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ORIGINAL ARTICLE

Phytochemical Profiling of *Passiflora alata* Using TLC and HPTLC Techniques

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ABSTRACT
Passiflora alata is widely recognized for its potential to treat central nervous system (CNS) disorders. However, comprehensive research on its phytochemical profile is scarce. Objectives: This study aimed to delineate the phytochemical profile of P. alata using thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) to better understand its therapeutic potential. TLC was performed using silica gel-coated plates to separate the chemical components, which were then visualized using specific reagents to produce colour distinctions and to calculate retention factor (Rf) values for comparison. HPTLC refined this analysis by applying precise sample volumes on specialized plates and densitometric analysis for the detailed quantification and visual profiling of chemical constituents. Preliminary screening identified phytochemical classes such as alkaloids, glycosides, and flavonoids. TLC revealed a spectrum of compound polarities, with n-hexane and methanol extracting nonpolar and polar compounds, respectively, indicating the complex nature of the extract. HPTLC fingerprinting identified specific compounds such as quercetin and isoorientin in some samples, although other compounds remained unidentified. Comprehensive phytochemical profiling of P. alata confirmed its complex biochemical makeup, highlighting its potential for therapeutic applications. Identifying specific bioactive compounds using advanced chromatographic techniques underscores the need for further pharmacological studies to explore the role and efficacy of these compounds in medical treatments.
Keywords: *Passiflora alata*, TLC, HPTLC, phytochemical profiling, therapeutic applications.

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INTRODUCTION

Passiflora alata, commonly known as the Winged-Stem Passionflower, is a perennial, evergreen vine indigenous to Brazil. This plant is distinguished by its strikingly beautiful flowers, edible fruits, and extensive use in traditional medicine [1]. Traditional healers have long used *P. alata* for its diverse medicinal properties, particularly in addressing digestion and nervous system issues. The significance of this plant in ethnomedicine is primarily attributed to its hypnotic and sedative effects, which are highly regarded in various cultural medicinal practices [2].

P. alata is frequently used in traditional medicine to treat anxiety, insomnia, gastrointestinal disorders, and neuralgia. The sedative properties of *P. alata* are well-documented and are believed to stem from its ability to modulate the central nervous system. Traditional preparations often involve using leaves, stems, and flowers brewed into tea or made into tinctures. These preparations promote relaxation, induce sleep, and alleviate pain. The hypnotic and sedative properties of *P. alata* have been recognized in various cultures. These effects are particularly interesting because they offer potential alternatives to synthetic pharmaceuticals, often resulting in side effects. The sedative effects of *P. alata* can be attributed to its rich phytochemical composition, which includes various bioactive compounds [3-5].

Phytochemically, *P. alata* is a rich source of diverse bioactive compounds that contribute to its broad pharmacological profile. Several of these compounds have been identified as particularly significant

because of their therapeutic potential, including quadranguloside, oleanolic acid-3-sophoroside, β-carboline alkaloids such as harmane and harmine, and steroids such as β-sitosterol. Quadranguloside and oleanolic acid-3-sophoroside are saponins with immunomodulatory and anti-inflammatory properties, respectively. These compounds enhance the ability to modulate immune responses, making *P. alata* useful in managing inflammatory conditions and supporting overall immune health. β-Carboline alkaloids, including harmane and harmine, are of significant interest owing to their psychoactive properties [6-9].

Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) are essential techniques for the qualitative and quantitative analysis of phytochemicals in *P. alata*. These chromatographic methods enable the identification and characterization of bioactive components and link the chemical constituents of plants to their pharmacological activities [10]. TLC is a widely used technique for the preliminary screening plant extracts, allowing the separation of different phytochemicals based on their polarity and affinity for the stationary phase [11]. By comparing the Rf) values of compounds with known standards, researchers can identify the presence of specific bioactive compounds in *P. alata*. HPTLC is an advanced form of TLC that offers a high resolution and sensitivity, enabling precise quantification of phytochemicals in complex mixtures [12] HPTLC helps standardize herbal preparations and ensure consistent quality and potency. This technique also facilitates a detailed exploration of the phytochemical profile of *P. alata*, providing insights into how individual components contribute to its medicinal properties [13].

