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Research Article

PHYTOCHEMICAL STUDIES AND HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY ANALYSIS OF *CALAMUS ROTANG* LINN LEAF EXTRACTS

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ABSTRACT

Objective: The present study was aimed at phytochemical screening, quantification, and high-performance thin-layer chromatography (HPTLC) analysis of hexane, chloroform and ethanol leaf extracts of *Calamus rotang*.

Methods: Leaf extracts were prepared according to the polarity of the solvents, i.e., hexane, chloroform, and ethanol. Preliminary phytochemical screening involved the qualitative methods to detect the presence of alkaloids, phenols, flavonoids, saponins, steroids, etc. Quantitative estimation of alkaloids using boldine as standard, phenols using gallic acid as standard, and flavonoids using quercetin as standard were done. HPTLC analysis was done with all three extracts along with quercetin and rutin standards using mobile phase for flavonoids, i.e., 90:10 ratio of chloroform and methanol solvents.

Results: Phytochemical screening showed the presence of phenols, flavonoids, alkaloids, etc. Hence, quantification was done for these phytochemicals. Alkaloids were present significantly more in hexane leaf extract, i.e., 2.54±0.216mg boldine equivalents/g. Phenols were present significantly more in ethanolic leaf extract, i.e., 49.04±0.364 mg gallic acid equivalents)/g. Flavonoids were present in significant amount in ethanolic leaf extract, i.e., 458.85±5.74 mg quercetin equivalents/g. HPTLC analysis of hexane, chloroform, and ethanolic extracts showed the presence of flavonoids such as quercetin, rutin, and some unknown flavonoid compounds.

Conclusion: Ethanolic leaf extract showed a high amount of phenols and flavonoids. Hence, the extract can be further exploited further for *in vitro* and *in vivo* research work.

Keywords: Leaves of *Calamus rotang*, Phytochemical screening, High-performance thin-layer chromatography analysis.

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INTRODUCTION

Plant-derived substances have recently become of great interest due to their versatile applications. The medicinal value of plants lies in some chemical constituents which produce a definite physiologic action in the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds [1]. Secondary metabolites from plants have important biological and pharmacological activities such as antioxidative, antiallergic, anti-inflammatory, antibiotic, hypoglycemic, and anticarcinogenic activities [2].

Today, there is a growing interest in the chemical composition of plant-based medicines. Several bioactive constituents have been isolated and studied for pharmacological activities. During the past two decades, the pharmaceutical industry has made a massive investment in pharmacological and chemical researchers all over the world in an effort to discover much more potent drugs [3].

According to the WHO guidelines, a herbal product needs to be standardized with respect to safety measures before releasing it into the market [4]. Standardization is a process that ensures a predefined amount of quantity, quality and therapeutic effect of ingredients in each dose. High-performance thin-layer chromatography (HPTLC) is a popular method for the analysis of herbal medicines. HPTLC fingerprint profile is the best choice for standardization followed by determination of specific active phytoconstituents of botanical materials [5]. HPTLC and HPLC, both emerged efficient tools for phytochemical evaluation, and enable the analysis of several samples simultaneously. It reduces both time and cost of analysis [6].

Calamus rotang is a common growing shrub in Bangladesh which belongs to the family Arecaceae. It is a native plant of south-west Asia. The basal part of the plant grows vertically for 10 m and horizontally for about 200 m or more. *Calamus* rotang are bitter and astringent in taste. They have acrid, depurative and expectorant qualities. Leaves are useful in conditions such as vitiated kapha, cough, skin diseases and pruritus.

Plant collection

Leaves of the plant were collected from forest region of Srikakulam division, Pathapatnam range, Antharaba beat, Srikakulam District, Andhra Pradesh. The plant was authenticated by Dr. Padal, taxonomist in the Department of Botany, Andhra University. The plant has given the authentication number 22204 and herbarium was prepared.

Extraction procedure

The leaves of *C. rotang* were carefully separated, cleaned, shade dried, mechanically grinded, and coarsely powdered. The powder was subjected to solvent extraction with Hexane, chloroform, ethanol using reflux method. The extracts were concentrated using the rotary evaporator (Heidolph Rotary Evaporator), and the yield of the extract was noted with respect to the dried plant material.

METHODS

Phytochemical screening

Phytochemical tests were carried out for all the extracts as per the standard method [7].

Test for carbohydrates

To 2 ml of plant extract, 1 ml of Molisch's reagent and a few drops of concentrated sulfuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates.

Test for tannins

To 1 ml of plant extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for saponins

To 2 ml of plant extract, 2 ml of distilled water was added and shaken in a graduated cylinder for 15 min lengthwise. Formation of a 1cm layer of foam indicates the presence of saponins.

