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Bioanalytical Profiling and Unveiling Phytopharmacological Potential of Psidium Guajava Using HPTLC

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ABSTRACT

The present study aimed to evaluate the phytochemical profile and anti-urolithiatic potential of Psidium guajava and Coccinia grandis leaf extracts using various in-vitro techniques. Successive extraction was carried out using solvents of increasing polarity (n-hexane, chloroform, ethyl acetate, methanol). Methanolic extract of C. grandis exhibited the highest yield (7.9% w/w), while phytochemical screening confirmed the presence of flavonoids, glycosides, terpenoids, phenols, and saponins. Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) validated the presence of rutin in P. guajava extract. Spectral analyses, including UV and FTIR, identified functional groups such as O-H, C=C aromatics, and C-O, indicating alcohols, phenols, and esters. Anti-urolithiatic activity was assessed via the crystal aggregation assay. Methanol extract of P. guajava demonstrated significant inhibition (68.18%) comparable to the standard drug Cystone (72.72%). These findings suggest the potential of P. guajava and C. grandis extracts, especially methanolic fractions, as effective herbal remedies for urolithiasis. Further in-vivo and clinical studies are needed to confirm their therapeutic efficacy.

Keywords: *Coccinia grandis, Psidium guajava, Anti-urolithiatic, TLC/HPTLC, Phytochemical screening*

INTRODUCTION

Urolithiasis, or kidney stone formation, is a common urological disorder with high recurrence and limited effective synthetic treatments.[1] Traditional medicine has long used plants like *Psidium guajava* and *Coccinia grandis* for treating urinary disorders. The current research investigates the phytochemical profile and anti-urolithiatic potential of these plants. *C. grandis*, commonly known as Ivy gourd, and *P. guajava*, or guava, are well-documented in ethnomedicine for various pharmacological effects.[2-4]

P. guajava leaf extract (2 and 5 g/kg) reduced the occurrence of cough induced by capsaicin aerosol by 35 and 54%, respectively, within 10 min after injection of the extract.[5-6] The growth of *Staphylococcus aureus* and beta–streptococcus group A, is inhibited by aqueous, methanol and chloroform extract of dry *P. guajava* leaves. Therefore *P. guajava* leaf extract may be recommended for cough.[7-11] have reported the antibacterial effect of *P. guajava* leaves extracts and found that they inhibited the growth of the *S. aureus*. The methanolic plant leaf extracts of *P. guajava* and barks of this plant have antimicrobial activity. The organism inhibited is *Salmonella* species, *Bacillus* species, and the concentrations vary according to the organisms.[12-17] The microbicidial activity of *P. guajava* is attributable to guajaverine and to psydiolic acid. The active flavonoid compound guaijaverin extracted from the leaves of the same plant is reported to have high potential antiplaque activity.[18-20]

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This study focuses on the isolation, phytochemical profiling, and in-vitro evaluation of anti-urolithiatic activity using the crystal aggregation method. By identifying the bioactive compounds and assessing their effects, this work bridges traditional claims with scientific validation. Phytochemical analysis, TLC, HPTLC, and spectroscopic methods were used to confirm the presence of key secondary metabolites. Through this integrated approach, the study aims to highlight the herbal potential of these plants in managing urolithiasis, encouraging their development into safer, cost-effective alternatives to synthetic drugs.

MATERIALS AND METHODS

Collection:

The whole plant of *Pisidum Guajava* was collected in the month of Feb 2025 from Sangulwadi, Dist- Sindudurg, India.

Fresh leaves of *Coccinia grandis* and *Psidium guajava* were collected and shade-dried. The plant material was powdered and subjected to Soxhlet extraction using solvents of increasing polarity (n-hexane, chloroform, ethyl acetate, methanol). Extractive yields were calculated. Qualitative phytochemical screening was conducted using standard reagents to detect alkaloids, flavonoids, glycosides, terpenoids, and phenolics.