P. alata, with its rich phytochemical composition and diverse pharmacological activities, holds significant promise as a therapeutic agent. The traditional uses of the plant are supported by scientific research that continues to uncover the mechanisms underlying its medicinal properties. Analytical techniques such as TLC and HPTLC play crucial roles in standardizing *P. alata* extracts and in linking bioactive compounds to their therapeutic effects. As research progresses, *P. alata* is poised to become a valuable component of traditional and modern medical practices, offering natural solutions for various health conditions. This comprehensive exploration of *P. alata* highlights the importance of integrating traditional knowledge with scientific research. By leveraging advanced analytical methods and conducting rigorous studies, we can unlock the full potential of this plant, paving the way for new therapeutic applications and improved global health outcomes.

MATERIAL AND METHODS

Plant material

The whole herb of *P. alata* was collected from Hyderabad and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati. The voucher specimen was (0779) preserved in the herbarium.

Extraction

One kilogram of fresh leaves, stems, and roots was shade-dried at 25-35 ºC for seven days. The dried plant parts were powdered using a grinder. The dried plant powder was subjected to Soxhlet extraction using n-hexane, Ethyl acetate, and methanol to get the respective extracts. Maceration of the marc was then performed with water for 24 h with continuous stirring at room temperature. Each extract was filtered using cotton plugs and Whatman No. 1 filter papers. The filtrate was concentrated, dried under reduced pressure in a rotary evaporator, and lyophilized to obtain a powder. The percentage of yield was calculated using the following formula: -

$$\text{Yield (g/100 g)} = (W_1 \times 100)/W_2$$

Where,

W_1 = weight of the crude extract residue obtained after solvent removal

W_2 = weight of plant powder packed in the extractor [14-17].

Phytochemical Screening

Preliminary phytochemical screening of all *P. alata* extracts was performed according to standard procedures [18-19].

TLC profiling

TLC analysis employs thin glass plates coated with a stationary phase, typically aluminium oxide or silica gel. The choice of mobile phase and solvent is dictated by the physicochemical characteristics of the components within the mixture. The foundational principle of TLC is based on the differential distribution of compounds between the stationary solid phase and mobile liquid phase as they traverse the solid surface. A minute sample was spotted near the base of the thin-layer chromatography (TLC) plate. The Rf values were consistent for each substance when the experimental conditions were replicated. Following

the development of the plates in a chosen solvent system and subsequent air drying, the plates were treated with 50% sulfuric acid and an anisaldehyde reagent (which includes 0.5 mL of anisaldehyde, concentrated sulfuric acid (1 mL), and acetic acid (50 mL), and heated until distinct colours appeared. The Rf values for the compounds are derived from three separate trials [20-22].

"Rf = Distance travelled by the solute / Distance travelled by the solvent"

After development, the plates were heated to 100°C to enhance the visibility of characteristic colours. The duration until the initial colour reaction, the appearance of the colours in natural light, after a ten-minute heating period, and under UV illumination (360 nm) were meticulously recorded. The Rf values were calculated following the completion of the three replicates [23].

HPTLC fingerprinting

HPTLC fingerprinting was performed using the method described by Wagner *et al*. Each 100 mg sample was solubilized in 1 mL of methanol, followed by centrifugation at 3000 rpm for 5 min to prepare the samples for HPTLC analysis. The HPTLC system used was a CAMAG setup from Muttenz, Switzerland, featuring an "Linomat 5 automatic sampler" with a 100 µL syringe, a twin trough chamber for plate development, a "Camag TLC Scanner 3, and winCATS software". A precise volume of 2 µL from standard and sample solutions was applied in 5 mm wide bands on silica gel 60 F254 HPTLC plates (4 × 10 cm, 250 µm thick) obtained from E. Merck (Mumbai, India). The applications were positioned at 8 mm from the bottom and 15 mm from the side margins of the plate. Before development, the plates were subjected to iodine vapor for 10 min as a pre-derivatization step. The development was then conducted in a twin-trough glass chamber pre-saturated with the chosen mobile phase for 20 min at a controlled temperature of 25±2°C. Chromatography was performed up to 90 mm from the plate base. The plates were then dried at 60°C for 5 min in an oven to evaporate the mobile phase completely. Densitometric analysis was performed with a "Camag TLC scanner set to reflectance mode", with the parameters set to a slit size of 6 mm by 0.45 mm, scanning speed of 20 mm/s, data resolution of 100 µm/step, employing a second-order optical filter and a Savitzky-Golay smoothing factor of 7. Scanning was performed at 254 nm, a wavelength determined experimentally to suit the unique absorption characteristics of the analyzed compounds within the 200–400 nm spectrum. The plates were then imaged using a "CAMAG REPROSTAR 3 system," capturing the chromatograms under both visible and UV light at 366 and 254 nm wavelengths, facilitating the assessment of peak metrics, visual profiles, and densitograms [24-26].