Test for flavonoids

To 2 ml of plant extract, 1 ml of 2 N sodium hydroxide was added. Appearance of yellow color indicates the presence of flavonoids.

Test for alkaloids

To 2 ml of plant extract, 2 ml of concentrated hydrochloric acid was added. Then, a few drops of Mayer's reagent were added. The presence of green color or white precipitate indicates the presence of alkaloids.

Test for quinones

To 1 ml of extract, 1 ml of concentrated sulfuric acid was added. Formation of red color indicates the presence of Quinones.

Test for glycosides

To 2 ml of plant extract, 3 ml of chloroform and few drops of 10% ammonia solution were added. Formation of pink color indicates the presence of glycosides.

Test for cardiac glycosides

To 0.5 ml of extract, 2 ml of glacial acetic acid and a few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulfuric acid. The formation of brown ring at the interface indicates presence of cardiac glycosides.

Test for terpenoids

To 0.5 ml of extract, 2 ml of chloroform was added and concentrated sulfuric acid was added carefully. Formation of red-brown color at the interface indicates the presence of terpenoids.

Test for triterpenoids

To 1.5 ml of extract, 1 ml of Liebermann–Burchard reagent (acetic anhydride+concentrated sulfuric acid) was added. Formation of blue-green color indicates the presence of triterpenoids.

Test for phenols

To 1 ml of the extract, 2 ml of distilled water followed by a few drops of 10% ferric chloride were added. Formation of blue or green color indicates the presence of phenols.

Test for coumarins

To 1 ml of extract, 1 ml of 10% NaOH was added. Formation of yellow color indicates the presence of coumarins.

Steroids and phytosteroids

To 1 ml of plant extract equal volume of chloroform was added and subjected with a few drops of concentrated sulfuric acid. Appearance of brown ring indicates the presence of steroids and appearance of the bluish-brown ring indicates the presence of phytosteroids.

Phlobatannins

To 1 ml of plant extract few drops of 2% HCl were added. Appearance of red color precipitate indicates the presence of phlobatannins.

Anthraquinones

To 1 ml of plant extract few drops of 10% ammonia solution were added, appearance pink color precipitate indicates the presence of anthraquinones.

Quantitative analysis of phytochemicals

Estimation of total phenols

The amount of total phenolics in extracts was determined according to the Folin–Ciocalteu procedure [8]. Samples (200µl) were introduced into test tubes. 1 ml of Folin–Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured. The total phenolic content was expressed as Gallic acid equivalents in milligrams per gram tissue as calculated from the standard gallic acid graph.

Estimation of total flavonoids

Total flavonoid content of the extracts was determined according to a modified colorimetric method [9]. Plant extract (1.0 ml) was mixed with 1 ml of distilled water and 75 µl of a 5% $NaNO_2$ solution. After 5 min, 75 µl of 10% $AlCl_3.H_2O$ solution was added. After 5 min, 0.5 ml of 1 M sodium hydroxide was added. The solution was mixed well and kept for 15 min. The increase in absorbance was measured at 510 nm using an ultraviolet (UV)-visible spectrophotometer. The total flavonoid content was calculated using standard quercetin calibration curve. The results were expressed as milligrams of quercetin equivalents per gram tissue.

Estimation of total alkaloids

Total alkaloid content was determined according to the method followed by Sree Vidya and Mehrotra [10]. A 5 ml amount of the extract/solution was taken, and the pH was maintained at 2–2.5 with dilute HCl. A 2 ml of Dragendorff's reagent was added to it and the precipitate formed was centrifuged. The centrifugate was checked for complete precipitation by adding DR (Dragendorff's reagent). After centrifugation, the centrifugate was decanted completely. The precipitate was further washed with alcohol. The filtrate was discarded, and the residue was then treated with 2 ml disodium sulfide solution. The brownish-black precipitate formed was then centrifuged. Completion of precipitation was checked by adding 2 drops of disodium sulfide. The residue was dissolved in 2 ml concentrated nitric acid, with warming if necessary. This solution was diluted to 10 ml in a standard flask with distilled water; 1 ml was then pipetted out, and 5 ml Thiourea solution was added to it. The absorbance was measured at 435 nm.

