IJBPAS, September, 2025, 14(9): 5127-5137

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PHYSICO- CHEMICAL AND PHYTOCHEMICAL ANALYSIS OF *Cosmostigma cordatum* (Poir.) M. R. Almeida

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Received 25th April 2024; Revised 28th Aug. 2024; Accepted 29th Sept. 2024; Available online 1st Sept. 2025

https://doi.org/10.31032/IJBPAS/2025/14.9.9434

ABSTRACT

Each plant has a specific quality and can be used to treat a disease. *Cosmostigma cordatum* is a climbing shrub belonging to the family Apocynaceae. It is a medicinal plant commonly found in India, Sri Lanka, and other parts of Southeast Asia. The plant has been used in traditional medicine for various therapeutic purposes. Pharmacognostic and phytochemical evaluations are necessary for drug authentication and for prediction and confirmation of pharmacological activities of any plant part. The present work deals with a preliminary physico- chemical and phytochemical analysis and HPTLC profiling of *C. cordatum*. This preliminary investigation shows presence of various secondary metabolites in the leaves of *C. cordatum* having significant medicinal properties. Hence conservation and exploration of this plant should necessary for future benefits.

Keywords: *Cosmostigma cordatum*, Pharmacognosy, Phytochemicals, HPTLC

INTRODUCTION

Herbal medicine is the oldest form of healing and treating various diseases. They can play a vital role in the discovery and development of new drugs. *Cosmostigma cordatum* is a species belonging to the family Apocynaceae, subfamily Asclepiadoideae. Apocynaceae family has been reported to be rich in bioactive phytochemicals like flavonoids and phenols etc. **[1]**. This plant is distributed in moist

deciduous forests **[2]**. And it is endemic to Western ghats **[3]**. It is said to be distributed in the South Indian states of Kerala, Tamilnadu and Karnataka and also in southern Maharashtra. It is a twining glabrous shrub. Leaves opposite, membranous, ovate or cordate. Flowers small, in axillary or lateral racemose cymes with rather long peduncles. *C. cordatum* is a climber with small greenish flowers with brown dots **[4]**. The flowers of *C. cordatum* is inflorescence of racemose to corymbose type; peduncle is between the petioles of leaf pair (interpetiolaris); peduncle is brightly green **[5]**.

The acceptance of herbal medicines has considerably increased during the past few decades. 80% of the people round the world use them as a part of primary healthcare. But scientific data regarding most of these plants are lacking. The species *C. cordatum* is also an important plant used in Indian traditional medicine. The leaves of this plants are used to cure ulcerous sores. Root bark is given internally as an efficient cholagogue in dyspepsia due to torpidity of the liver. The glycosides extracted from the seeds are active on heart failure. The trunk bark or the leaves are occasionally used as a purgative. A preliminary investigation of the roots had revealed the presence of some crystalline fatty acid, a glucosidal acid resin, a sugar similar to dextrin and compound suspected to be an alkaloid **[6]**. But no such investigation had ever reported on *C. cordatum*. The physico- chemical and phytochemical investigation of the secondary metabolites of leaves of *C. cordatum* is significant in these perspectives.

MATERIALS AND METHODS

Collection and preparation of plant material

The whole plant of *Cosmostigma cordatum* was collected from Konni, Pathanamthitta, Pramadom, Pandalam, Kollam, Kariavattom. The samples were authenticated for its botanical identity at the Department of Botany, University of Kerala, Kariavattom campus, Thiruvananthapuram (BOT/KUBH/167/24). The leaves of *C. cordatum* were dried in shade and stored in air tight container.

Preparation and yield of extract [7]

About 2 g of the powdered plant material was subjected to extraction by Soxhlet apparatus using 100 ml each of the three solvents, methanol, ethyl acetate and distilled water. The extract was concentrated under reduced pressure and preserved in refrigerator until further use. The percentage of the crude extract was determined using the following equation.

$$\text{Percentage yield (\%)} = \frac{\text{Weight of the crude extract}}{\text{Weight of the sample}} \times 100$$

Preliminary physicochemical analysis

Preliminary physicochemical analysis of leaves of *C. cordatum* was done

as per standard procedures. The parameters such as LOD at 105^0C, total ash, acid insoluble ash, water soluble ash, sulphated ash, pH of water extract (4% aqueous solution), alcohol soluble extractives, water soluble extractives, volatile oil, swelling index ml/g and foaming index were determined **[7, 8, 9]**.