RESULTS

Preliminary Phytochemical screening

Table 1 shows the percentage yields of the extracts obtained from different parts of *P. alata* using four solvents: n-hexane, ethyl acetate, methanol, and water. The yield represents the amount of extract obtained as a percentage of the weight of the plant material used for extraction (Table 1).

Table 1: Percentage yield of *P. alata*

Extract	n-hexane extract	Ethyl acetate extract	Methanol extract	Aqueous extract
Leaves	2.42	5.76	12.39	3.45
Stems	1.15	3.58	10.05	1.67
Roots	0.61	0.83	2.37	0.91

Preliminary phytochemical screening results provided an initial insight into the types of phytochemicals present in different parts of the plant, which can be helpful in identifying potential bioactive compounds. The results indicate the presence or absence of several classes of phytochemicals, including alkaloids, glycosides, flavonoids, terpenoids, steroids, saponins, tannins, lipids, proteins, and carbohydrates (Table 2).

Table 2: Preliminary phytochemical screening results of prepared extracts

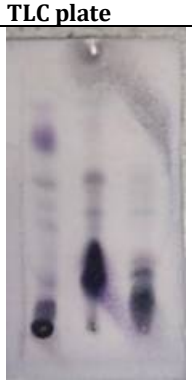
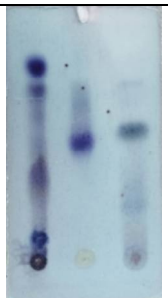
Phytochemicals	Leaves				Stem				Root			
	HE	EAE	ME	AQ	HE	EAE	ME	AQ	HE	EAE	ME	AQ
Alkaloids	-	++	+++	-	-	-	++	-	-	+	++	-
Glycosides	-	++	+++	+++	-	++	++	+	-	+	+++	+
Flavonoids	+++	++	+	-	+	+	-	-	+	+	-	-
Terpenoids& steroids	-	-	+	++	-	-	+	+++	-	-	+	++
Saponins	-	-	++	+++	-	-	++	+++	-	-	+	+
Tannins	+	-	-	-	+	-	-	-	+	-	-	-
Lipids	-	-	-	+	-	-	-	-	-	-	-	-
Proteins	-	-	-	++	-	-	-	-	-	-	-	+
Carbohydrates	-	++	+++	-	-	-	++	-	-	+	++	-