HPTLC analysis of C. rotang leaf extract

About 5 mg of leaf extract was dissolved in 5 ml of the respective solvents, and the solution was filtered with Whatman no.1 filter paper, and the solutions were used for HPTLC analysis as test solution. The samples (2 µl) were spotted in the form of bandwidth 5 mm with a Camag microliter syringe on precoated silica gel glass plate 60F–254 (10 cm×10 cm) with 250 µm thickness (E-Merck, Darmstadt, Germany) using a Camag Linomat IV (Switzerland). The plates were prewashed with methanol and activated at 60°C for 5 min before chromatography. The sample loaded plate was kept in TLC twin through developing chamber after saturated with solvent vapor with respective mobile phase and the plate was developed in the respective mobile phase up to 90 mm. The toulene:acetone:water (20:40:1) was employed as a mobile phase for secondary metabolites. Linear ascending development was carried out in (10 cm × 10 cm) twin trough glass chamber (Camag, Mutenz, Switzerland) saturated with the mobile phase and the chromatoplate was developed twice with the same mobile phase to get good resolution of phytochemical contents. The optimized chamber saturation time for mobile phase was 30 min at room temperature ([25±2]°C). The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in the photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images under UV light at 254 and 366 nm. The plate was photodocumented at UV 366 nm and daylight using photodocumentation (CAMAG REPROSTAR 3) chamber. Finally, the plate was fixed at scanner stage and scanning was done at 254 and 366 nm. Densitometric scanning was performed on Camag TLC Scanner III and operated by CATS software (V 3.15, Camag).

RESULTS

Phytochemical screening of the leaf extract of *C. rotang* with hexane, chloroform, and ethanol showed the presence of various secondary metabolites such as phenols, flavonoids, alkaloids, and steroids. (Table 1).

Quantitative phytochemical analysis

Estimation of phenols

Quantitative phytochemical analysis of phenols in the leaf extracts of hexane, chloroform, and ethanol (three concentrations) was done. Of the three leaf extracts, ethanolic leaf extract showed a high amount of phenols than chloroform and hexane extract at all the three concentrations tested (Table 2).

Estimation of flavonoids

Quantitative estimation of flavonoids was done with three solvent leaf extracts of *C. rotang*. Of the three leaf extracts, the ethanolic leaf extract showed a high amount of flavonoids followed by chloroform and hexane leaf extracts, respectively, at all the three concentrations tested (Table 3).

Estimation of alkaloids

Quantitative estimation of alkaloids in hexane, chloroform, and ethanolic leaf extracts of the plant was carried out. Of the three extracts, hexane leaf extract of the plant showed the highest amount of alkaloids followed by chloroform and ethanolic extracts at all the three concentrations tested (Table 4).

Table 1: Phytochemical screening of *Calamus rotang* leaf extract

Phytochemical test	Hexane extract	Chloroform extract	Ethanolic extract
Mayer's test	+ve	−ve	+ve
Wagner's test	+ve	+ve	−ve
$Fecl_3$ test	+ve	−ve	−ve
Lead acetate test	+ve	−ve	+ve
Sulfuric acid test	−ve	−ve	−ve
Steroids	+ve	−ve	+ve
Coumarins	+ve	−ve	−ve
Saponins	+ve	−ve	−ve
Tannins	−ve	−ve	−ve
Quinines	−ve	−ve	−ve
Glycosides	−ve	−ve	−ve
Phlobatannins	−ve	−ve	−ve
Cardiac glycosides	−ve	−ve	−ve
Oxalates	+ve	+ve	+ve
anthraquinones	−ve	−ve	−ve
Amino acids	+ve	+ve	+ve

+Present, −Absent. *C. rotang: Calamus rotang*

Table 2: Quantitative estimation of phenols

Phenol concentration in μg/ml				p
Concentration of leaf extracts	250 μg/ml	500 μg/ml	1000 μg/ml	
Hexane	3.15±0.44	6.41±0.11	12.75±0.48	0.045
Chloroform	6.11±0.09	12.01±0.55	24.62±3.35	
Ethanol	12.31±0.46	24.77±0.7	49.04±0.36	

*p≤0.05 indicates significant value. All the values represented are the averages of three observations. Data presented as the mean±standard deviation

Table 3: Quantitative estimation of flavonoids

Flavonoid concentration in μg/ml				p
Concentration of leaf extracts	250 μg/ml	500 μg/ml	1000 μg/ml	
Hexane	28.76±1.05	57.82±2.29	111.74±4.32	0.05
Chloroform	56.71±3.34	115.33±1.81	231.62±6.09	
Ethanol	114.98±1.85	230.35±3.55	458.85±5.74	

*p≤0.05 indicates significant value. All the values represented are the averages of three observations. Data presented as the mean±standard deviation

HPTLC analysis of *C. rotang*

Three dimensional display of HPTLC chromatogram of plant leaf solvent extracts and standards was read at 254 nm (Figs. 1 and 2) and 366 nm (Fig. 3). The chromatogram showed many peaks which indicates the