TLC and HPTLC were used to isolate and confirm phytocomponents; the mobile phase was optimized to Toluene:Ethyl Acetate:Methanol:Formic Acid (5:4:2:0.5). UV and FTIR spectroscopy analyzed functional groups of the isolated compounds. For anti-urolithiatic activity, a crystal aggregation assay was used. Methanol and pet ether extracts were incubated with calcium oxalate crystals at different time intervals (5–25 minutes), and absorbance was recorded to determine percent inhibition. The standard drug used was Cystone (5 mg/mL). All procedures were repeated in triplicate to ensure reproducibility.

Pharmacological Screening: - Anti- Urolithiatic Activity

Crystal Aggregation Assay

The rate of aggregation of the CaOx crystals was determined by the method of Atmani and Khan with slight modifications. The COM crystals were prepared by mixing both the solutions of calcium chloride and sodium oxalate at 50 m mol/L. The solutions were equilibrated to 60° C in water bath, cooled to 37ºC and kept overnight. Then the solution was centrifuged and evaporated at 37 oC. CaOx crystals were used at a final concentration of 0.8 mg/mL, buffered with 0.05 mol/L Tris- HCl and 0.15 mol/L sodium chloride at pH 6.5. The experiment was conducted at 37ºC in the presence and absence of Ethanol extract at concentration range of 100, 200, 400, 600, 800 and 1000 µg /mL. The absorbance was recorded at 620 nm for a period of one hour for every 10 minutes time interval. All samples were assayed in triplicate. Cystone was used as positive control. Percentage inhibition of aggregation rate was then calculated by comparing the turbidity slope of different concentrations of cystone /Ethanol extract with the turbidity slope of the control by the following formula.

$$[1-(Tsi / Tsc)] \times 100$$

Where Tsi was the turbidity slope of aggregation in the presence of inhibitor sample i.e, cystone/ plant extract (Ethanol) and Tsc was the turbidity slope of aggregation in the absence of inhibitor.

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RESULTS

Qualitative Chemical Analysis

Qualitative chemical analysis of phytoconstituents of the leaf extracts Coccinia grandis tabulated in Table 1.

Table no: 1 Phytochemical screening of Coccinia grandis

Sr.No	TEST	n-Hexane	Chloroform	Ethyl acetate	Methanol
1	Alkaloids	-	-	+	-
2	Carbohydrate	-	-	+	-
3	Glycosides	-	+	-	+
4	Phytosterol	-	-	-	+
5	Fixed oils and Fats	-	-	-	-
6	Tannins	-	-	-	-
7	Phenols	-	-		+
8	Proteins	-	-	+	-
9	Gums and Mucilages	-	-	-	-
10	Flavonoids	-	-	-	+
11	Terpenoids	-	-	-	+
12	Steroids	-	+	-	-
13	Saponins	-	-	+	+

Note: + ve indicates positive result, whereas – ve indicates negative resul

Qualitative preliminary phytochemical analysis of Coccinia grandis was performed initially with different chemical reagents to detect the nature of phytoconstituents and their presence in each extract. Chloroform extract showed the presence of glycosides and steroids. Ethyl acetate extract was found to contain, Alkaloids, carbohydrates, proteins and saponins, Methanolic extract showed the presence of glycosides, phytosterol, terpenoids, flavonoids, phenols and saponins.

Thin Layer Chromatography

Table no: 2 The TLC studies of methanol extracts of Coccinia grandis.