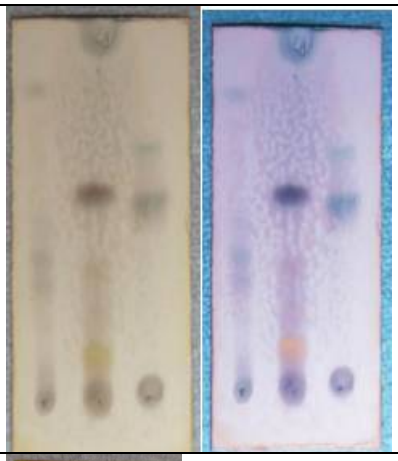
(+) present, (-) absent

TLC profiles of extracts

TLC profiling of *P. alata* revealed a rich and complex chemical composition indicative of the plant's potential pharmacological benefits. The variation in Rf values across different samples and conditions highlights many phytochemicals with differing polarities and solubilities. For instance, the higher Rf values observed with mobile phases containing a higher proportion of ethyl acetate suggest the presence of less polar compounds that are more soluble in the organic phase. The use of specific spraying reagents such as FeSO4 and LB Reagent, which revealed spots indicative of tannins and steroids/terpenoids, suggests that *P. alata* contains bioactive compounds known for their therapeutic properties, such as antioxidant and anti-inflammatory activities. The variability in spot patterns between different samples (PLM, PLE, and PSM) may reflect the efficacy of the extraction methods or chemical diversity in different plant parts. For example, the absence of spots with Dragendorff's reagent for alkaloids suggests either the absence or low concentration in the tested samples, underscoring the importance of selecting appropriate analytical conditions based on the target compounds. Overall, the TLC results emphasize the need for methodical optimization of phytochemical analysis to uncover the full therapeutic potential of *P. alata*, paving the way for further isolation and characterization of its active compounds (Table 3).

Table 3: TLC profile of *P. alata* extracts

Description	TLC plate	Distance travelled	Rf Values
Mobile phase:Ethylacetate: n-Hexane=1.5:8.5 (15%EA+Hex) Spraying reagent= Anisaldehyde.H2SO4 Sample 1= PLM Sample 2=PLE Sample 3=PSM		**Distance travelled by** The solvent =3.3 cm **Sample=1** Spots Name with numbering from top to bottom PA 1=2.4cm PA 2= 1.9cm PA 3=1.7cm PA 4=1.5cm PA 5=0.6cm PA 6=0.4cm **Sample=2** PA 1=2.0cm PA 2= 1.5cm PA 3=1.0cm PA 4=0.6cm **Sample=3** PA 1=0.7cm PA 2= 0.4cm	 0.72 0.57 0.51 0.45 0.18 0.12 0.60 0.45 0.30 0.18 0.21 0.12
Mobile phase:Ethylacetate: n-Hexane=3:7 (30%EA+Hex) Spraying reagent= Vanillin.H2SO4 Sample 1= PLM Sample 2=PLE Sample 3=PSM		**Distance travelled by** The solvent **=3.2 cm** **Sample=1** PA 1=2.6cm PA 2= 2.3cm PA 3=1.1cm PA 4=0.3cm **Sample=2** PA 1=2cm PA 2= 1.5cm **Sample=3** PA 1=2.2cm PA 2= 1.6cm	 0.81 0.69 0.33 0.09 0.60 0.45 0.66 0.50

Mobile phase:Ethylacetate: n-Hexane=3:7 (30%EA+Hex) Spraying reagent= Anisaldehyde.H2SO4 Sample 1= PLM Sample 2=PLE Sample 3=PSM		**Distance travelled by** The solvent **=3.2 cm** **Sample=1** PA 1=2.9cm PA 2= 2.1cm PA 3=0.9cm PA 4=0.6cm **Sample=2** PA 1=1.7cm PA 2= 1.2cm **Sample=3** PA 1=1.6cm PA 2= 1.0cm	 0.90 0.65 0.28 0.18 0.53 0.37 0.50 0.31
Mobile phase:Ethylacetate: n-Hexane=3:7 (30%EA+Hex) Spraying reagent= Dragendorff's reagent for Alkaloids Sample 1= PLM Sample 2=PLE Sample 3=PSM		No spots were observed	-
Mobile phase:Ethylacetate: n-Hexane=3:7 (30%EA+Hex) Spraying reagent= FeCl3 for Polyphenolic compounds Sample 1= PLM Sample 2=PLE Sample 3=PSM		**Distance travelled by** The solvent=3.2 cm **Sample=1** PA 1=2.1cm PA 2= 0.9cm **Sample=2** PA 1=1.2cm PA 2= 1.2cm PA 3= 0.4cm **Sample=3** No spots were observed	 0.65 0.28 0.37 0.37 0.12 --
Mobile phase:Ethylacetate: n-Hexane=3:7 (30%EA+Hex) Spraying reagent= FeSO4 for tannins Sample 1= PLM Sample 2=PLE Sample 3=PSM		**Distance travelled by** The solvent=3.2 cm **Sample=1** PA 1=3.1cm PA 2= 1.4cm PA 3=1.6cm **Sample=2** PA 1=1.7cm PA 2= 1.5cm PA 2= 0.4cm **Sample=3** PA 1=2.0cm PA 2= 1.5cm	 0.96 0.43 0.5 0.53 0.46 0.12 0.62 0.46

