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RESEARCH ARTICLES

Qualitative and quantitative phytochemical screening and chemical fingerprint analysis of *Conocarpus lancifolius* plant using HPTLC

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Abstract

Most of the traditional medicinal plants in India need to be scientifically validated. Scientific evaluation and traditional knowledge are essential to obtain effective drugs for commercial purposes. This study used a *Conocarpus lancifolius* plant of the Combretaceae family and the *Conocarpus* genus. The present study was aimed at phytochemical screening, thin layer chromatography (TLC), and high-performance thin layer chromatography (HPTLC) analysis of hexane, chloroform, and ethanol extracts of *Conocarpus lancifolius*. Leaf extracts were prepared according to the polarity of the solvents, i.e., hexane, chloroform, ethanol. Preliminary phytochemical screening involved qualitative methods to detect the presence of carbohydrates, flavonoids, saponins, alkaloids, phenols, etc. The present study establishes the chemical fingerprint through TLC and HPTLC studies carried out as per standard method with different wavelengths in ethanolic extract. The results of qualitative phytochemical screening confirm the presence of carbohydrates, phenols, flavonoids, etc. The HPTLC and TLC analysis was developed to help correctly identify and quantify marker compounds. The phytochemical analysis of the plant is very important commercially, and pharmaceutical companies are very interested in producing new drugs to cure various diseases. The essential phytochemical properties recognized by our study in the indigenous medicinal plant are expected to be beneficial in curing various diseases.

Keywords *Conocarpus lancifolius* · Phytochemicals analysis · Total flavonoid content · Thin layer chromatography · HPTLC

Abbreviations

C. lancifolius	*Conocarpus lancifolius*
TFC	Total flavonoid content
TLC	Thin layer chromatography
HPTLC	High-performance thin-layer chromatography
HCL	Hydrochloric acid
H_2SO_4	Sulfuric acid
RF	Retention factors

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Introduction

Plants are living chemical factories for the biosynthesis of a diverse range of secondary metabolites (SMs). These metabolites are the foundation for many commercial pharmaceutical drugs and herbal remedies derived from medicinal plants (Zeidali et al. 2021). Many SMs, alkaloids, terpenoids, and phenylpropanoids are being considered for drug development. The various chemical constituents of medicinal plants have biological activities that can benefit human health through the pharmaceutical and food industries, in addition, they have significant value in the perfume, agrochemical, and cosmetic industries.

Conocarpus lancifolius is a plant in the genus *Conocarpus* and the family Combretaceae (Oves et al. 2022). *Conocarpus lancifolius* is a riverine tree native to Somalia, Djibouti, Yemen, the Horn of Africa, the Arabian Peninsula, and South Asia. This tree was not found in the Kuwait desert before the 1991 Gulf War (Zafar et al. 2022). It is responsible for greenery projects undertaken by the Kuwait government. *C. lancifolius* was introduced into Kuwait (Redha et al. 2012).

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The plant has thrived in the harsh environment of the Kuwait desert (Al-Surrayai et al. 2009), and it can now be found along all of the city's main boulevards and parks, as well as a green fence around private homes (Hussain and Abbas 2022). It is also trimmed in various appealing shapes that add to its grace. It grows quickly, has long hairy roots, is widely available, is well-liked by the locals, and provides significant amounts of shading material due to its large biomass (Redha et al. 2021). *C. lancifolius* is an ornamental plant that thrives in semiarid environments (Al-Kandari et al. 2009). *C. lancifolius* has potential medicinal value in its native countries. Its leaf extract treats anemia, conjugative inflammatory disease, catarrh, diarrhea, diabetes, fever, bleeding, skin ulcers, and syphilis orchitis (Oves et al. 2022).

C. lancifolius has antioxidant and antibacterial activity in vitro, with the crude methanolic extract obtained by simple maceration exhibiting excellent anti-a-glucosidase activity (Saadullah et al. 2014). TLC is a valuable tool for analyzing various biological compounds and natural products because it allows for the simple separation and convenient visualization of multiple samples in parallel in a relatively short period (Sobstyl et al. 2020). Hence the present investigation was taken up to study the physicochemical characteristics of leaves of *C. lancifolius* extract with ethanol, chloroform, and hexane, phytochemical constituents, total flavonoid content, and thin layer chromatography (TLC) analysis of leaves of *Conocarpus lancifolius* extracted with ethanol and also to perform qualitative and quantitative analysis of some secondary metabolites and minerals present in this plant (Al-Kandari et al. 2009).