S.No	Name of the Extract	Solvent system	No of spots	R_f Values
1	Methanol (CGM)	Methanol : Ethyl acetate: Water (6:3:1)	03	0.27 0.33 0.88

Isolation and Purification

Table No. 3 Details of Ether TLC of Ether

Adsorbent :	Silica gel G
Thickness :	0.3 mm
Plate size :	10 x 20 cms
Activation temperature :	110 ± 1oC for one hour

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Mobile phase :	Ethyl acetate: Methanol (9:1)
Detecting reagent :.	Iodine vapours
Detecting apparatus :.	UV TLC Viewer chamber at 240 nm and 360 nm

Fig. No.1. TLC of isolated compound

Table No. 4- TLC analysis of *p guajava* leaves pet ether extract (Ethyl acetate: Methanol 9:1)

		R_f	Colour developed
Spot 1	**4.9cm/8cm**	**0.62**	brownish

Table No. 5- Details of Methanol TLC of Ether

Adsorbent :	Silica gel G
Thickness :	0.3 mm
Plate size :	10 x 20 cms
Activation temperature :	110 ± 1oC for one hour
Mobile phase :	Ethyl acetate: Methanol (9:1)
Detecting reagent :.	Iodine vapours
Detecting apparatus :.	UV TLC Viewer chamber at 240 nm and 360 nm

Fig. No. 2. - TLC of isolated compound

Table No. 6- TLC analysis of *p guajava* leaves methanol extract (Ethyl acetate: Methanol 9:1)

		R_f	Colour developed
Spot 1	**4.9cm/8cm**	**0.62**	brownish

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TLC plate was developed in the solvent system Ethyl acetate and methanol (9: 1) and single dark brownish smearing spot, was observed for ethanol extract at the same R_f value 0.62 with the help of iodine vapours.

UV SPECTRUM

Fraction In Pet Ether

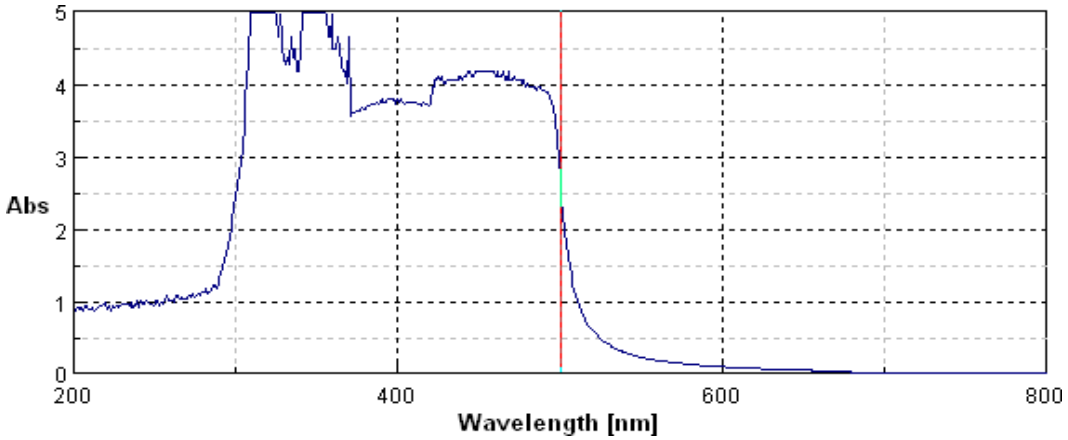

Fig. No. 3. UV spectra of isolated compound from p guajava was performed using methanol fraction in pet ether at λ= 321, 352, 413.

The UV spectra of isolated compound from *p guajava* was performed using methanol. The UV spectrum showed characteristic bands of fraction in pet ether at λ= 321, 352, 413 The UV spectra of standard showed one characteristic peak at λ= 321.

