THEORETICAL
BIOLOGY FORUM

TBF : 45–54 (2022)
RESEARCH ARTICLE

Phytochemical Studies in *Capparis divaricata Lam.* leaf extract using Fourier Transform Infrared Spectroscopy (FTIR) and HPTLC Fingerprinting

K. Swetha[1], G.Prabhakar[2], Kandula Jayapaul[3], B. Kiran Kumar[1]*

[1] Department of Botany, UCS, Osmania University, Hyderabad, Telangana State- 500007, India
[2]Sri Gp Avens Life Sciences Pvt Ltd, Medical Biotechnology Complex, AIC-CCMB, ANNEX-2IDA Uppal, Hyderabad, Telangana -500 039, India
[3]Department of Botany, Govt. Degree College, Alair, Telangana State, India

ABSTRACT

Traditional medicines are being used in India from ancient times for various disease/ disorder conditions. Importantly, most of the traditional medicinal plants do not have scientific validation. Scientific evaluation of medicinal properties along with traditional knowledge is essential to obtaining effective drugs for commercial purposes. This study aimed to determine the phytocomponents present in the leaves of Cappris divaricata by Fourier transform infrared spectroscopy (FTIR) and HPTLC fingerprinting. Cappris divaricate belonging to the family Capparaceae, a traditional medicinal plant species commonly known as "caper brush" in Telugu and found throughout India. The plant is used in Siddha and veterinary medicine for trypanosomiasis. The extraction process was carried out using different solvents in increasing order of polarity and the resulting extracts were analysed using standard procedures. FTIR spectroscopy and HPTLC fingerprinting were used to determine the composition, profile, and structure of the organic compounds in the leaf extract. The phytochemical analysis in the leaf extracts revealed the presence of various compounds including alkaloids, carbohydrates, flavonoids, tannins, saponins, phenols, steroids, terpenoids, glycosides, and resins, in the hexane, chloroform, ethyl acetate, acetone, and methanolic extracts. FTIR data indicated the presence of phenolic compounds, hydroxyl groups, alkanes, alkenes, amines, aromatic amines, and aliphatic groups in C. divaricata. HPTLC analysis of the methanolic extract of the leaves showed the presence of 13 spots at 254 nm and 12 spots at 366 nm, with a spot at Rf 0.90 and a maximum concentration of 66.23% at 366 nm, which may serve as biomarkers and fingerprint parameters for identification of the plant

Keywords: *Capparis divaricata, Phytochemicals, leaf extracts, FTIR, HPTLC*

ARTICLE HISTORY:
Received: 02 August 2022
Revised: 10 September 2022
Accepted: 28 November 2022
Available Online: 10 December 2022

DOI:
https://doi.org/10.5281/zenodo.7629976

CORRESPONDING AUTHOR:
Dr. B. Kiran Kumar

E-MAIL ID:
kiran.nrcpb@gmail.com

INTRODUCTION

Medicinal plants have a long history of use in folk medicine and continue to be used in many countries for the treatment of various diseases. With the growing awareness of the importance of optimal nutrition and health, people are increasingly turning to plants as a source of food and medicine. [2,3] Medicinal plants are becoming increasingly important in the pharmaceutical industry because of their active phytochemicals, which can provide diverse health benefits. (Anwar et al., 2007). Herbal and plant-based products are often seen as less hazardous to human health and the environment since they include bioactive compounds that have several uses. It is estimated that 70-80% of the world's population, especially in developing countries, relies on herbal medicine to prevent and cure diseases. Additionally, it has been reported that about 25% of synthesised drugs are derived from medicinal plants. [1]

The *Capparis* genus is made up of around 80 different species, which are classified under the family Capparaceae. In many parts of the world, the *Capparis* plant and its parts have a long history of being consumed as food and used medicinally. The plant contains various compounds that have

potential medicinal and therapeutic properties, such as alkaloids, flavonoids, vitamins, proteins, and amino acids. Studies have reported anti-cancer activity of the leaves of *Capparis divaricata* [4], and various other medicinal properties such as analgesic activity [5], the development and evaluation of an herbal fast-dissolving tablet [6], and the use of bark paste and leaf juice with milk to cure dysentery, stomach problems, and infertility [6]. [7] also reported that the bark extract of the plant, when pounded with leaves of Erythrina Variegata, ginger, garlic and turmeric in goat's milk and given orally, can be used for medicinal purposes [9].