Mobile phase:Ethylacetate: n-Hexane=3:7 (30%EA+Hex) Spraying reagent= LB Reagent for steroids and terpenoids Sample 1= PLM Sample 2=PLE Sample 3=PSM		**Distance travelled by** The solvent **=3.2 cm** **Sample=1** PA 1=2.9cm PA 2= 2.4cm PA 3=1.6cm PA 4=1.3cm PA 5=0.4cm PA 6=0.3cm **Sample=2** PA 1=2.3cm PA 2= 1.8cm PA 3= 1.5cm **Sample=3** PA 1=1.3cm PA 2= 0.8cm PA 3= 0.4cm	 0.90 0.75 0.50 0.40 0.125 0.09 0.71 0.56 0.46 0.40 0.25 0.12
Mobile phase:Ethylacetate: n-Hexane=3:7 (30%EA+Hex) Spraying reagent= NaOH for Flavonoids Sample 1= PLM Sample 2=PLE Sample 3=PSM		**Distance travelled by** The solvent **=3.2 cm** **Sample=1** PA 1=1.6cm PA 2= 1.1cm PA 3=0.7cm **Sample=2** PA 1=1.3cm PA 2= 0.9cm PA 3= 0.6cm **Sample=3** No spots were observed	 0.5 0.34 0.21 0.40 0.28 0.18 --
Mobile phase:Ethylacetate: n-Hexane=3:7 (30%EA+Hex) Spraying reagent= Ninhydrin for aminoacids Sample 1= PLM Sample 2=PLE Sample 3=PSM		**Distance travelled by** The solvent **=3.2 cm** **Sample=1** PA 1=1.6cm PA 2= 1.8cm **Sample=2** PA 1=1.9cm PA 2= 1.3cm PA 3= 0.9cm **Sample=3** No spots were observed	 0.50 0.56 0.59 0.40 0.28 --

HPTLC fingerprinting

From the results of TLC analysis, three samples were selected for further chromatographic analysis using HPTLC. The HPTLC data showed that the three samples were good regarding phytochemical spots, and the Rf values were compared with reference standards (Table 4 and 5). The results suggest that the phytochemicals present in PLM and PSM were matched with a few references and identified as quercetin and isoorientin, and the remaining compounds are unknown, including the phytochemicals in PSE (Figure 1-3).

Table 4: HPTLC Details

Instrument	CAMAG TLC Scanner 3 "Scanner3_071124" S/N 071124 (1.14.30),
Executed by	IICT, NPL, Monday, June 20, 2022 12:18:21 PM
Number of tracks	10
Position of first track X	15.0 mm
Distance between tracks	21.2 mm
Scan start pos. Y	5.0 mm
Scan end pos. Y	75.0 mm
Slit dimensions	4.00 x 0.30 mm, Micro
Optimize optical system	Light
Scanning speed:	20 mm/s
Data resolution:	100 um/step