Materials and methods

Plant materials

This study included the Combretaceae family's *Conocarpus lancifolius* leaves (Supplementary material 3).

Chemicals

Fehling solution A and Fehling solution B, ethanol, acetone, distilled water, aqueous HCl, methanol, chloroform, concentrated sulphuric acid, ammonia solution, picric acid, hexane, sulfuric acid, Wagner's reagent.

Sample collection

The medicinal plant was collected locally from Gujarat University, Ahmedabad, in three seasons: summer, winter, and rainy. The plants were botanically identified at Ahmedabad Gujarat University's Department of Botany, Bioinformatics, and Climate Change Impacts Management. The plant was employed for the phytochemical examination, and the chosen plant's young, fresh leaves were used for the analysis.

Preparation of plant extract

The leaves of the chosen plant were removed from the plant and washed under running tap water to remove dust. After air drying the plant sample for a few days, the leaves were crushed into powder and stored in polythene bags for later use. 10 g plant powdered material was subjected to 100 ml solvent extraction with increasing polarity using hexane, ethanol, and chloroform after 24 h at room temperature with each solvent separately. Each extract was filtered through Whatman filter Paper no. 1 to remove the solvent and obtain the dried extract.

The yield of each dried extract was recorded and calculated using Eq. (1). All of the sections were refrigerated until they were used in phytochemical analyses.

$$\text{Extraction yield (\%): mass of extracted product} \,/\text{mass of raw material} \times 100 \quad (1)$$

Qualitative phytochemical analysis

Phytochemical analysis of *Conocarpus lancifolius* extracts in hexane, ethanol, and chloroform was performed sequentially. Thirty-five grams of *Conocarpus lancifolius* plant extract from each season were mixed with 35 ml of each solvent (hexane, ethanol, chloroform). These solutions were subjected to phytochemical analysis according to accepted methods. The following procedures were used to analyze secondary compounds in *Conocarpus lancifolius* with the standard system according to Banu and Cathrine (2015), Medeo et al. (2015) shown in Supplementary material 1.

Total flavonoids content

The total content of flavonoid compounds was determined using spectrophotometric analyses. The experiments were carried out using the Saadullah et al. (2016) protocol with minor modifications, with quercetin serving as the standard. Ethanol was used to make a standard quercetin solution at a concentration of 1 mg/1 ml (Nandini et al. 2020). Different season plant extracts were prepared using ethanol, 0.2 ml of 5% $AlCl_3$, and 0.1 ml of sodium hydroxide and incubated for 30 min to obtain a standard calibration curve. A spectrophotometer was used to measure absorbance at

510 nm against a blank. All measurements were performed in triplicate, and the TFC was calculated using the standard quercetin calibration curve (Saadullah et al. 2016).

Thin layer chromatography

The thin layer chromatographic analysis of the extracts was carried out on silica gel 60 F254 (20 × 20 cm) stationary phase using mobile phases:

Toluene:acetone:formic acid (4.5:4.5:1) protocol with minor changes (Sobstyl et al. 2020) and standard quercetin was prepare with the ratio of quercetin:ethanol (1:1).

Procedure

The solvent system was created using the abovementioned ratio toluene:acetone:formic acid (4.5:4.5:1). TLC plates were spotted with the sample solution using a capillary 1 cm above the plate's bottom. The plate was then placed in the solvent system and covered with a lid. Four spots were made, one for standard quercetin and three for different season plant samples, and were watched for areas going upwards until 1 cm remained on the upper side of the plate, at which point the TLC plate was removed from the solvent system and dried. The R_f value was calculated and observed in UV, where the short wavelength is 254 nm and the long wavelength is 365 nm.

The formula for calculating the R_f value

$$R_f = (\text{Distance travelled by the solute} / \text{distance travelled by the solvent})$$

HPTLC profile (high-performance thin layer chromatography)

Developing solvent system

For extract, a variety of solvent systems were tested. The phytochemical constituent flavonoids were successfully resolved in toluene:ethyl acetate:formic acid (5:4:0.2).