UV SPECTRUM:- Fraction In Pet Methanol

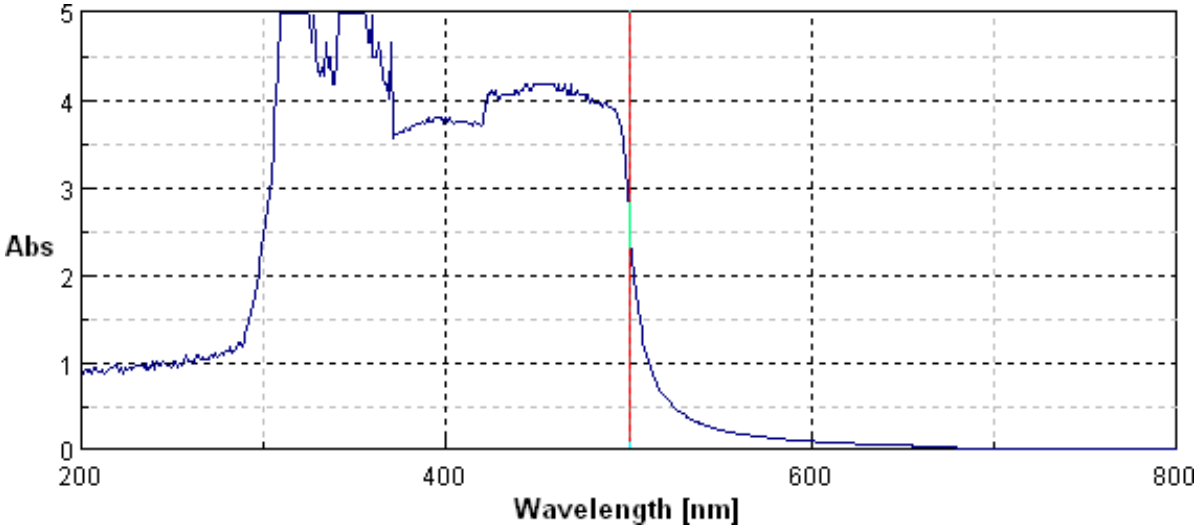

Fig. No. 4. The UV spectrum showed characteristic bands of fraction in methanol at λ= 321, 352, 413

The UV spectra of isolated compound from *p guajava* was performed using methanol. The UV spectrum showed characteristic bands of fraction in methanol at λ= 321, 352, 413 The UV spectra of standard showed one characteristic peak at λ= 321.

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INTERPRETATION OF ETHER

Interpretation of Ether

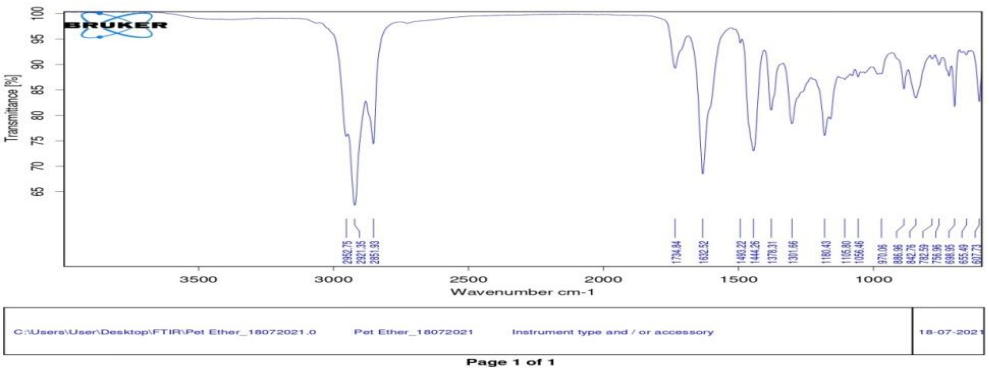

Fig No. 5- IR Spectra of Isolated Compound compound like Ether

Table No. 7- Interpretation of FTIR of Isolated compound like Ether

Peak rang	Functional group
3949 cm-1and 3772 cm-1	strong O-H bonding
3422 cm-1 and 2239 cm-1	O-H stretching
1636 cm-1, and 1564 cm-1	C=C aromatic

The spectrum shows peaks at 3949 cm-1and 3772 cm-1 alcoholic (strong O-H bonding) which indicates the presence of –O-H stretching of the carboxyl group. These peaks indicate the presence of bonded hydroxyl groups. Further, the peaks observed at 3422 cm-1(O-H stretching) 2239 cm-1 represents the stretching bonds of alkenes. The peak observed at 1636 cm-1, and 1564 cm-1 represent the C=C aromatic conjugates.