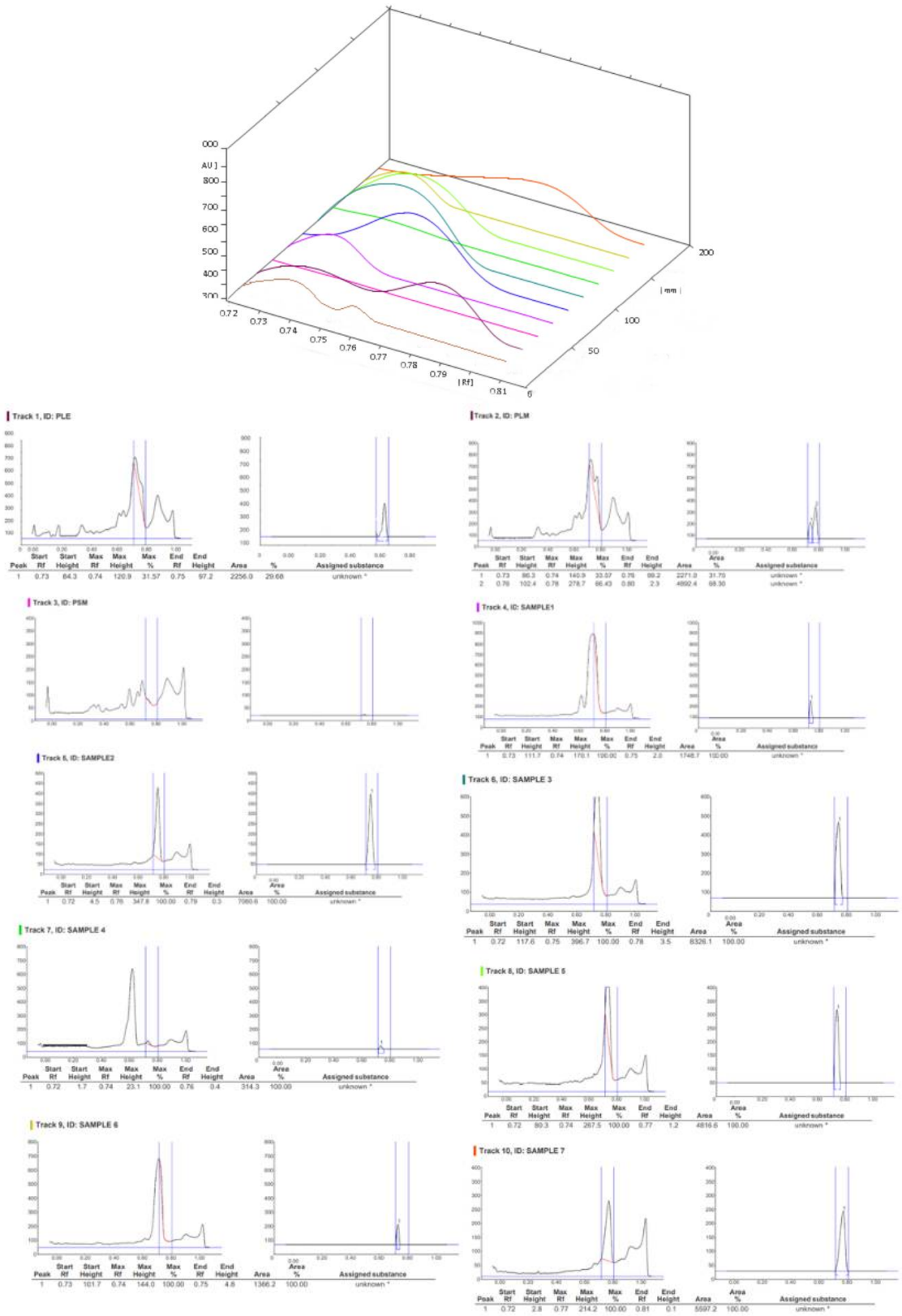


Fig. 1: Densitograms of *P. alata*

Table 5: Visualization Method at 254 and 366nm

Illumination instrument	CAMAG Visualizer: 230851 (Visualizer_230851)	CAMAG Visualizer: 230851 (Visualizer_230
Digital camera type : snr & Lens	DXA252 : 769611416, Computer, 12 mm, f4.0	DXA252 : 769611416, Computer, 12 mm, 14
Created by : on	IICT,NPL : Monday, June 20, 2022 12:18:21 PM	IICT,NPL : Monday, June 20, 2022 12:18:21
Resolution	Full	Full
Plate border size	-2 mm	-2 mm
Automatic capture	Off	Off
Save mode	Uncompressed	Uncompressed
Exposure mode	Automatic, digital level: 80 %, Band	Automatic, digital level: 85 %, Band
Capture settings:	1459 Pxl x 715 Pxl (0.13 mm/Pxl)	1459 Pxl x 715 Pxl (0.13 mm/Pxl)
Image size:	121.21 ms gain: 1.00	1995.94 ms gain: 1.00
Exposure :	R: 1.40, G: 1.00, B: 1.20	R: 1.40, G: 1.00, B: 1.20
White balance	254 nm remission: Default correction	366 nm remission: Default correction
Illumination type / correction type : **Display settings:**		
White balance:	R: 1.00 G: 1.00 B: 1.00	R: 1.00 G: 1.00 B: 1.00
Contrast enhancement:	1	1
Brightness:	0	0
Accentuation:	0.8	0.8
Color saturation:	1.30*	1.30 *
Blank plate compensation :	N/A	N/A