Sample application

Using a spray technique, bands of extract (4 mm in length and 1ul in concentration for leaf) were applied. The sample was applied in duplicate on pre-coated silica gel 60F254 aluminium sheets (15 × 10 cm) using a Linomat 5 applicator connected to a CAMAG HPTLC system programmed with VISION CATS software.

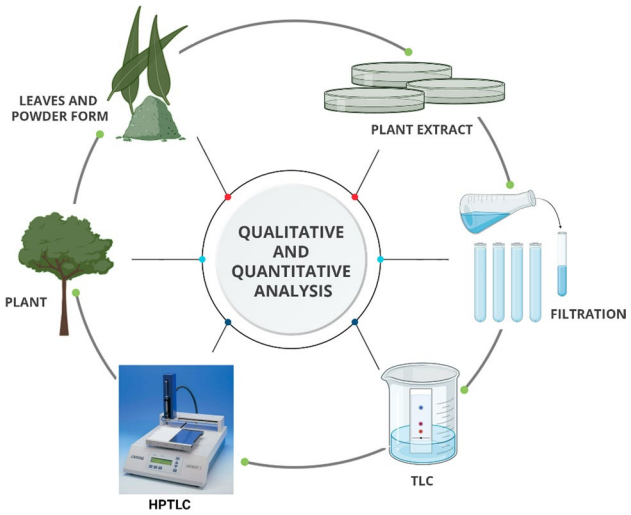

Fig. 1 Graphical abstract

Detection of spots

The air-dried plates were examined in ultraviolet and mid-day light (Fig. 1). After spraying with anisaldehyde sulphuric acid, the chromatograms were scanned with a densitometer at 366 nm and 254 nm. VISION CATS software was used to record the R_f values and fingerprint data.

Results

The extraction, screening, and identification of medicinally active substances found in plants are referred to as phytochemical analysis. Table 1 and Supplementary material 2 shows the results of their extraction yield and qualitative phytochemical screening of *Conocarpus lancifolius* leaf. In hexane, ethanol, and chloroform extracts, 12 phytochemicals were tested. alkaloids, flavonoids, saponins, tannins, phenol, coumarins, steroids, proteins, carbohydrates, cardiac glycosides, quinone, and anthraquinone glycosides are all found in the leaves of *Conocarpus lancifolius*. This suggests that the leaf of this plant contains a high concentration of bioactive secondary metabolites.

During the phytochemical screening process, all available bioactive secondary metabolites produced different results in different solvents and seasons. Supplementary material 1 shows that using ethanol solvent, the leaves extract yielded 13.83% in the rainy season, 9.11% in the summer, and 9% in the winter. The leaf extract of plant shows a higher percentage yield of 3.53% in the rainy

Table 1 Extraction yield (%) of the leaf of *Conocarpus lancifolius* with various solvents and different seasons

Sr. no.	Solvent	Color	% Extraction yield in summer	% Extraction yield in rainy	% Extraction yield in winter
1	Hexane	Light green	1.83	2.45	2.62
2	Ethanol	Dark green	9.11	13.83	9
3	Chloroform	Yellowish brown	2.7	3.53	2.47

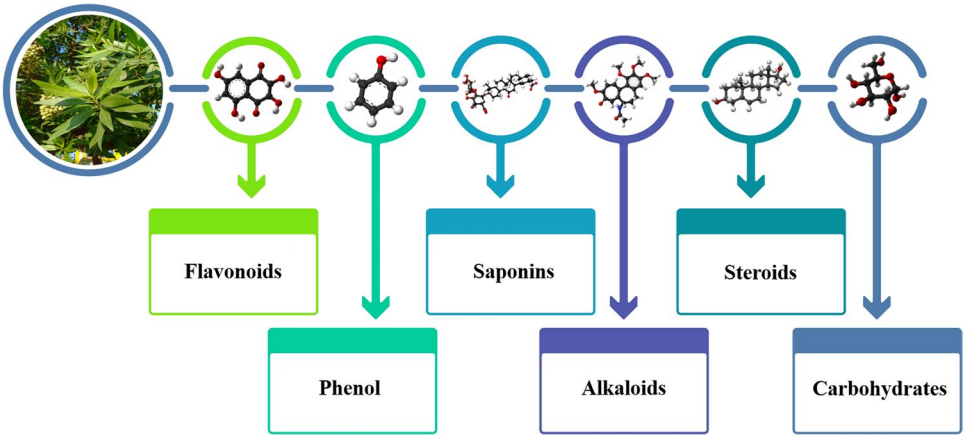

Fig. 2 Group of compounds present in *Conocarpus lancifolius*

season with chloroform solvent, followed by 2.62% in the winter season with Hexane solvent (Table 1).

