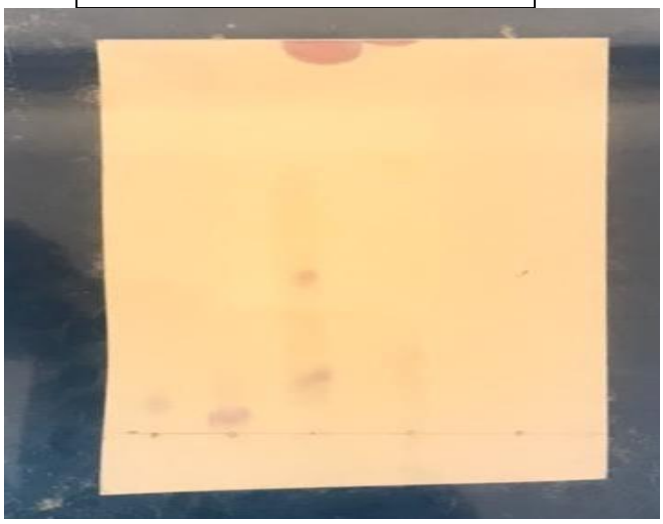


Fig 7:TLC plate under UV light

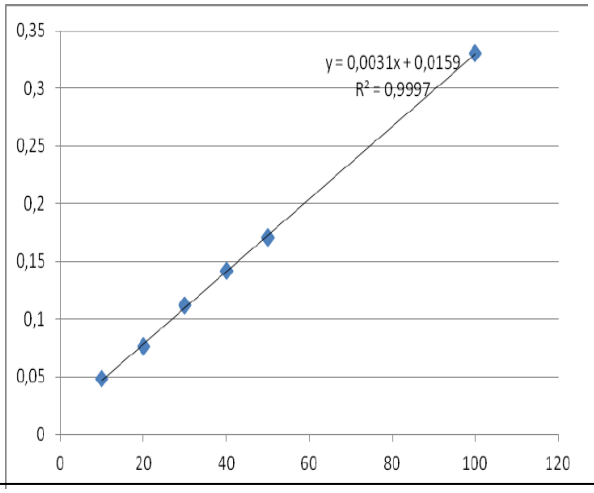


Fig 9:Standard quercitin curve

RESULTSAND CALCULATIONS

Rfvalue=(Distance moved by solute)/(Distance moved by solvent)

TABLE 1:SAMPLE AND THE DISTANCE MOVED BY SOLUTE FROM BASE LINE

SAMPLE NO.	COMPOUND 1	COMPOUND 2	COMPOUND 3
1	0.7	-	3.2
2	1.25	5.5	0.5
3	1.5	5.2	3.1
4	1.2	5.6	3.4

Based on the above table , the following can be calculated

TABLE 2:SAMPLES AND THEIR CORRESPONDING Rf VALUES

SAMPLE NO.	Rf value	Rf value	Rf value
1	0.113	-	0.52
2	0.202	0.88	0.08
3	0.242	0.84	0.5
4	0.19	0.9	0.548

Based on the above 2 tables it can clearly be concluded that the sample 1 has only compound 1 and 3 whereas sample 2,3 and 4 have all the 3 compounds .

The TLC plate was visualised under UV light and under normal conditions as well and the distance moved by each solute and the distance moved by the solvent was noted as well for calculating the Rf value.

SAMPLE	TOTAL PHENOLICS CONTENT(mg of gallic acid/g of ample
SAMPLE 1	211.76
SAMPLE 2	2.18
SAMPLE 3	165.22
SAMPLE 4	24.3

TABLE 3:TOTAL PHENOLICS CONTENT

SAMPLE NO.	TOTAL FLAVANOIDS CONTENT(mg of quercitin/g of sample)
1	852.071
2	4.44
3	188.33
4	0.08563

TABLE 4 : TOTAL FLAVANOIDS CONTENT

Phenolics content was highest in *Tinospora cordifolia* and least in *Ocimum tenuiflorum.*

Flavanoids content was highest in *Tinospora cordifolia* and least in *Bacopa monnieri*

*E*ach colour on the TLC plate corresponds to a particular compound

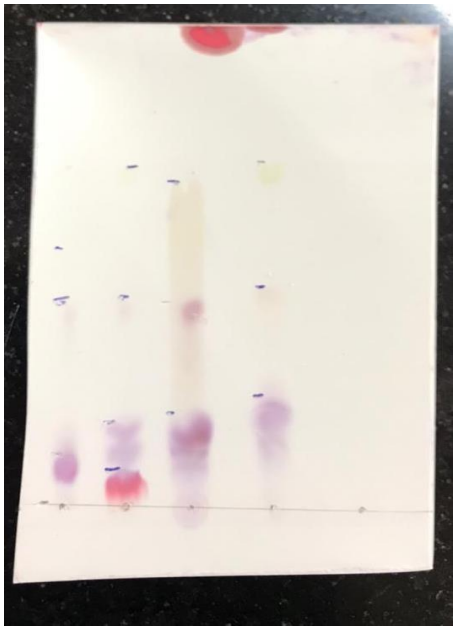


Fig 8:TLC plate

CONCLUSIONS

From the leaf extracts of 4 medicinally important plants secondary metabolites were extracted and the sample with the highest and lowest phenolics and flavonoids content was found out.

Using TLC,purification of these metabolites was seen and each colour corresponded to a particular metabolite.

Title: Downstream Processing of Secondary Metabolite Extraction from Medicinal Plants (Tinospora cordifolia, Ocimum tenuiflorum, Asparagus racemosus, Bacopa monnieri): Optimization and Equipment Utilization

The downstream processing of secondary metabolite extraction from medicinal plants involves a well-orchestrated series of steps, each optimized for efficiency. The utilization of specific equipment, such as Soxhlet apparatus, ultrasonic processors, rotary evaporators, and chromatography setups, plays a pivotal role in achieving successful extraction, separation, and purification. This comprehensive approach enhances our ability to isolate bioactive compounds from medicinal plants, paving the way for further pharmacological studies and potential drug development.

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