The sharp peak at 1415 $cm^{-}1$ and 1115 cm-1is assigned to O-H is stretching and C-O stretching (primary alcohol and ester). The peak observed at 659 cm-1, and 486 cm-1 represent the presence of different functional groups like Alkanes (-C-H- stretching).

Interpretation of Methanol

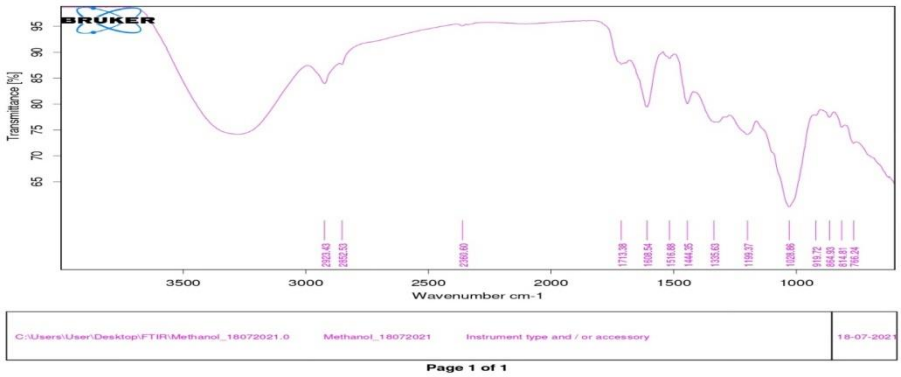

Fig No. 6- IR Spectra of Isolated Compound compound like Methanol

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Table No. 8.- Interpretation of FTIR of Isolated compound like Methanol

Peak rang	Functional group
3949 cm-1and 3772 cm-1	strong O-H bonding
3422 cm-1 and 2239 cm-1	O-H stretching
1636 cm-1, and 1564 cm-1	C=C aromatic
1415 cm⁻1 and 1115 cm	O-H is stretching and C-O stretching
659 cm-1, and 486 cm-1	-C-H- stretching

The spectrum shows peaks at 3949 cm-1and 3772 cm-1 alcoholic (strong O-H bonding) which indicates the presence of –O-H stretching of the carboxyl group. These peaks indicate the presence of bonded hydroxyl groups. Further, the peaks observed at 3422 cm-1(O-H stretching) 2239 cm-1 represents the stretching bonds of alkenes. The peak observed at 1636 cm-1, and 1564 cm-1 represent the C=C aromatic conjugates.

The sharp peak at 1415 cm⁻1 and 1115 cm-1is assigned to O-H is stretching and C-O stretching (primary alcohol and ester). The peak observed at 659 cm-1, and 486 cm-1 represent the presence of different functional groups like Alkanes (-C-H- stretching).

HPTLC Analysis

Table No. 9 Optimization of Mobile Phase for Rutin Separation

Trial	Mobile Phase Composition	Ratio	Observation
1	Toluene:Ethyl Acetate:Methanol:Formic Acid	4:2:4:0.5	Separation noted
2	Toluene:Ethyl Acetate:Methanol:Formic Acid	4:1:5:0.5	Improved separation
3	Toluene:Ethyl Acetate:Methanol	4:1:5	Baseline drift
4	Toluene:Ethyl Acetate:Methanol	4:2:4	Moderate resolution
5	Toluene:Ethyl Acetate:Methanol:Formic Acid	5:4:2:0.5	Final optimized condition

This table outlines the different mobile phase combinations tried to optimize Rutin separation via HPTLC. The final selected system was **Toluene:Ethyl Acetate:Methanol:Formic Acid (5:4:2:0.5)**, offering sharp, reproducible peaks and good resolution for both standard and sample.