The presence of phytochemicals, which are considered active medicinal chemical constituents, was discovered in this study. The samples contained important medicinal phytochemicals such as steroids, coumarins, flavonoids, alkaloids, saponins, carbohydrates, and tannins, as shown in Fig. 2. The phytochemical analysis results show that the plant is abundant in at least one of the alkaloids, flavonoids, and terpenoids present in Hexane solvent during the summer. The phytochemical screening and qualitative estimation of plants in three different seasons using different solvents revealed that the leaves were high in steroids, flavonoids, alkaloids, and tannins, as well as phenolic, coumarin, and carbohydrates (Supplementary material 3).

Supplementary material 3 shows alkaloids' presence in hexane and chloroform solvents during the summer. Flavonoid and phenol levels are increased in ethanol solvents during the winter, summer, and rainy seasons, and saponin levels are high in all seasons. Carbohydrates are more prevalent in ethanol extract during the summer season. Coumarin is found in chloroform extract in summer and ethanol solvent during the rainy and winter seasons. Anthraquinone glycosides are not found in any season or solvent. In the Millions Test, protein concentrations in ethanol are higher during the summer and rainy seasons. In all extracts, quinone is present in hexane during the summer but not during the rainy and winter seasons. Every season contains a steroid.

Table 2 Total flavonoid content in ethanol solvent

Sr. no.	Season	Flavonoid content (QE/ml)
1	Summer	0.183 ± 0.057
2	Rainy	0.176 ± 0.066
3	Winter	0.181 ± 0.047

Total flavonoid content

Supplementary material 3 displays the quantitative results of the Visible-UV spectrophotometer analyses of the various extracts. The method used is Uzair's one (Saadullah et al. 2016). In the survey, the absorbance of a set of quercetin concentrations resulted in standardization curves with equations and correlation coefficients, respectively:

$$y = 0.1034x + 0.1456$$

$$R^2 = 0.9917 \text{ for quercetin}$$

Flavonoids have a polyphenolic structure and are phytochemicals. They have been shown to have a wide range of biological activities, including antitumor, antimicrobial, antioxidant, and anticancer properties (Mwamatope et al. 2021; Patil et al. 2021). Because of its sensitivity and dependability, an aluminium chloride assay was used to analyze TFC in plants (Morreeuw et al. 2021). As shown in