MATERIAL AND METHODS

Collection and authentication of plant material

The *Capparis divaricata* Lam. leaves were harvested in the months of August and September 2020 in Sriramagiri Village, Nellikudur Mandal, Mahabubabad District, Telangana State, India. The plant was verified as legitimate by the Department of Botany, Osmania University and the specimen was deposited to the Herbarium, Hyderabadens, O.U., Hyderabad (Accession number: OU-0465).

Drying

The leaves were cleaned before being chopped into pieces (0.5—1.5×1×0.2 cm3) using scissors and knives. They were allowed to dry on the newspapers for ten days in the shade. Before beginning the extraction process, the fruit pieces were then dried for an hour in a hot air oven at 40°C to remove any moisture.

Successive extraction using Soxhlet apparatus

Dried leaves were powdered using a mechanical grinder and sieved to ensure a consistent size. In the Soxhlet apparatus, the fruit powder was extracted sequentially with hexane at 69 °C, chloroform at 61°C, ethyl acetate at 77°C, acetone at 56°C, and methanol at 65°C. To allow for a faster rate of cycling of fresh solvent, the extraction temperatures were adjusted to the boiling points of the solvent. Each solvent received six hours of time for hot, continuous, and consecutive extraction. The extracted materials were subsequently concentrated and dried in a 45°C oven. The dried extracts were then used for phytochemical screening [11].

Calculation of percentage yield

The dried extracts obtained with each solvent were weighed and yield was calculated concerning the air-dried weight of the plant material.

$$\text{Percentage Yield} = \frac{\text{Weight of the crud(mg/g)}}{\text{Weight of the plant material}} \times 100$$

Screening of Phytochemicals

A preliminary analysis of secondary metabolites in leaf extracts was conducted using standard methods for phytochemical screening. Various compounds were tested for, including alkaloids, carbohydrates, proteins, amino acids, flavonoids, saponins, steroids, terpenoids, phenols, tannins, glycosides, coumarins, anthraquinones, quinones, resins, gums, and leuco-anthocyanins. Several qualitative assays have been undertaken to determine the presence or absence of bioactive substances [13,to22].

Detection of Alkaloids

5 ml of diluted hydrochloric acid was added to 50 mg of solvent-free extract before being agitated and filtered. Using a variety of reagents, the filtrate was thoroughly examined for alkaloids.

Mayer's test: A few drops of Mayer's reagent were added by the side of the test tube to 3 ml of filtrate. The presence of alkaloids was indicated by the formation of a white or creamy precipitate.

Wagner 's test: To a few ml of filtrate, few drops of Wagner's reagent was added by the side of the test tube. A reddish-brown precipitate indicates the presence of alkaloids.

Hager s' test: To a few ml of filtrate, one or two ml of Hager's reagent (saturated aqueous solution of picric acid) was added. A prominent yellow precipitate indicates the test as positive.

Dragendorff's test: To a few ml of filtrate, one or two ml of Dragendroff's reagent was added. A prominent yellow precipitate indicates the test as positive.

Detection of Carbohydrates

100 mg of the extract was dissolved in 5 ml of distilled water and filtered. The filtrate was subjected to the following tests.

Molisch's test: In a test tube, 3 ml of filtrate was added, followed by a few drops of an alcoholic solution of -naphthol, shaken well, and 1 ml of concentrated sulphuric acid was added slowly along the walls of the test tube and allowed to stand. Forming a violet ring at the junction of liquids indicates the presence of carbohydrates.

Benedict's test: To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added and heated on a boiling water bath for 2 minutes. The formation of an orange-red colour precipitate confirmed the presence of reducing sugars.

Fehling's test: One ml of filtrate was taken in a test tube, which was boiled on a water bath with 1 ml each of Fehling solutions A and B. Brick-red precipitate formation indicates the presence of reducing sugars.

Barford's test: To 1 ml of filtrate, 1 ml of Barford's reagent was added and heated on a boiling water bath for 2 minutes. A red precipitate indicates the presence of sugars.