Fig. 1: *Calamus rotang* plant

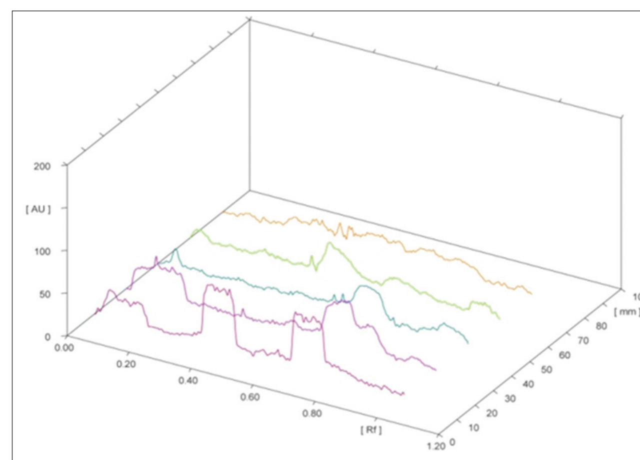

Fig. 2: 3-dimensional display for flavonoid profile of hexane, chloroform, ethanolic leaf extract along with their standards rutin and quercetin at ultraviolet 254 nm

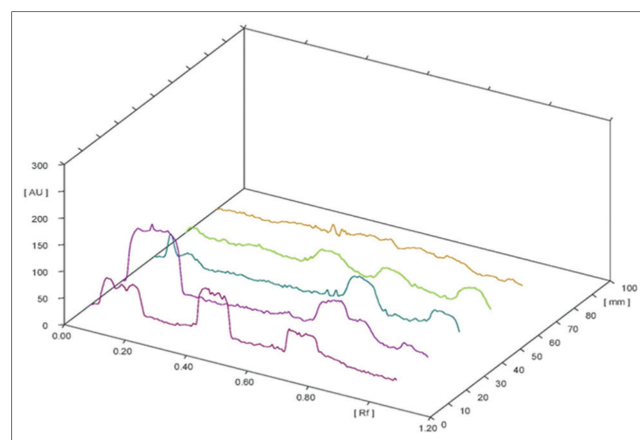

Fig. 3: 3-dimensional display for flavonoid profile of hexane, chloroform, ethanolic leaf extract along with their standards rutin and quercetin at and ultraviolet 366 nm

presence of flavonoid, quercetin, rutin, and other unknown flavonoids when separated in a flavonoid mobile phase chloroform-methanol solvents in 90:10 ratio).

HPTLC analysis of hexane leaf extract at 254 nm

HPTLC data of hexane leaf extract at UV 254 nm showed the presence of 7 peaks (Fig. 4) in which max retention factor (Rf) value, i.e., 0.38 matched with the max Rf value of quercetin. This indicates that the compound may be quercetin (Table 5).

HPTLC analysis of chloroform leaf extract at UV 254 nm

HPTLC chromatogram of chloroform leaf extract at 254 nm showed the presence of 5 peaks (Fig. 5) out of which max Rf value, i.e., 0.67 was matched with Max Rf value of rutin standard. This indicates that the compound may be rutin as shown in Table 6.

Table 4: Quantitative estimation of alkaloids

Alkaloid concentration in μg/ml				p
Concentration of leaf extracts	**250 μg/ml**	**500 μg/ml**	**1000 μg/ml**	
Hexane	0.62±0.10	1.38±0.096	2.54±0.216	0.05
Chloroform	0.34±0.06	0.67±0.08	1.41±0.04	
Ethanol	0.17±0.08	0.28±0.04	0.57±0.098	

*p≤0.05 indicates significant value. All the values represented are the averages of three observations. Data presented as the mean±standard deviation

HPTLC data of ethanolic leaf extract at UV 254 nm

HPTLC data of ethanolic leaf extract at 254 nm showed the presence of 5 peaks (Fig. 6) out of which one Max Rf value, i.e., 0.67 was matched with the Max Rf value of rutin that indicates the compound may be rutin as shown in Table 7.

HPTLC analysis of hexane extract at UV 366 nm

HPTLC data of hexane extract at 366 nm showed the presence of 6 peaks (Fig. 7) out of which one peak Max Rf value, i.e., 0.38 was matched with the Max Rf value of quercetin standard that indicates that the compound may be quercetin as shown in Table 8.

HPTLC analysis of chloroform leaf extract at UV 366 nm

HPTLC data of chloroform leaf extract at UV 366 nm showed the presence of 7 peaks (Fig. 8) in which no Max Rf value was matched with either quercetin or rutin standards (Table 9).