Qualitative analysis of phytochemicals [8]

Preliminary phytochemical analysis was done with Methanol, ethyl acetate, and aqueous extract of *C. cordatum* leaf powder for detection of presence of various secondary metabolites as, saponins, tannins, terpenoids, phenols, steroids, quinones, glycosides, alkaloids and flavonoids.

HPTLC

Developing solvent system

A number of solvent systems were tried and a system which gave the maximum resolution was selected as the solvent system for the extract. The optimum separations of constituents were achieved using the solvent system: -

Sample application

The extracts were applied as different tracks of different concentrations of width 8 mm each on silica gel 60 F_{254} pre-coated aluminium sheets through CAMAG micro litre syringe using Automatic TLC Sampler 4 (ATS4).

Development of chromatogram

After sample application the plate was introduced vertically in a CAMAG developing chamber (10 cm × 10 cm) pre-saturated with the mobile phase selected.

Documentation

The developed chromatogram was air dried to evaporate solvents from the plate and the plate was kept in CAMAG Vizualizer and the images were captured under UV light at 254 nm and 366 nm.

Densitometry

The plate was scanned at 254 nm and 366 nm using TLC Scanner 4 and the finger print profiles were documented. The R_f values and finger print data were recorded with win CATS software associated with the scanner.

Post chromatographic derivatisation

The plate was derivatised using vanillin-sulphuric acid reagent, heated at 105^0 C by placing on CAMAG TLC plate heater till the colour of the bands appeared. Then the plate was visualized under white light and the chromatograms were documented. The plate was scanned at 575 nm and the R_f values and finger print data were documented.

RESULTS

Phytochemical screening

Yield of extract

The methanol, ethyl acetate and aqueous extracts was prepared by Soxhlet extraction. The yield of the methanol, ethyl acetate and aquous extracts was 63%, 42% and 18%.

Results of preliminary physicochemical evaluation of leaves of *Cosmostigma cordatum*

Table 1: Preliminary physicochemical evaluation of leaves of *C. cordatum*

Sl. No.	Parameters	Observations
1.	LOD at 105^0C	5.23%
2.	Total Ash	9.7%
3.	Acid insoluble ash	4.72%
4.	Water soluble ash	11.27%
5.	Sulphated ash	3.90%
6.	pH of water extract (4% aqueous solution)	6.13
7.	Alcohol soluble extractives	28.80%
8.	Water soluble extractives	11.91%
9.	Volatile oil	Nil
10.	Swelling index ml/g	7.5
11.	Foaming index	>100

Results of preliminary phytochemical analysis of leaves of *Cosmostigma cordatum*

Table 2: Preliminary phytochemical analysis of leaves of *C. cordatum*

Sl No.	Phytochemicals	Methanol	Ethyl acetate	Distilled Water
1	Saponins	+	+	+
2	Tannins	+	+	+
3	Terpenoids	+	+	+
4	Phenols	+	+	-
5	Steroids	+	+	-
6	Quinones	+	+	-
7	Glycosides	+	+	+
8	Alkaloids	+	+	+
9	Flavanoids	+	+	-

+ Presence, - Absence

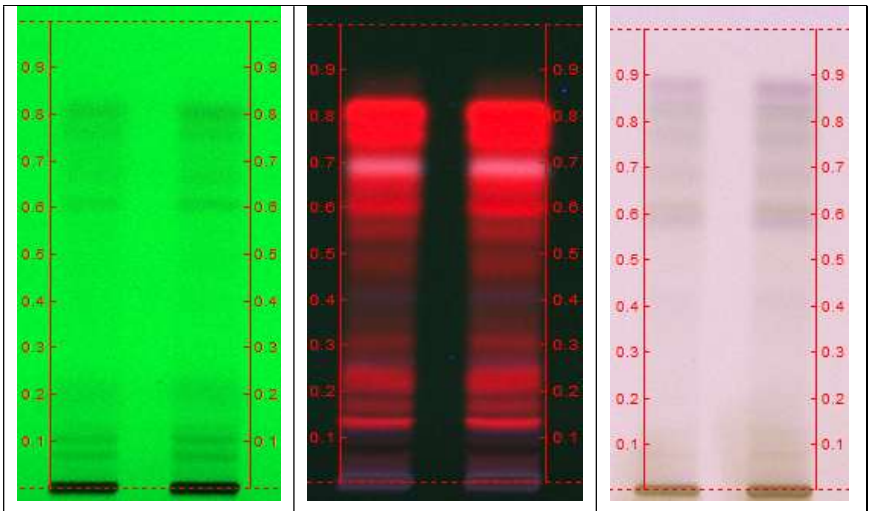
Results of HPTLC profiling

HPTLC analysis of hydromethanolic extract of leaves of *C. cordatum* was done with the solvent system Toluene: ethyl acetate: methanol in the ratio 8:3:1.