Detection of Proteins

100mg of extract was dissolved in 10ml of distilled water and filtered through Whatman No.1 filter paper and the filtrate was subjected to tests for proteins.

Millon's test: To 2 ml of filtrate, a few drops of Millon's reagent were added. A white precipitate indicates the presence of proteins.

Biuret test: An aliquot of 2 ml of filtrate is treated with one drop of a 2% copper sulphate solution. To this, 1 ml of 95% ethanol is added, followed by an excess of potassium hydroxide pellets. The pink colour of the ethanolic layer indicates the presence of proteins.

Detection of Amino acids

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) were added to two ml of aqueous filtrate. A characteristic purple colour indicates the presence of amino acids.

Detection of Flavonoids

Alkaline reagent test: Extracts were treated with a few drops of sodium hydroxide solution. The formation of an intense yellow colour, which becomes colourless with the addition of a few drops of dilute acid, indicates the presence of flavonoids.

Lead acetate test: Extracts were treated with a few drops of a 10% lead acetate solution. The formation of a yellow-coloured precipitate indicates the presence of flavonoids.

Shinoda's test: To the test, solution of mixture of zinc dust and conc. hydrochloric acid was added. It gave magenta colour after a few minutes, indicating the presence of flavonoids.

Detection of Saponins

The extract (50 mg) was diluted with distilled water and made up to 20 mL. The suspension is shaken in a graduated cylinder for 15 minutes, and the formation of a 2-cm layer of foam indicates the presence of saponins.

Steroids and Terpenoids

Liber Mann-Buchard's test: The crude extract was mixed with a few drops of acetic anhydride, boiled, and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration or the formation of a deep red colour in the lower layer would indicate a positive test for steroids and triterpenoids, respectively.

Detection of Phenols

Ferric chloride test: The extract (50 mg) is dissolved in 5 ml, of distilled water, to which a few drops of a neutral (5%) ferric chloride solution are added. A dark green colour indicates the presence of phenolic compounds.

Gelatin test: The extract (50mg) is dissolved in 5ml of distilled water and 2ml of 1% solution of gelatin containing 10% sodium chloride is added to it. White precipitate indicates the presence of phenolic compounds.

Lead acetate test: The extract (50 mg) is dissolved in distilled water, and to this, 3 ml of a 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

Detection of Tannins

Ferric chloride test: Treat the extract with ferric chloride solution; the blue or green colour of the extract indicates the presence of tannins.

Potassium Ferricyanide test: A small quantity of the test solution was treated with a 2% potassium ferricyanide and ammonia solution. A deep red colour indicated the presence of tannins.

Potassium Dichromate test: To the test solution, 2% potassium dichromate solution was added. A yellow precipitate indicated the presence of tannins.

Detection of Glycosides

The test solution was prepared by dissolving the extract in alcohol or boiling with the hydro-alcoholic solution.

Baljet's test: The test solution was treated with 2% sodium picrate. The appearance of a yellow-orange colour indicated the presence of glycosides.

Legal's test: The test solution was treated with pyridine and made alkaline; the addition of 2% sodium nitroprusside gave a pink to red color, indicating the presence of glycosides.

Keller-Killiani test: The extract (100 mg) was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution. This was then layered with 1 ml of concentrated H2SO4. A brown ring obtained at the interface indicates the presence of glycosides.

Detection of Coumarins

Place one millilitre of extract in a test tube and cover with filter paper moistened with a dilute sodium hydroxide solution. For several minutes, place the covered test tube in a water bath. Remove the paper and expose it to ultraviolet (UV) light; the paper fluoresces green, indicating the presence of coumarins.

Detection of Anthraquinones

Borntrager's test: The test solution was treated with 5ml of 10% sulphuric acid for 5min., filtered while hot, cooled, and the filtrate was shaken gently with an equal volume of benzene. The benzene layer was separated and treated with half of its volume with a solution 10% ammonia allowed to separate it, the rose-pink colour in the ammonial layer indicated the presence of anthraquinones.

Detection of Quinones

1 ml of extract was added to the 2 ml of dilute NaOH. The formation of blue green or red coloration confirms the presence of quinones.