HPTLC analysis of ethanolic leaf extract at UV 366 nm

HPTLC data of ethanolic leaf extract at UV 366 nm showed the presence of 6 peaks (Fig. 9) in which one of the Max Rf value, i.e., 0.57 was matched with Max Rf value of quercetin indicates that the compound may be quercetin as shown in Table 10.

Table 5: HPTLC data of hexane leaf extract at UV 254 nm

Peak	Start Rf	Start height	Max Rf	Max height	Max%	End Rf	End height	Area	Area%	Assigned substance
1	0.02	4.5	0.05	33.0	9.67	0.08	23.2	905.4	12.16	Unknown
2	0.12	22.4	0.14	27.0	7.93	0.18	1.7	721.2	9.69	Unknown
3	0.34	10.3	0.38	70.71	20.73	0.39	68.1	1906.5	25.60	Quercetin
4	0.43	63.0	0.44	70.6	20.70	0.48	4.9	1093.0	14.68	Unknown
5	0.51	0.0	0.55	10.5	3.07	0.56	5.1	200.8	2.70	Unknown
6	0.63	6.0	0.65	64.5	18.93	0.66	63.1	817.1	10.97	Unknown
7	0.70	57.7	0.71	64.6	18.97	0.75	15.2	1802.1	24.20	Unknown

Rf: Retention factor, HPTLC: High-performance thin-layer chromatography, UV: Ultraviolet

Table 6: HPTLC data of chloroform leaf extract at UV 254 nm

Peak	Start Rf	Start height	Max Rf	Max height	Max%	End Rf	End height	Area	Area%	Assigned substance
1	0.01	0.0	0.05	30.3	14.13	0.07	29.0	797.6	14.42	Unknown
2	0.07	29.4	0.10	46.9	21.86	0.11	34.1	1022.2	18.48	Unknown
3	0.17	32.9	0.17	34.3	16.00	0.20	4.3	472.2	8.53	Unknown
4	0.62	10.6	0.67	49.1	22.88	0.68	47.0	1296.2	23.43	Rutin
5	0.68	47.1	0.72	53.9	25.13	0.75	28.3	1944.3	35.14	Unknown

Rf: Retention factor, HPTLC: High-performance thin-layer chromatography, UV: Ultraviolet

Table 7: HPTLC data of ethanolic leaf extract at 254 nm

Peak	Start Rf	Start height	Max Rf	Max height	Max%	End Rf	End height	Area	Area%	Assigned substance
1	0.02	1.4	0.06	21.9	18.71	0.08	3.4	394.0	10.61	Unknown
2	0.55	7.3	0.57	17.6	15.07	0.58	10.3	258.5	6.96	Unknown
3	0.59	10.4	0.60	21.0	17.94	0.60	11.7	181.4	4.89	Unknown
4	0.61	12.1	0.67	37.9	32.37	0.74	10.0	2288.8	61.65	Rutin
5	0.90	7.9	0.92	18.6	15.91	0.96	11.9	589.9	15.89	Unknown

Rf: Retention factor, HPTLC: High-performance thin-layer chromatography, UV: Ultraviolet

Table 8: HPTLC data of hexane extract at UV 366 nm

Peak	Start Rf	Start height	Max Rf	Max height	Max%	End Rf	End height	Area	Area%	Assigned substance
1	0.02	3.3	0.05	56.9	16.82	0.08	40.6	1560.4	18.05	Unknown
2	0.12	41.6	0.14	56.5	16.68	0.18	3.3	1588.5	18.37	Unknown
3	0.33	6.2	0.38	84.7	25.00	0.40	74.9	2879.0	33.30	Quercetin
4	0.43	71.2	0.44	78.4	23.16	0.47	2.4	1099.5	12.72	Unknown
5	0.63	2.1	0.65	47.1	13.91	0.68	40.8	1040.0	12.03	Unknown
6	0.77	12.5	0.78	15.1	4.45	0.84	3.4	478.4	5.53	Unknown

Rf: Retention factor, HPTLC: High-performance thin-layer chromatography, UV: Ultraviolet