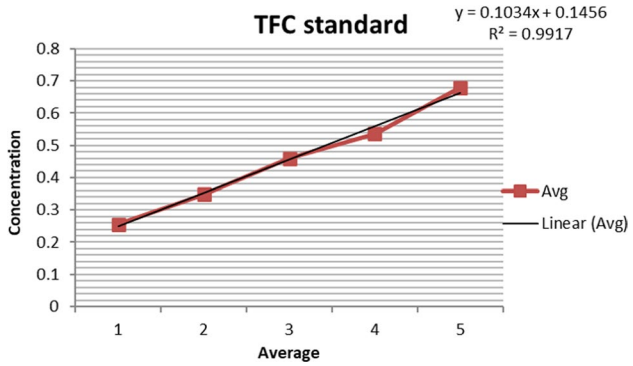

Fig. 3 Standard quercetin curve

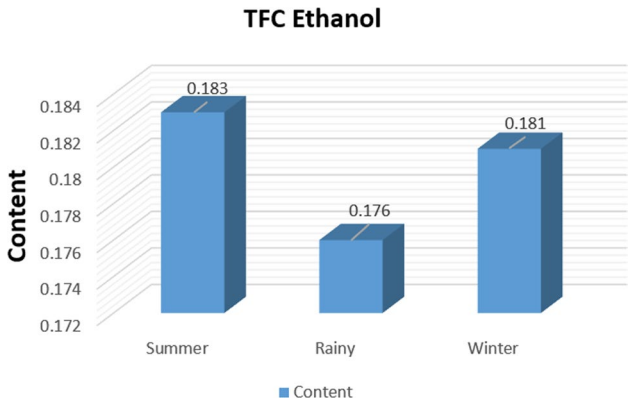

Fig. 4 Comparison of three seasons in TFC

Table 2, the flavonoid contents of plant ethanol extract varied according to seasonal changes, with all samples exhibiting high contents in the summer season and lower contents in the rainy season in Fig. 3. TFC levels were increased in *Conocarpus lancifolius* leaves (0.183 ± 0.057 QE/ml). TFC decreased in ethanol solvent significantly in the following order: summer > winter > rainy in Fig. 4. These high flavonoid contents reported during the summer are consistent with previous research, indicating a high total flavonoid content (Kadhom et al. 2020).

Thin layer chromatography

TLC fingerprinting of TS extract yields impressive results regarding the presence of various bioactive compounds. The observation shows that separating polar compounds varies with the different combinations of organic solvents (Azeem et al. 2019). 1st spot standard (quercetin); 2nd spot summer season, 3rd spot winter season, and 4th spot rainy season of *C. lancifolius* plant leave ethanol extract in Fig. 5. Ethanol extracts were chosen for TLC studies based on preliminary phytochemical studies. A thin layer chromatogram of the ethanol extract of *Conocarpus lancifolius* is given in Fig. 4. TLC of the ethanol extract of *C. lancifolius* revealed the presence of a spot having R_f value of 0.696, 0.913 in the summer season, 0.608, 0.942 in the Rainy season, and 0.724, 0.971 in winter season when a solvent phase of toluene, acetone, and formic acid in the ratio of (4.5:4.5:1) a solvent system was used (Table 3).

Fig. 5 Thin layer chromatography results

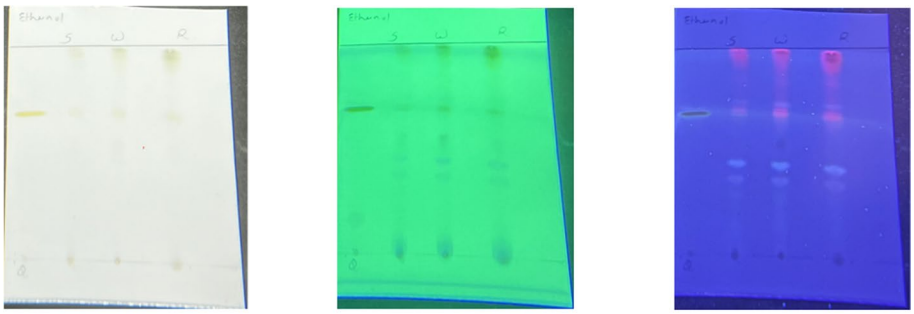

Table 3 Thin layer chromatography

Compound	Season	Mobile phase	Ratio	R_f value
Flavonoids	Summer	Toluene:acetone:formic acid	4.5:4.5:1	0.696, 0.913
	Rainy			0.608, 0.942
	Winter			0.724, 0.971

For the flavonoid compound, qualitative analysis of ethanol extract using TLC was performed using a mixture of toluene:acetone:formic acid (4.5:4.5:1 v/v) used as the mobile phase. Deshpande et al. (2022) and Sobstyl et al. (2020) yielded an excellent resolution of quercetin with bands at $R_f = 0.66$ Fig. 5. Quercetin was identified as a phytochemical marker in flavonoid compound analysis and quantified at visible light and wavelength 254 and 365 nm before derivatization (Fig. 5). The results revealed that methanol extracts at 1 g/10 ml of 100% Ethanol contained quercetin following TFC analysis, where the presence of flavonoids in separated bands on the chromatogram developed in toluene:acetone:formic acid (4.5:4.5:1) solvent system was visualized (Fig. 5). Taking into account that phytochemical analysis revealed less amount of flavonoids in chloroform and Hexane extracts of tested plant materials, therefore, these two solvent systems were not used for TLC separation. Further TLC analysis showed that ethanol extracts of *C. lancifolius* exhibited high flavonoids and were also detected by some bands separated in the summer season (R_f values of active bands: 0.696, 0.913), in winter season R_f values (0.724, 0.971), and rainy season R_f values (0.608, 0.942).