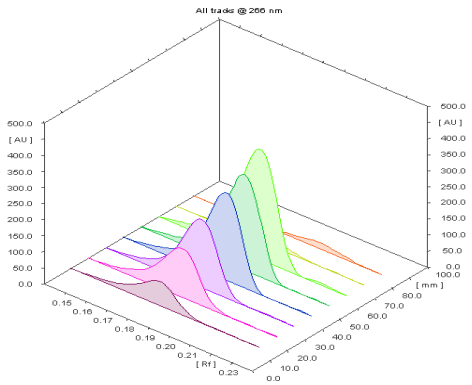

Fig No. 7 3D Spectral Overlay of Rutin at Various Wavelengths

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This figure displays the 3D spectra generated during wavelength optimization for rutin detection. The overlay confirmed 300 nm as the optimal detection wavelength due to maximum absorbance and sharpness of peak.

Table No. 10 Rutin present at various volumes

Track	Application position	Application volume	Vial	Sample ID	Active
1	15.0 mm	0.2 µl	1	RUTIN	☑
2	25.0 mm	0.4 µl	1	RUTIN	☑
3	35.0 mm	0.6 µl	1	RUTIN	☑
4	45.0 mm	0.8 µl	1	RUTIN	☑
5	55.0 mm	1.0 µl	1	RUTIN	☑
6	65.0 mm	1.2 µl	1	RUTIN	☑
7	75.0 mm	0.4 µl	2	EXTRACT E.A	☑
8	85.0 mm	0.6 µl	2	EXTRACT E.A	☑

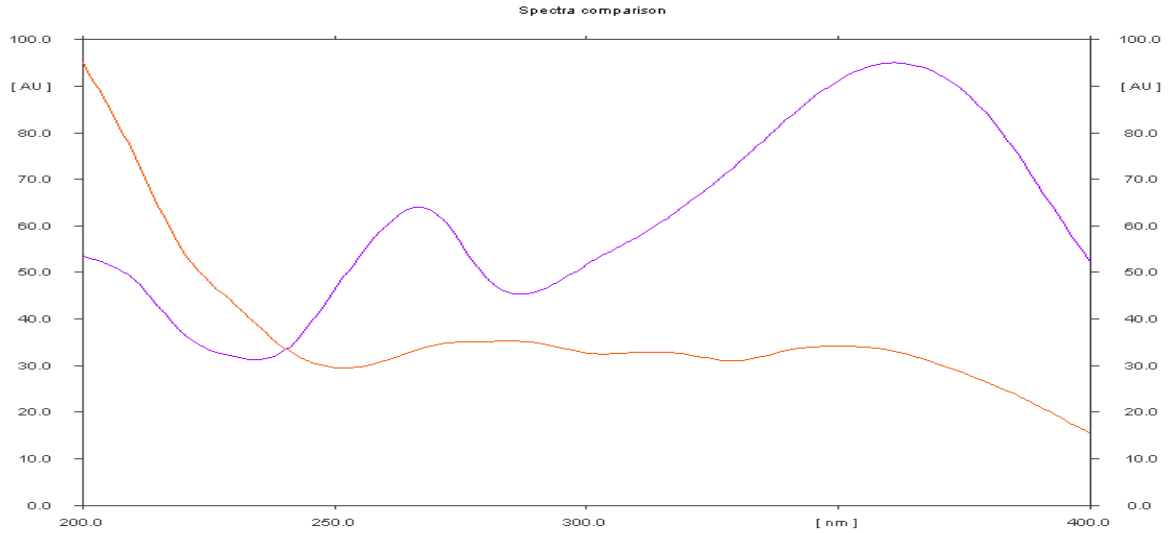

Fig No. 8 HPTLC Chromatogram of Standard Rutin + Extract (0.6 µl)

Table No. 11 HPTLC Chromatogram of Standard Rutin + Extract (0.6 µl)