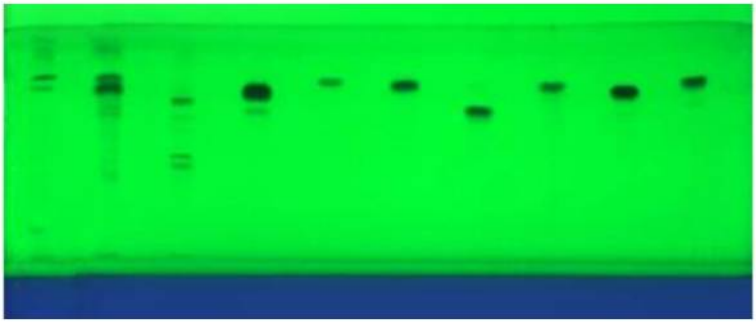


Fig. 2: HPTLC Chromatogram at 254 nm

Fig. 3: HPTLC Chromatogram at 366 nm

DISCUSSION

This study examined the yield of extracts from various parts of *P. alata*, using four solvents: "n-hexane, ethyl acetate, methanol, and water." The yield was expressed as a percentage of the initial weight of the plant material used. Preliminary phytochemical screening indicated the presence or absence of several phytochemical classes, including alkaloids, glycosides, flavonoids, terpenoids, steroids, saponins, tannins, lipids, proteins, and carbohydrates.

The findings from the TLC analysis provided insights into the polarity of the compounds present in the different extracts. It was determined that the n-hexane extract predominantly contained nonpolar

compounds. In contrast, the methanol extract exhibited spots indicative of more polar compounds. Interestingly, the Ethyl acetate extract presented a combination of spots encapsulating polar and nonpolar compounds, suggesting a multifarious nature.

A detailed understanding of the polarity of phytochemicals is crucial because it provides crucial information regarding their possible interactions, solubility, and bioavailability in biological systems. The wide range of compounds in the TLC profiles highlighted the complexity and heterogeneity of the phytochemicals present in *P. alata*, each of which may have distinct therapeutic functions. The observed polarity variations also suggest that these compounds are differentially soluble in different solvents, which may affect the effectiveness of the solvent extraction and the consequent yield of bioactive chemicals. A similar experiment was conducted in 2017 by Wosch, who studied the TLC profiles of Passiflora taxa using various mobile phases.[25] Our results also agree with those of Menghini et al. (1988), who studied flavonoid accumulation in *P. incarnata* leaves [28].

HPTLC fingerprinting analysis was conducted on three samples based on TLC and *in vitro* studies. The HPTLC data revealed discernible phytochemical spots, which were subsequently compared with the reference standards. This comparison facilitated the identification of several phytochemicals, including quercetin and isoorientin, in the PLM and PSM samples; however, some phytochemicals in the PSE samples and others remained unidentified. Identifying specific phytochemicals is instrumental as it offers insights into the potential bioactive compounds in *P. alata*, thereby paving the way for further exploration of their therapeutic properties and mechanisms of action. Furthermore, these findings suggest that *P. alata* is a rich source of diverse phytochemicals, some of which have yet to be explored and understood. The HPTLC results agree with the research carried out by Pereira *et al*., 2004 and Foudah *et al*., 2019, who performed the HPTLC fingerprinting profile of other species of Passiflora [29-30].

CONCLUSION

The TLC and HPTLC studies showed that the *P. alata* extracts were rich in secondary metabolites. The results suggest that the phytochemicals present in PLM and PSM were matched with a few references and identified as quercetin and isoorientin, and the remaining compounds, including the phytochemicals in PSE, are unknown.

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