HPTLC

A comparison between HPTLC plates, which have been visualized under visible light or UV 254 nm and UV 366 nm, is demonstrated in Fig. 6.

Figure 7 shows the HPTLC chromatogram of *C. lancifolius*. HPTLC chromatogram of the standard flavonoids profile of *C. lancifolius* was depicted. This confirmed the presence of flavonoids in leaves parts of *C. lancifolius*.

In the winter season, the results of HPTLC fingerprint scanning at wavelength 366 nm for ethanolic extract of *Conocarpus lancifolius* leaf. Figure 8 depicts the corresponding HPTLC. There are seven polyvalent phytoconstituents with corresponding ascending R_f values ranging

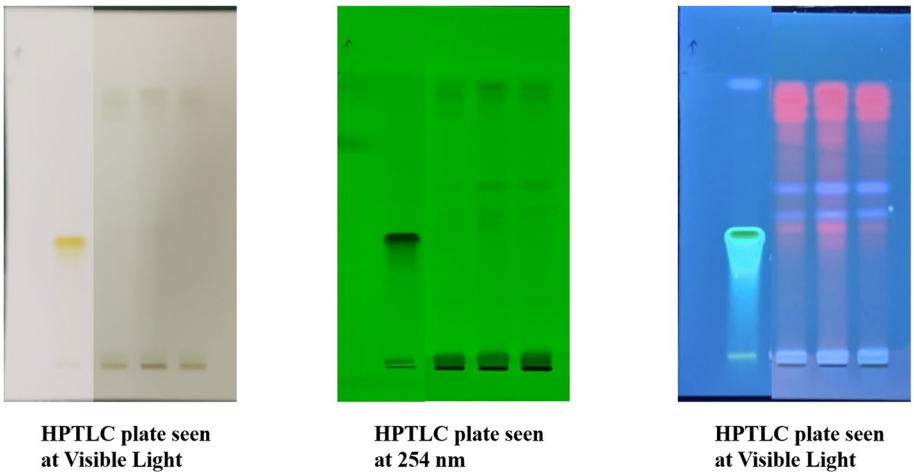

Fig. 6 HPTLC profile of leaf extract of *Conocarpus lancifolius*

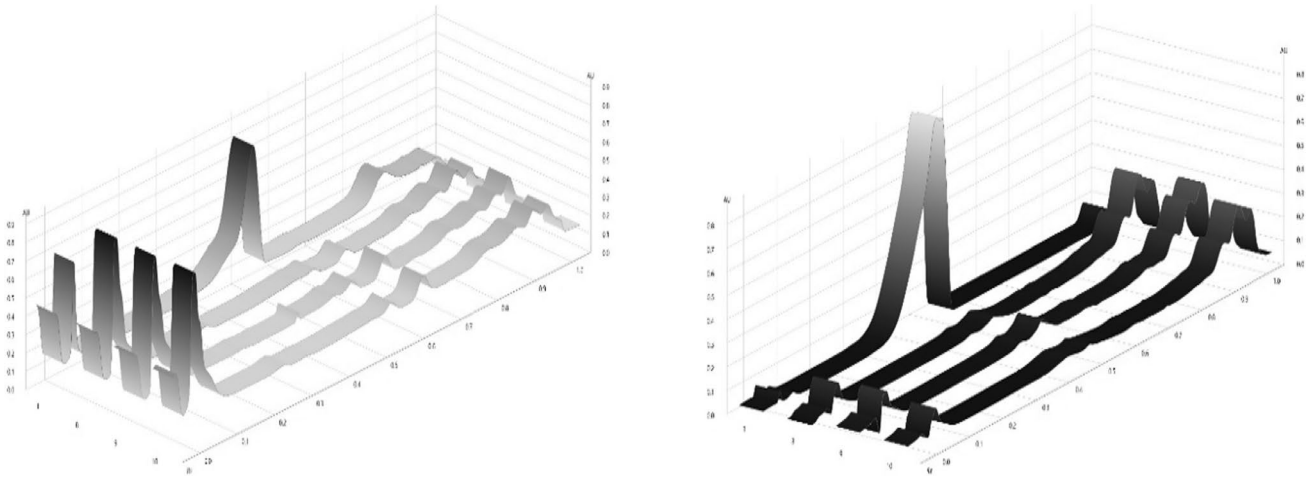

Fig. 7 Ethanol solvent in three seasons (summer, winter, rainy) with derivatives in 366 nm and 254 nm