Table 9: HPTLC data of chloroform leaf extract at UV 366 nm

Peak	Start Rf	Start height	Max Rf	Max height	Max%	End Rf	End height	Area	Area%	Assigned substance
1	0.01	0.7	0.05	104.7	21.91	0.07	101.9	2909.5	18.81	Unknown
2	0.07	102.2	0.10	119.0	24.92	0.11	108.6	2718.1	17.57	Unknown
3	0.13	109.4	0.15	113.7	23.81	0.21	2.5	3852.6	24.90	Unknown
4	0.49	9.0	0.54	19.5	4.08	0.56	12.0	581.1	3.76	Unknown
5	0.62	15.1	0.72	65.1	13.63	0.75	34.7	3983.8	25.75	Unknown
6	0.77	34.4	0.78	39.2	8.21	0.85	4.1	1207.2	7.80	Unknown
7	0.90	0.8	0.92	16.3	3.42	0.93	12.9	218.0	1.41	Unknown

Rf: Retention factor, HPTLC: High-performance thin-layer chromatography, UV: Ultraviolet

Table 10: HPTLC data of ethanolic leaf extract at UV 366 nm

Peak	Start Rf	Start height	Max Rf	Max height	Max%	End Rf	End height	Area	Area%	Assigned substance
1	0.03	3.1	0.05	49.5	23.87	0.08	11.6	816.7	11.4	Unknown
2	0.08	11.7	0.11	22.1	10.68	0.17	0.8	779.7	10.89	Unknown
3	0.54	8.5	0.57	19.3	9.31	0.58	9.6	256.0	3.58	Quercetin
4	0.58	10.3	0.60	22.9	11.04	0.60	12.8	235.8	3.29	Unknown
5	0.61	16.1	0.67	60.3	29.07	0.75	6.5	3624.7	50.65	Unknown
6	0.90	11.7	0.94	33.2	16.03	0.98	28.4	1443.9	20.18	Unknown

Rf: Retention factor, HPTLC: High-performance thin-layer chromatography, UV: Ultraviolet

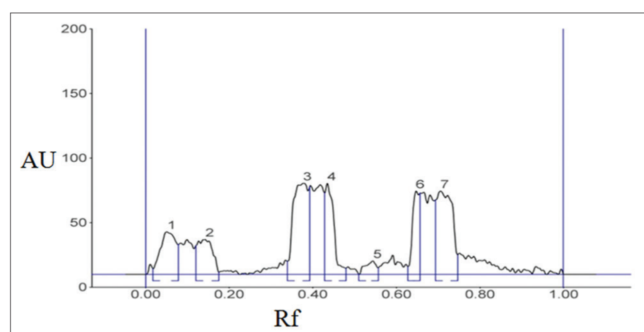

Fig. 4: High-performance thin-layer chromatography (HPTLC) chromatogram of hexane leaf extract at ultraviolet (UV) 254 nm. HPTLC chromatogram of flavonoids in hexane leaf extract of *Calamus rotang* at UV 254 nm

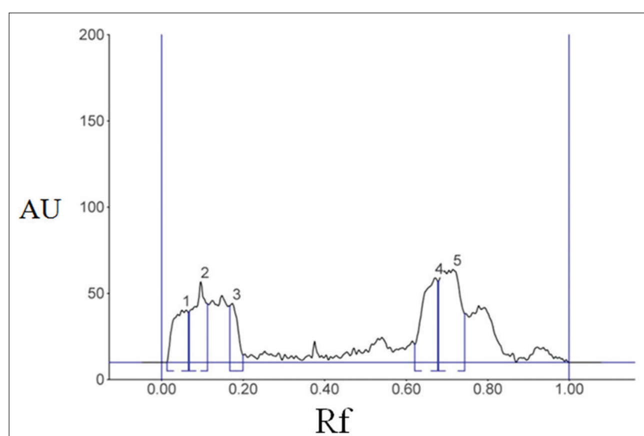

Fig. 5: High-performance thin-layer chromatography (HPTLC) chromatogram of chloroform leaf extract at ultraviolet (UV) 254 nm. HPTLC chromatogram of flavonoids in chloroform leaf extract of *Calamus rotang* at UV 254 nm

HPTLC fingerprint profile of leaf extracts

HPTLC fingerprint profile of different solvent extracts of the leaf of *C. rotang* along with standards at UV 254 nm and UV 366 nm was shown in Figs. 10 and 11.

DISCUSSION

Preliminary qualitative screening of *C. rotang* leaf extracts showed the presence of phenols, flavonoids, and alkaloids, etc. The presence of these secondary metabolites suggests that the plant might be of medicinal importance. As reported in earlier studies, flavonoids and phenolic compounds exhibited a wide range of biological activities such as antioxidant and lipid peroxidation inhibition properties [11,12]. The presence of phenolic compounds provides pharmacological activities such as anticancer [13], antioxidant [14], antimicrobial [15], and anti-inflammatory activities [16]. Alkaloids were also detected in the leaf extracts of *C. rotang*. These alkaloids are toxic against the cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer [17].