Alcohol Extract

Solvent system: Toluene: Ethyl acetate: Formic acid (5:3:0.1)

Track 1-5µl, Track 2- 7µl



Under UV short	Under UV long	Under white light

Figure 1: HPTLC profiling under (a) UV short (b) UV long (c) White light

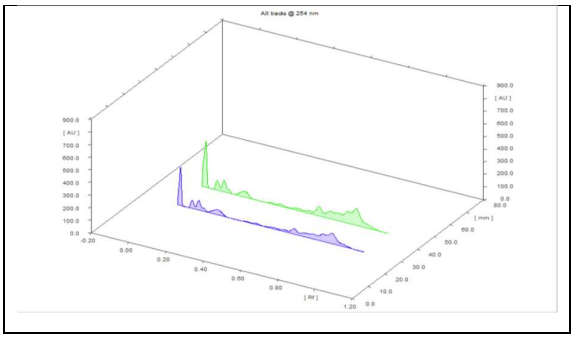
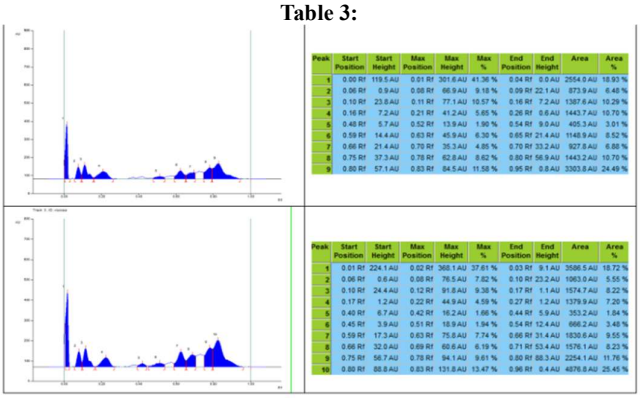


Figure 2: Graphical representation under 254 nm

Table 3:



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	119.5 AU	0.01 Rf	301.6 AU	41.36 %	0.04 Rf	0.0 AU	2554.0 AU	18.93 %
2	0.06 Rf	0.9 AU	0.08 Rf	66.9 AU	9.18 %	0.09 Rf	22.1 AU	873.9 AU	6.48 %
3	0.10 Rf	23.8 AU	0.11 Rf	77.1 AU	10.57 %	0.16 Rf	7.2 AU	1387.6 AU	10.29 %
4	0.16 Rf	7.2 AU	0.21 Rf	41.2 AU	5.65 %	0.26 Rf	0.6 AU	1443.7 AU	10.70 %
5	0.48 Rf	5.7 AU	0.52 Rf	13.9 AU	1.90 %	0.54 Rf	9.0 AU	405.3 AU	3.01 %
6	0.59 Rf	14.4 AU	0.63 Rf	45.9 AU	6.30 %	0.65 Rf	21.4 AU	1148.9 AU	8.52 %
7	0.66 Rf	21.4 AU	0.70 Rf	35.3 AU	4.85 %	0.70 Rf	33.2 AU	927.8 AU	6.88 %
8	0.75 Rf	37.3 AU	0.78 Rf	62.8 AU	8.62 %	0.80 Rf	56.9 AU	1443.2 AU	10.70 %
9	0.80 Rf	57.1 AU	0.83 Rf	84.5 AU	11.58 %	0.95 Rf	0.8 AU	3303.8 AU	24.49 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	224.1 AU	0.02 Rf	368.1 AU	37.61 %	0.03 Rf	9.1 AU	3586.5 AU	18.72 %
2	0.06 Rf	0.6 AU	0.08 Rf	76.5 AU	7.82 %	0.10 Rf	23.2 AU	1063.0 AU	5.55 %
3	0.10 Rf	24.4 AU	0.12 Rf	91.8 AU	9.38 %	0.17 Rf	1.1 AU	1574.7 AU	8.22 %
4	0.17 Rf	1.2 AU	0.22 Rf	44.9 AU	4.59 %	0.27 Rf	1.2 AU	1379.9 AU	7.20 %
5	0.40 Rf	6.7 AU	0.42 Rf	16.2 AU	1.66 %	0.44 Rf	5.9 AU	353.2 AU	1.84 %
6	0.45 Rf	3.9 AU	0.51 Rf	18.9 AU	1.94 %	0.54 Rf	12.4 AU	666.2 AU	3.48 %
7	0.59 Rf	17.3 AU	0.63 Rf	75.6 AU	7.74 %	0.66 Rf	31.4 AU	1830.6 AU	9.55 %
8	0.66 Rf	32.0 AU	0.69 Rf	60.6 AU	6.19 %	0.71 Rf	53.4 AU	1576.1 AU	8.23 %
9	0.75 Rf	56.7 AU	0.78 Rf	94.1 AU	9.61 %	0.80 Rf	88.3 AU	2254.1 AU	11.76 %
10	0.80 Rf	88.8 AU	0.83 Rf	131.8 AU	13.47 %	0.96 Rf	0.4 AU	4876.8 AU	25.45 %