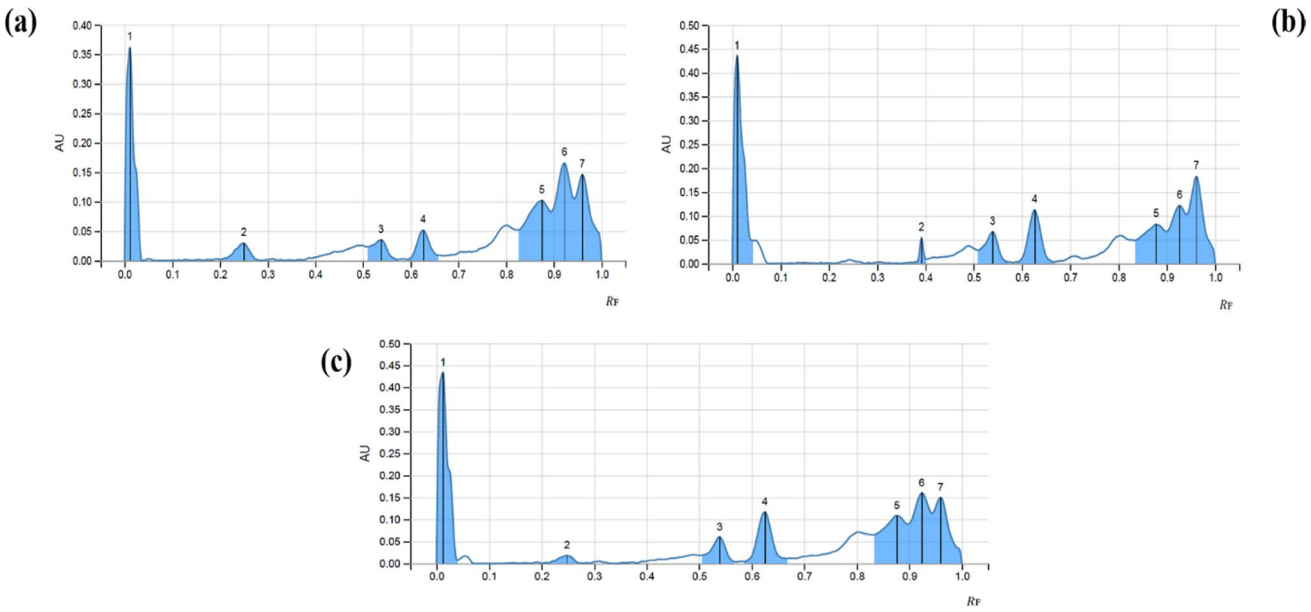

Fig. 8 Densitogram of ethanolic extract of *Conocarpus lancifolius* leaf **a** winter, **b** summer, and **c** rainy season

from 0.036 to 1.000, with the highest concentration of phytoconstituents being 40.63% and its corresponding R_f value being 0.036, as shown in Supplementary material 4. The results of the HPTLC fingerprint scanned at wavelength 366 nm for ethanolic extract of *Conocarpus lancifolius* leaf in the summer season revealed seven polyvalent phytoconstituents and corresponding ascending order of R_f values ranging from 0.043 to 1.000, with the highest concentration of phytoconstituents being 41.29% and its corresponding R_f value being 0.043, displayed in Supplementary material 4 and Fig. 8 illustrates the corresponding HPTLC Densitogram. The results of an HPTLC fingerprint scan at 366 nm for an ethanol extract of *Conocarpus lancifolius* leaf in the rainy season. There are seven polyvalent phytoconstituents with ascending R_f values ranging from 0.040 to 1.000, with the highest Conc. The phytoconstituents were found to be 41.42%, and the corresponding R_f value was found to be 0.040, according to Supplementary material 4. Figure 8 illustrates the corresponding HPTLC Densitogram.