Detection of Resins

Take one gm of aqueous extract boiled the extract and then add a few drops of concentrated H_2SO_4 observed for reddish brown colour indicates the presence of resins.

Detection of Gums

The extract (100 mg) is dissolved in 10 ml of distilled water, and to this, 25 ml of absolute alcohol is added with constant stirring. A white or cloudy precipitate indicates the presence of gums.

Detection of Leuco-anthocyanins

2 ml of the extract was treated with 2 ml of 2N hydrochloric acid, and ammonia was added to it. The appearance of a pink-red colour turning blue-violet indicates the presence of leuco-anthocyanins.

Fourier transform infrared spectroscopy (FTIR)

A dry leaf powder of *C. divaricata* was taken and subjected to Fourier transform infrared (FTIR, School of Chemistry, University of Hyderabad) spectroscopy measurement using the potassium bromide (KBr) pellet technique diffuse reflection mode at a resolution of $4cm^{-1}$. The powder was mixed with KBr and exposed to an infrared source of 500 to 4000 cm^{-1}. A similar process was used for the FTIR studies of *C. divaricata* extract before and after bioreduction. [23][24]. The frequency of the vibrational peak (ν) depends on two factors

i.e., force constant and reduced mass, which can be explained by the following equation.

$$v=1/2\pi c \sqrt{(k/\mu)}$$

Here, c is the speed of light, k is the force constant and μ is reduced mass.

HPTLC fingerprinting analysis

The concentrated methanolic extract was spotted in the form of bands of 5 mm length with a 25-L syringe on a pre-coated silica gel aluminium plate 60 F254, (5 x 10 cm with 0.25 mm thickness; Merck, Darmstadt, Germany), and the plates were washed with methanol before use. The TLC development chamber was saturated with mobile phase using filter paper. The sample and standard solutions were applied as bands of 6 mm wide and 10 mm apart using an Automatic TLC Sampler 4 applicator (CAMAG, Muttenz, Switzerland, supplied by Anchrom Technologies, Mumbai) fitted with a 25-μL Hamilton syringe supplied with nitrogen flow. A constant application rate of 15μL/sec was employed. The space between the bands was fixed at 20 mm/sec. The slit dimension was kept at 4×0.20 mm, and a scanning speed of 20 mm/sec was employed. The mobile phase consisted of a saturated mixture of toluene. Ethyl acetate: Methanol: Glacial acetic acid 7:2:1 v/v were found to be a suitable mobile phase for separation of the phytoconstituents in the species studied, and chromatography was performed using 10 mL of mobile phase in a 10×10 cm twin-trough glass chamber (Camag, Muttenz, Switzerland) with linear ascending development [24]. The optimised chamber saturation time for the mobile phase was 20 minutes at room temperature with a chromatographic run length of 8.5 cm. After development, the TLC plates were dried in a current of air with the help of a hot air dryer in a wooden chamber with adequate ventilation. Densitometric scanning was performed with a (CAMAG TLC Scanner III) in the absorbance–reflectance mode at 254 nm and 366 nm with a slit dimension of 4×0.20 mm and a scanning speed of 20 mm/sec. All the instruments were operated by winCATS software (v. 1.4.3 CAMAG) resident in the system. The source of radiation utilised was a deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm, and the concentrations of the compounds chromatographed were determined from the intensity of diffusely reflected light. Further, for digital documentation, the Digi Store 2 documentation system (CAMAG) consisting of the illuminator, Reprostar 3, and digital camera Power Shot G2 (Canon, Tokyo, Japan) was used.

RESULTS

Plant Profile:

[26-30]

Classification of *Capparis divaricata* Lam.