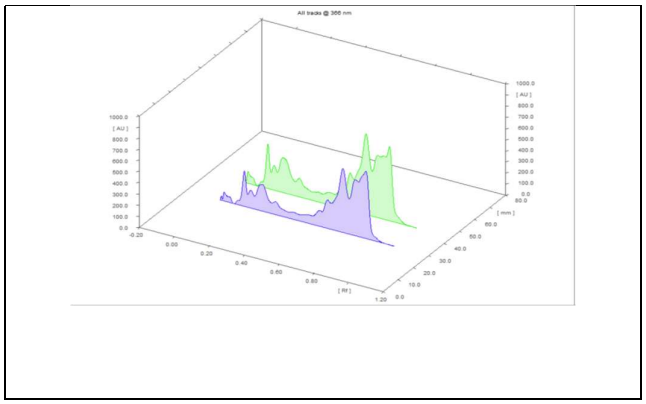
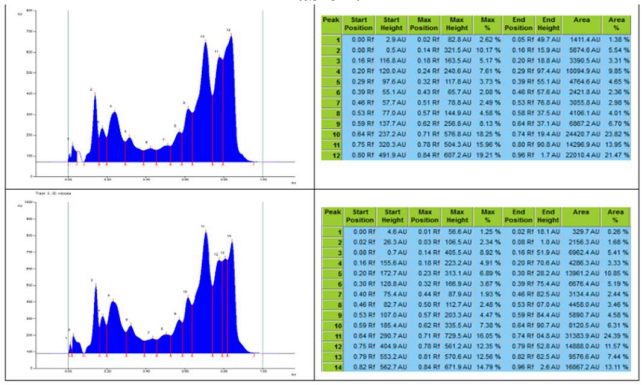


Figure 3: Graphical representation under 366nm

Table 4:



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	2.9 AU	0.02 Rf	82.8 AU	2.62 %	0.05 Rf	49.7 AU	1411.4 AU	1.38 %
2	0.06 Rf	0.5 AU	0.14 Rf	321.5 AU	10.17 %	0.16 Rf	15.9 AU	5674.6 AU	5.54 %
3	0.16 Rf	116.8 AU	0.18 Rf	163.5 AU	5.17 %	0.20 Rf	18.8 AU	3390.5 AU	3.31 %
4	0.20 Rf	120.0 AU	0.24 Rf	240.6 AU	7.61 %	0.29 Rf	97.4 AU	10094.9 AU	9.85 %
5	0.29 Rf	97.6 AU	0.32 Rf	117.6 AU	3.73 %	0.39 Rf	55.1 AU	4764.8 AU	4.65 %
6	0.39 Rf	55.1 AU	0.43 Rf	65.7 AU	2.08 %	0.46 Rf	57.6 AU	2421.8 AU	2.36 %
7	0.46 Rf	57.7 AU	0.51 Rf	78.8 AU	2.49 %	0.53 Rf	76.8 AU	3055.8 AU	2.98 %
8	0.53 Rf	77.0 AU	0.57 Rf	144.9 AU	4.58 %	0.58 Rf	37.5 AU	4106.1 AU	4.01 %
9	0.59 Rf	137.7 AU	0.62 Rf	256.8 AU	8.13 %	0.64 Rf	37.1 AU	6867.2 AU	6.70 %
10	0.64 Rf	237.2 AU	0.71 Rf	576.8 AU	18.25 %	0.74 Rf	19.4 AU	24420.7 AU	23.82 %
11	0.75 Rf	320.3 AU	0.78 Rf	504.3 AU	15.96 %	0.80 Rf	90.8 AU	14296.9 AU	13.95 %
12	0.80 Rf	491.9 AU	0.84 Rf	607.2 AU	19.21 %	0.96 Rf	1.7 AU	22010.4 AU	21.47 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	4.6 AU	0.01 Rf	56.6 AU	1.25 %	0.02 Rf	10.1 AU	329.7 AU	0.26 %
2	0.02 Rf	26.3 AU	0.03 Rf	106.5 AU	2.34 %	0.08 Rf	1.0 AU	2156.3 AU	1.68 %
3	0.08 Rf	0.7 AU	0.14 Rf	405.5 AU	8.92 %	0.16 Rf	51.9 AU	6962.4 AU	5.41 %
4	0.16 Rf	155.6 AU	0.18 Rf	223.2 AU	4.91 %	0.20 Rf	75.6 AU	4286.3 AU	3.33 %
5	0.20 Rf	172.7 AU	0.23 Rf	313.1 AU	6.89 %	0.30 Rf	28.2 AU	13961.2 AU	10.85 %
6	0.30 Rf	126.8 AU	0.32 Rf	166.9 AU	3.67 %	0.39 Rf	75.4 AU	6676.4 AU	5.19 %
7	0.40 Rf	75.4 AU	0.44 Rf	87.9 AU	1.93 %	0.46 Rf	82.5 AU	3134.4 AU	2.44 %
8	0.46 Rf	82.7 AU	0.50 Rf	112.7 AU	2.48 %	0.53 Rf	97.0 AU	4458.9 AU	3.46 %
9	0.53 Rf	107.0 AU	0.57 Rf	203.3 AU	4.47 %	0.59 Rf	84.4 AU	5890.7 AU	4.58 %
10	0.59 Rf	185.4 AU	0.62 Rf	335.5 AU	7.38 %	0.64 Rf	90.7 AU	8120.5 AU	6.31 %
11	0.64 Rf	290.7 AU	0.71 Rf	729.5 AU	16.05 %	0.74 Rf	64.8 AU	31383.9 AU	24.39 %
12	0.75 Rf	404.9 AU	0.78 Rf	561.2 AU	12.35 %	0.79 Rf	52.8 AU	14888.0 AU	11.57 %
13	0.79 Rf	553.2 AU	0.81 Rf	570.6 AU	12.56 %	0.82 Rf	62.5 AU	9576.6 AU	7.44 %
14	0.82 Rf	562.7 AU	0.84 Rf	671.9 AU	14.79 %	0.96 Rf	2.6 AU	16867.2 AU	13.11 %

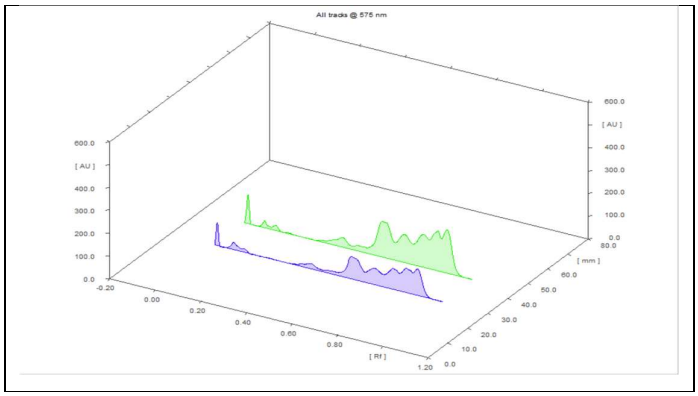
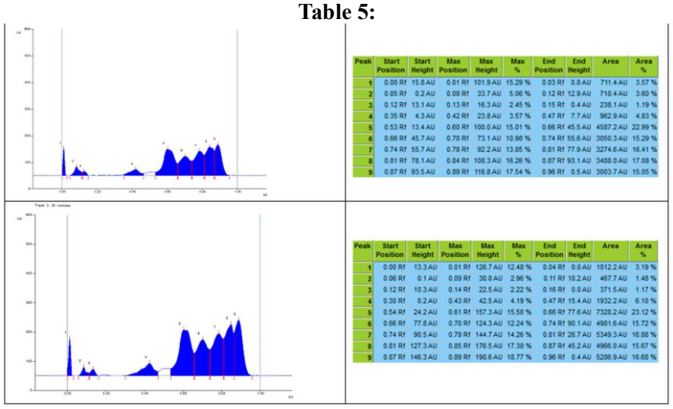