In this study, the total phenolic content (49.04 mg/g) of ethanolic extract of leaves of *C. rotang* was found to be more than methanolic leaf extract (14.53 mg/g) [18]. Phenolic content in methanolic leaf extract of *Acorus calamus* [19] was reported as 34.4 mg/g, and methanolic leaf extract of *Phoenix paludosa* [19] was reported as 16 mg/g. The total phenol content (49.04 mg/g) in ethanol leaf extract of *C. rotang* was <70% ethanolic extract of leaves of *Acorus calamus* [19] (66.25 mg/g). Flavonoid content was also high in ethanolic leaf extract of *C. rotang* (458.85 mg/g) when compared with methanolic leaf extract of *C. rotang* (2.68 mg/g) [18]. The flavonoid content in methanolic extract of leaves of *Acorus calamus* was 22 mg/g, whereas in 70% ethanolic extract of *A. calamus* it was reported to be 1.48 mg/g [19], and in methanolic extract of leaf of *Phoenix paludosa* was 9 mg/g [20]. The total phenol and flavonoid contents in ethanolic leaf extract of *C. rotang* (49.04 mg/g and 458.85 mg/g) were found to be more than the total phenol and flavonoid contents in methanolic leaf extract of *Colocasia esculenta* (27 mg/g and 11 mg/g), respectively [21]. The total phenolic and flavonoid contents were more in ethanolic extract of *C. rotang* than in hexane and chloroform extracts. Alkaloids were present in relatively less amount. The alkaloid content was high in hexane extract than in chloroform and ethanol extracts. From quantitative studies, ethanolic extract showed the presence of good amount of phenol and flavonoid content.

HPTLC fingerprint profile showed the presence of flavonoids in the three extracts using quercetin and rutin as standards. Some of the Max Rf values of the extracts were matched with the Max Rf values of the standards.

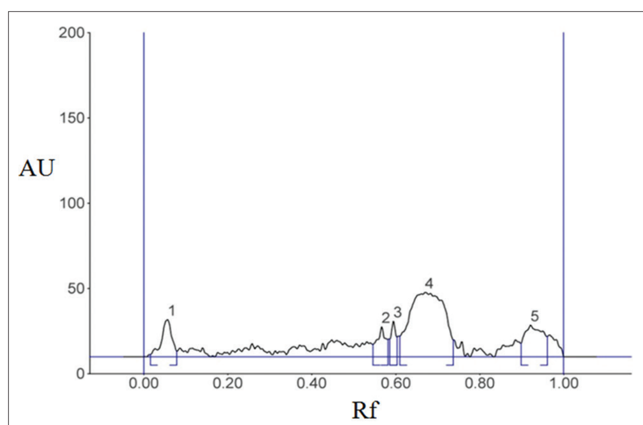

Fig. 6: High-performance thin-layer chromatography (HPTLC) chromatogram of ethanolic leaf extract at ultraviolet (UV) 254 nm. HPTLC chromatogram of flavonoids in ethanolic leaf extract of *Calamus rotang* at UV 254 nm

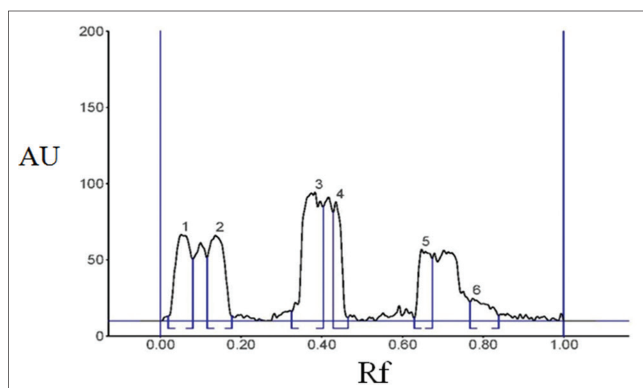

Fig. 7: High-performance thin layer chromatography (HPTLC) chromatogram of hexane leaf extract at ultraviolet (UV) 366 nm. HPTLC chromatogram of flavonoids in hexane leaf extract of *Calamus rotang* at UV 366 nm