Discussion

The study was conducted on the chosen medicinal plant in three different seasons, revealing that phytochemical constituents such as terpenoids, flavonoids, alkaloids, steroids, and alkaloids are present or absent in this plant, as shown in Table 1. Our research discovered that alkaloids and flavonoids are more abundant in the summer and rainy seasons than in the winter. In contrast, quinone, anthraquinone glycosides, and proteins are absent in all seasons. Previously, it was reported that flavonoids, alkaloids, and tannins were present in the plant's aqueous extract (Raheema and Shoker 2020). At the same time, alkaloids are absent in all seasons in ethanol and chloroform. The results of the recent and previous research studies differed, which could be attributed to differences in location, season, and solvents. Tannins and saponins were discovered in all seasons using an ethanol extract. This study revealed that all extracts contain flavonoids and tannins in all seasons. Previous research found alkaloids, saponins, steroids and triterpenoids, flavonoids, cardiac glycosides, anthraquinones, and tannins in *Conocarpus lancifolius* dichloromethane and methyl alcohol extracts (Saadullah et al. 2020). In response to external changes, the phytochemical composition of the plant undergoes qualitative and quantitative changes, which may result in the accumulation or absence of metabolites in different seasons (Ribeiro et al. 2020). Secondary metabolites with enjoyable pharmacological activities found in plants can be isolated for drug development. Due to their diverse functional properties, phytochemicals have medical, biological, chemical, and agricultural applications (Justin et al. 2014). The aqueous and methanolic extracts of *Conocarpus lancifolius* contained alkaloids, tannins, glycosides, and phenol (Kadim and Al-azawi 2021). Anthraquinone glycosides were not found in our research study. An earlier study isolated ellagic acid and kaempferol derivatives responsible for *C. lancifolius*'s anti-diabetic potential (Al-Taweel et al. 2016). TFC analysis of *Conocarpus lancifolius* extract revealed that ethanolic extract in the summer season contained the most flavonoids. The high flavonoid content was found to play a role in

biological activities such as DPPH scavenging (Obando-Camino et al. 2021). Flavonoids are phenolic compounds that play various roles in plants, including defence against insects, fungi, viruses, and bacteria and protection against ultraviolet radiation (Jan et al. 2021). The chemical structures of flavonoids are primarily responsible for their biochemical activities (Obando-Camino et al. 2021). However, the bioactivity of *Conocarpus* spp. is derived from bioactive, demonstrating nine alkaloids, five saponins, and eight total phenolic compounds in *C. lancifolius* leaf extract using thin layer chromatography (TLC) (Touqeer et al. 2015). The phytochemical analysis of *C. lancifolius* HMEL revealed a variety of compounds, including carbohydrates, amino acids, alkaloids, tannins, steroids, and saponins (Moni et al. 2023). TLC is an important technique for the quality control of herbal products and natural drug analysis (Durón et al. 2009).

Conclusion

Medicinal plants play an essential role in disease prevention. The phytochemical analysis is critical for evaluating a plant's potential medicinal properties and determining the active principles responsible for the plant's known biological activities. According to this study, *Conocarpus lancifolius contains alkaloids*, *cardiac glycosides*, *steroids*, *flavonoids*, *tannin*, *phenol*, *saponin*, *coumarin*, and *carbohydrates*. According to quantitative analysis, ethanol extracts have the highest concentrations of total flavonoid content in the summer (0.183 ± 0.057) compared to the winter and rainy seasons. Profile of TLC showed the presence of Flavonoids, and the R_f value of the plant sample was also calculated, and it shows a 0.913 value. Following the separation of active constituents, it is concluded that *Conocarpus lancifolius* reveals the content of flavonoids in plants and that the best season for obtaining more flavonoids is summer. The initial study was conducted with HPTLC, and the results showed many compounds in *Conocarpus lancifolius*. From the HPTLC studies, it has been found that Ethanol extracts contain not a single compound but a mixture of compounds, so it is established that the pharmacological activity shown by them is due to the cumulative effect of all the compounds in the composite. As a result, we hope that the important phytochemical properties discovered in plants using different solvents and seasons will help deal with various diseases.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42535-024-00892-6.

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Author contributions Poojaben Prajapati: Writing the article. Bharat B. Maitreya: Writing- Editing and Review, Research concept and Final approval of article, and Rakesh M. Rawal: Writing- Editing and Review, Research concept and Final approval of article.

Data availability Not applicable for that section.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval Not applicable.

Consent for publication Not applicable.

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