Track	Rf	Assigned Substance	Max. Signal	Display
1	0.18	rutin	142 AU @ 362 nm	☐
2	0.18	rutin	283 AU @ 360 nm	☐
3	0.19	rutin	366 AU @ 361 nm	☑
4	0.19	rutin	458 AU @ 361 nm	☐
5	0.19	rutin	487 AU @ 362 nm	☐
6	0.19	rutin	541 AU @ 362 nm	☐
7	0.19	rutin	114 AU @ 200 nm	☐
8	0.20	rutin	181 AU @ 200 nm	☑

This figure shows the HPTLC profile of the standard Rutin mixed with *Psidium guajava* extract applied at 0.6 µl. Distinct peaks at the same Rf values confirm the presence of Rutin in the extract, indicating its phytochemical potential.

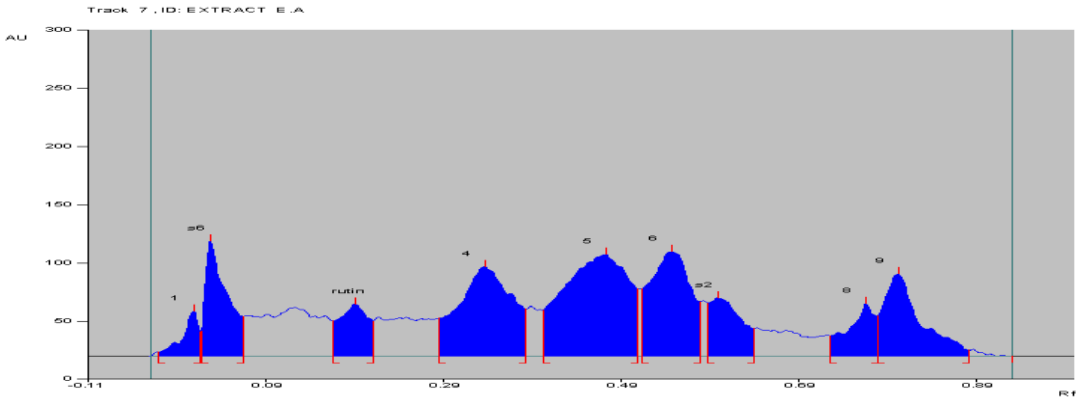

Fig No. 9 HPTLC Chromatogram of Rutin + Extract (0.4 µl)

Table No. 12 HPTLC Chromatogram of Rutin + Extract (0.4 µl)

Track 7, ID: EXTRACT E.A

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.03 Rf	3.4 AU	0.01 Rf	38.6 AU	6.40 %	0.02 Rf	21.0 AU	579.1 AU	2.70 %	unknown *
2	0.02 Rf	21.7 AU	0.03 Rf	98.7 AU	16.37 %	0.06 Rf	34.3 AU	2020.3 AU	9.41 %	s6
3	0.17 Rf	30.4 AU	0.19 Rf	44.9 AU	7.44 %	0.21 Rf	30.1 AU	1236.3 AU	5.76 %	rutin
4	0.28 Rf	32.6 AU	0.34 Rf	77.1 AU	12.78 %	0.38 Rf	40.4 AU	3834.7 AU	17.86 %	unknown *
5	0.40 Rf	39.4 AU	0.47 Rf	87.6 AU	14.52 %	0.51 Rf	58.0 AU	5361.9 AU	24.97 %	unknown *
6	0.51 Rf	58.1 AU	0.55 Rf	89.9 AU	14.92 %	0.58 Rf	46.7 AU	3421.1 AU	15.93 %	unknown *
7	0.59 Rf	45.4 AU	0.60 Rf	50.0 AU	8.29 %	0.64 Rf	24.0 AU	1490.5 AU	6.94 %	s2
8	0.72 Rf	17.6 AU	0.77 Rf	45.5 AU	7.54 %	0.78 Rf	34.4 AU	1106.1 AU	5.15 %	unknown *
9	0.78 Rf	34.6 AU	0.80 Rf	70.7 AU	11.73 %	0.88 Rf	5.2 AU	2420.2 AU	11.27 %	unknown *

This chromatogram shows a lower volume (0.4 µl) application of Rutin and extract. It confirms dose-dependent visibility of the Rutin peak and validates its consistency within the extract.