Figure 4: Graphical representation under 575 nm

Table 5:



DISCUSSIONS

The determination of physicochemical parameter is important in determination of adulterants and improper handling of drugs. Depending on the preliminary phytochemical test, quantitative determination of phytoconstituents was carried out by various standard methods. The evaluation of physicochemical and phytochemical parameters helps in the identification, authentication, and safety of medicinal plants **[10]**. Here all the analysis are done according to the guidelines of Ayurvedic Pharmacopoeia of India and WHO.

The moisture content was determined with reference to air-dried sample by loss on drying method which detects the net weight of a substance after drying at a specified temperature. The moisture content of *C. cordatum* in the present study were low (<14%) indicating that it could discourage bacterial, fungal or yeast growth **[11]**. Although the loss in weight, in the samples so tested, principally is due to water, small amounts of other volatile materials will also contribute to the weight loss.

The ash value is determined by four different methods, which measures total ash, acid-insoluble ash, water soluble ash and

sulphated ash. The total ash is particularly important in evaluation of purity and identity of drugs mainly the presence or absence of foreign inorganic matter such as metallic salts /silica **[12]**. The percentage of total ash was 9.7.%. Acid-insoluble ash is the part of total ash which is insoluble in dil. HCl and measures the amount of silica present. Water soluble ash is the water soluble portion of total ash. The ash values of the plant revealed a high percentage of water soluble ash. Sulphated ash present in this plant is very least, only 3.90%. The pH of the 4% aqueous solution of species indicated the acidic nature of the drug powder which may be due to the presence of acidic salts **(Table 1)**.

In the present study, alcohol soluble extractive values were more when compared to water soluble extractives in *C. cordatum*. And also, preliminary physico chemical results shows that the highest parameter present in *C. cordatum* is alcohol soluble extractives. Volatile oil is absent in *C. cordatum* **(Table 1)**. Swelling index value was low for *C. cordatum*, which may probably be due to the absence of mucilage. Foaming index is a significant characteristic for the presence of saponins in the crude drug. It was found to be more than 100 units, which suggests the presence of saponin glycosides in *C. cordatum* **(Table 1)**.

The presence/ absence of specific compounds was determined by specific-coloured products produced in reactions on the addition of specific chemicals. Various pharmacological activities are expressed by medicinal plants based on the type and amount of biologically active secondary metabolites such as terpenoids, flavonoids, alkaloids and glycosides **[13]**.

The powdered plant material of *C. cordatum* were subjected to soxhlet extraction using three solvents such as, methanol, ethyl acetate and distilled water. The methanol extract gave the highest yield in *C. cordatum*, whereas aqueous extract gave the least yield **(Table 2)**. Compared to other solvents, methanol has been known to be more effective in dissolving active compounds within cells, since methanol easily penetrates the cellular membrane to extract the intracellular ingredients from plant materials **[14]**. Saponins, tannins, terpenoids, phenols, steroids, quinones, glycosides, alkaloids and flavanoids were detected in *C. cordatum*.

Saponins have great pharmaceutical importance as they have antioxidant, anticancer, anti-inflammatory, anti-fungal, anti-diarrhoeal, anti-hepatotonic, wound healing, expectorant, spasmolytic, antiviral, antimicrobial properties and are used to treat hypercholesterolemia, cardiovascular diseases and hyperglycemia. Saponins was present in three extracts of *C. cordatum*. Tannins have received considerable attention in the field of nutrition, health and

medicine, due to their antioxidant, antimicrobial, anthelmintic, antiviral, anti-diarrhoeal, antiparasitic, analgesic, anti-irritant, anti-secretolytic, antiphlogistic, anticancer, wound healing and anti-inflammatory properties **[15]**. Tannins was present in all the three extracts of *C. cordatum* **(Table 2)**.