Kingdom	**Plantae**
Phylum	**Tracheophyta**
Class	**Magnoliopsida**
Order	**Capparales**
Family	**Capparaceae**
Genus	***Capparis***
Species	***Capparis divarcata* Lam.**

Morphology

Capparis divaricata Lam., also known as caper brush, is a common plant found throughout India. It is a bushy shrub or small tree, reaching up to 5m in height, with branches that are either glabrous or hoary. The stipular thorns are variable and can be straight or curved upwards or downwards. The bark is rough and deeply cleft, with a thickness of about 1 inch. The leaves are thick, coriaceous, and can be linear to linear-oblong or elliptic to oblong, with a size of 4 to 6 × 1 to 2.5 cm. The base of the leaves is cuneate, the margin is entire, and the apex is mucronate. Petioles are around 3.5 cm long and the stipular thorns are stout, straight or slightly curved, and 2 to 3 cm in length. The flowers are yellow, with a diameter of 5.7 cm and are found axillary and solitary. The sepals are 4, ovate, acute, elliptic-orbicular, thick, and pubescent on both sides, with the outer pair being boat-shaped and the inner pair being petaloid and equaling the petals. Petals are 4, greenish-yellow, creamy or white, linear, oblong, obovate-spathulate or strap-shaped. Stamens are 45 to 65, filaments are yellowish, and anthers are 3mm long. The Gynophores are 2 to 2.5 cm long, ovary is ovoid and 6mm long. The fruit is a berry that is globose, 5x2 cm in diameter, ribbed, apiculate, reddish, and beaked above. The seeds are 6 to 8 and embedded in white or creamy pulp. It flowers and fruits from March to September. (Fig:1A-D)

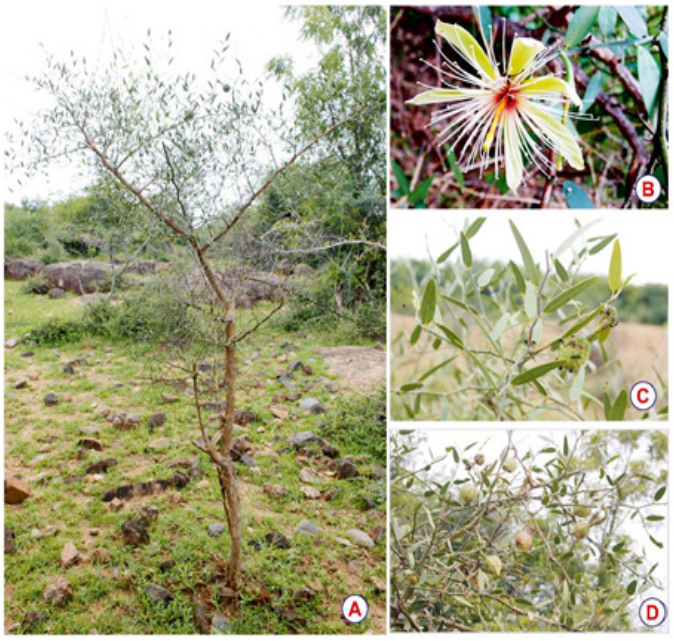


Fig: 1A-D Entire plant along with, flower and fruits

Phytochemical screening

The leaf extracts of *C. divaricata* were analyzed for the presence of secondary metabolites using phytochemical screening. A qualitative assay of the fruit of *C. divaricata* revealed a diverse range of phytochemicals. The results of the analysis were presented in Table-1 and Figures 2 and 3 to allow for a comparison of the solvents used for extraction. The screening revealed the presence of alkaloids in the chloroform, ethyl acetate, and methanol extracts, while carbohydrates were present in all extracts except for the hexane extract. Proteins, amino acids, leuco-anthocyanins, anthraquinones, quinones, and gums were not found in any of the extracts. Flavonoids and tannins were present in the ethyl acetate and methanol extracts, while saponins and phenols were only detected in the methanol extract. Steroids and terpenoids were found in the chloroform and ethyl acetate extracts only, while glycosides were present in the ethyl acetate and methanol extracts. Coumarins were identified in all extracts except for the chloroform extract, and resins were found in the petroleum ether and ethyl acetate extracts only (Table-1). The percentage yield of the hexane, chloroform, ethyl acetate, acetone, and methanol crude extracts were 11.8%, 11.1%, 12.7%, 12.3%, and 31.7% respectively.