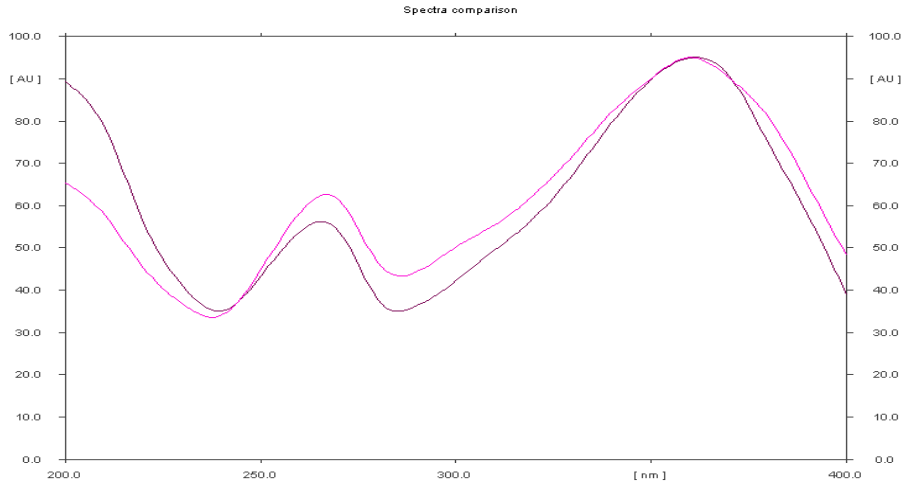

Fig No. 10 Overlay Spectra of Standard Rutin and Extract

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The overlay spectra clearly match the standard Rutin and the *Psidium guajava* extract at 300 nm, confirming phytochemical identity and successful bioanalytical profiling.

Anti- Urolithiatic Activity

Crystal Aggregation Assay

Table No. 13 Std drug Cystone tab conc.(5mg/ml)

Std drug Cystone tab conc.(5mg/ml)	O. D.	% inhibition
Control	0.22	
5min	0.10	54.54
10min	0.07	68.18
15 min	0.07	68.18
20min	0.07	68.18
25 min	0.06	72.72

Table No. 14 Pet ether extract Pisidum guujava 10mg/ml

Pet ether extract Pisidum guujava 10mg/ml	O.D	% inhibition
Control		
5min	0.18	18.18
10min	0.17	22.72
15 min	0.15	31.81
20min	0.11	50.00
25 min	0.09	59.09

Table No. 15 Methanol extract Pisidum guujava 10mg/ml

Methanol extract Pisidum guujava 10mg/ml	O.D	% inhibition
Control		
5min	0.13	40.90
10min	0.11	50.00
15 min	0.09	59.09
20min	0.08	63.63
25min	0.07	68.18

As compared to standard the methanol extract showed good activity

CONCLUSION

The study validates the traditional use of *Coccinia grandis* and *Psidium guajava* for their anti-urolithiatic properties through a combination of phytochemical, chromatographic, and biological methods. Methanol extracts showed the highest yield and were rich in bioactive compounds such as flavonoids, glycosides, and phenols.

TLC and HPTLC confirmed the presence of rutin in *P. guajava*, and UV and FTIR spectroscopy supported the presence of key functional groups. In vitro crystal aggregation assay demonstrated strong anti-urolithiatic activity, with *P. guajava* methanol extract achieving 68.18% inhibition—closely matching the standard drug Cystone. These results indicate that both plants, particularly *P. guajava*, possess significant potential for development into herbal formulations targeting urolithiasis.

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The integrated approach supports the scientific basis of their use in folk medicine and highlights the need for further in-vivo and clinical studies to confirm safety, efficacy, and dose standardization for potential therapeutic application.

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