Terpenoids inhibit cholesterol synthesis and exhibits analgesic, antiviral, anti-malarial, antiseptic, stimulant, carminative, diuretic, anthelmintic, anti-rheumatic, anticancer, antimicrobial, anti-diarrhoeal and anti-inflammatory activities. Terpenoids was present in methanol, ethyl acetate and distilled water **(Table 2)**. Phenols are of great importance as they protect the human body from the oxidative stress, which cause many diseases, including cancer, cardiovascular problems and ageing. Phenolic compounds were present in methanol and ethyl acetate **(Table 2)**. Steroids are also of importance and interest in pharmacy due to their relationship with sex hormones. They reduce stress, inflammation, cholesterol levels, activate immune system, enhance memory and learning, prevent tumor growth and enhances intestinal absorption of Na+ and water **[16]**. Steroids were present in methanol and ethyl acetate extracts **(Table 2)**.

Quinones possess antimicrobial activity. They have the ability to bind with adhesions, complexes with cell wall and inactivate the enzymes. Quinones was present only in methanol extracts **(Table 2)**. Glycosides have a sedative and relaxant effect on the heart and muscles when taken in small doses. Furthermore, glycosides are known to have laxative, diuretic and antiseptic properties. Glycosides was seen in methanol, ethyl acetate and aqueous extracts **(Table 2)**. The activities of alkaloids have been widely studied for their potential use in the elimination and reduction of human cancer cell lines **[17]**. They possess antimicrobial, anti-inflammatory, antineoplastic, anthelmintic, antispasmodic, sedative, cytotoxic and anti-diarrhoeal properties. Here alkaloids were present in all the three extracts **(Table 2)**. Flavonoids were present in methanol and ethyl acetate extracts **(Table 2)**. Flavonoids also being one of the major constituents in plants exhibits several pharmacological, therapeutic, and nutraceutical properties like antioxidant, anti-cancer, anti-inflammatory, anti-allergic, etc. **[18]**. The methanol and ethyl acetate extracts of *C. cordatum* were observed to contain most of the phytochemicals tested when compared to aqueous extracts **(Table 2)**. Hence this plant should be evaluated further to assess its phytotherapeutic properties.

The HPTLC (high-performance thin layer chromatography) is an advanced form of TLC as it provides high resolution and

much accurate data. The HPTLC analysis of *C. cordatum* revealed the presence of various phytochemicals. The chromatograms **(Figure 2, 3, and 4)** were obtained upon scanning at UV 254 nm, 366 nm and 575nm and peak tables were generated. The Rf values, peak height, peak area, and percent area of the unknown substances are depicted in the tables **(Tables 3, 4 and 5)**. The HPTLC performed on the alcoholic extract of *C. cordatum* showed the presence of various phytoconstituents in different concentrations as illustrated in figures and tables. Figure 1 represents the HPTLC profiling under UV short, UV long and White light. The chromatogram scanned at 254 nm **(Figure 2)** represents 9 and 10 peaks for track 2 and track 3, respectively, whereas the chromatogram scanned at 366 nm **(Figure 3)** indicates 12 and 14 peaks for track 2 and track 3, respectively. And the chromatogram scanned at 575nm **(Figure 4)** represents 9 peaks for track 2 and 3 respectively. The number of peaks indicates the presence of different phytoconstituents present in the sample. The Rf values **(Tables 3, 4 and 5)** calculated for the phytoconstituents present in the tested sample would be helpful in the identification of the unknown compounds by comparing them with the reference standards, and from the values of peak area, the concentration of the compounds can be determined. The bands of separated compounds can be seen **(Figure 1)** on the TLC plates visualized under UV short, UV long and white light of wavelengths 254 nm, 366 nm and 575nm respectively.

CONCLUSION

Pharmacognostic studies and phytochemical screening can helps to the identification and investigation of a plant. *C. cordatum* is a plant having numerous medicinal properties. The present study attempted to record the preliminary physicochemical and phytochemical analysis of *C. cordatum*. These findings are helpful for future studies.

ACKNOWLEDGEMENT

The authors are sincerely thankful to Dr. Sandhya P, Head, Department of Botany, N.S.S College, Pandalam, Pathanamthitta for providing the required facilities to complete the work. We express our sincere gratitude to University of Kerala, Thiruvananthapuram for financial assistance for the study.

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