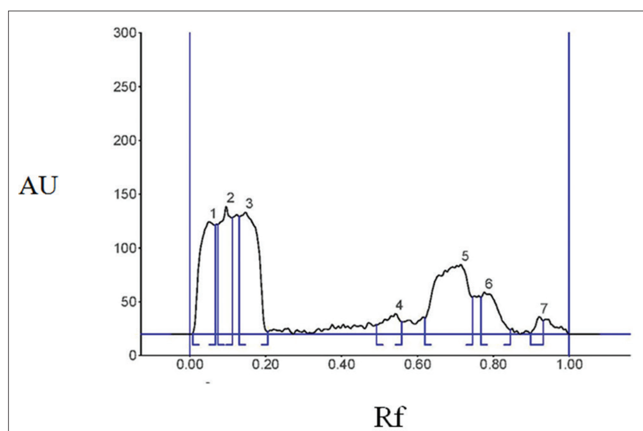

Fig. 8: High-performance thin-layer chromatography (HPTLC) chromatogram of chloroform leaf extract at ultraviolet (UV) 366 nm. HPTLC chromatogram of flavonoids in chloroform leaf extract of *Calamus rotang* at UV 366 nm

CONCLUSION

The results of the phytochemical analysis showed that the ethanolic extract contained a considerable amount of phenols and flavonoids.

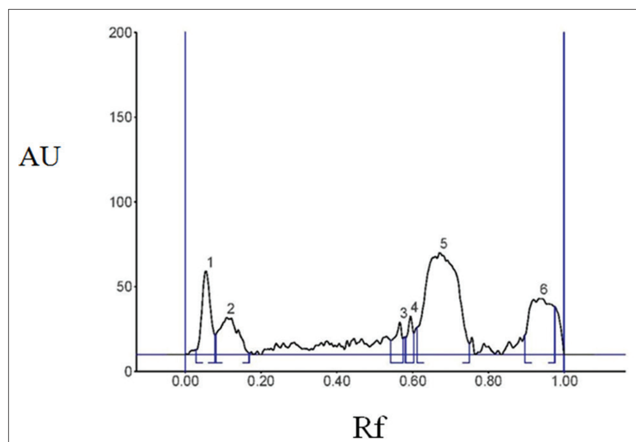

Fig. 9: High-performance thin-layer chromatography (HPTLC) chromatogram of ethanol leaf extract at ultraviolet (UV) 366 nm. HPTLC chromatogram of flavonoids in ethanolic leaf extract of *Calamus rotang* at UV 366 nm

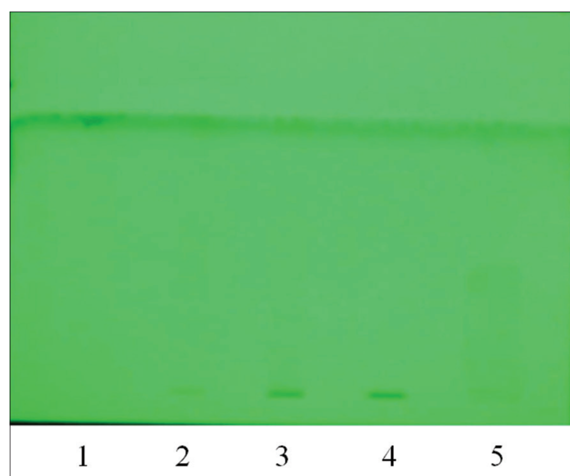

Fig. 10: High-performance thin-layer chromatography fingerprint profile at ultraviolet 254 nm. Track 1 - Hexane extract, Track 2 - Chloroform extract, Track 3 - Ethanolic extract, Track 4 - Rutin, Track 5 - Quercetin

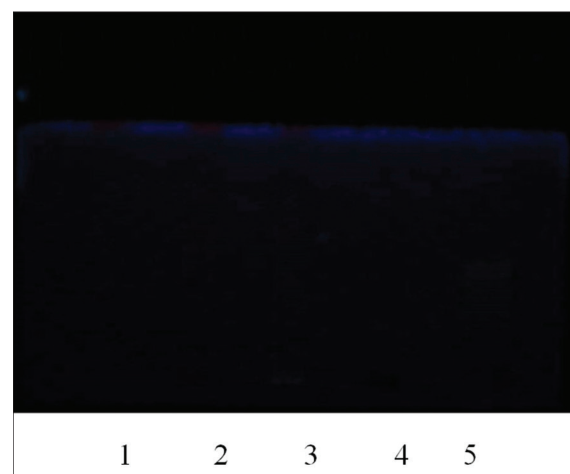

Fig. 11: High-performance thin-layer chromatography fingerprint profile at ultraviolet 366 nm. Track 1 - Hexane extract, Track 2 - Chloroform extract, Track 3 - Ethanolic extract, Track 4 - Rutin, Track 5 - Quercetin

Thus, the plant studied can be seen as a potential source of the new useful drug.

HPTLC profile is a rapid, precise and powerful procedure for analyzing the presence or absence of chemical constituents. In this study, HPTLC analysis of the three extracts of the plant showed the presence of flavonoids.

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