Fig:2 Phytochemical extracts

Fig:3 Phytochemical test tube tests

Table-1: Phytochemical analysis of *C. divaricata*

S. NO	Pyto. Name	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol
1	Alkaloids	-	++	+	-	+
2	Carbohydrates	-	+	+	+	++
3	Proteins	-	-	-	+	-
4	Amino acids	-	-	-	+	-
5	Flavonoids	-	-	++	-	-
6	Saponins	-	-	-	-	++
7	Steroids & Terpenoids	-	+++	++	-	-
8	Phenols	-	-	-	-	+
9	Tannins	-	-	++	-	+
10	Glycosides	-	-	+	-	++
11	Coumarins	+	-	+	+	+
12	Anthraquinones	-	-	-	-	-
13	Quinones	-	-	-	-	-
14	Resins	+	-	+	-	-
15	Gums	-	+	-	-	-
16	Leuco-anthocyanins	-	-	-	-	-

"+" = present; "-"= absent

FTIR analysis

Characterization of the biochemical molecules extracted from *Capparis divaricata* leaves depending on FTIR spectrum analysis is

represented in Figure 4. The FTIR result revealed the presence of hydroxyl group (OH) by the peak at 3283.cm^{-1}, while frequency peak at 2915.93cm^{-1} 2848.78cm^{-1},1627.56 cm^{-1} refers to stretching of C-H alkene alkane and medium stretching C=C alkane group, 1732.93cm^{-1}, refers to strong stretching δ- lactone,1371.58 cm^{-1}, refers to medium stretching of C-H$_3$ phenolic group, strong stretching C-N aromatic amine at,1315.85cm^{-1} , 1229.32 cm^{-1} medium stretching frequency peak recorded at amine group C-N, 1026.75 cm^{-1} to the presence strong stretching C-N, the aliphatic group recorded.(Table-2 & Figure-4)

Table-2 *FTIR* of leaf extract of *C. divaricata*

Sr no.	Peek	Bonds	Bond strength	Bond vibrations	Functional groups
1.	3283.96	O-H	Strong	Stretching	Alcohol
2.	2915.93	C-H	Medium	Stretching	Alkene
3.	2848.78	C-H	Medium, strong	Stretching	Alkane
4.	1732.93	C=O	Strong	Stretching	δ- lactone
5.	1627.56	C=C	Medium	Stretching	Alkene
6.	1371.58	C-H3	Medium	Stretching	Phenol
7.	1315.85	C-N	Strong	Stretching	Aromatic amine
8.	1229.32	C-N	Medium	Stretching	Amine
9.	1026.75	C-N	Strong	Stretching	Aliphatic

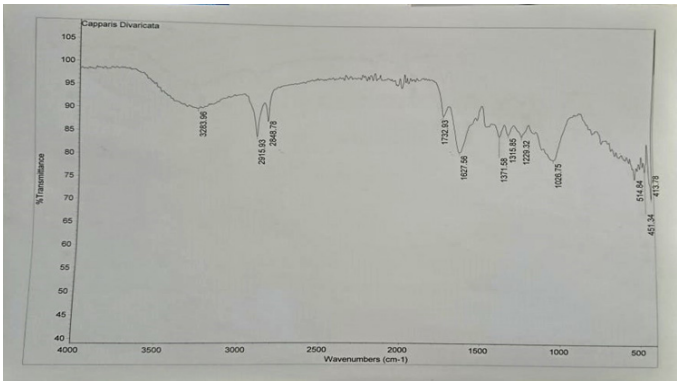
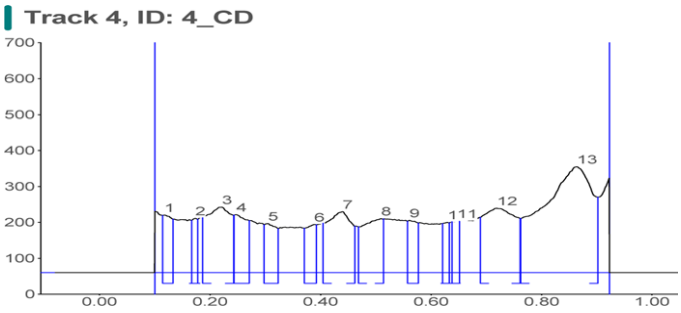


Fig.4: FTIR report of leaf extract of *C.divaricata*

Profile of HPTLC finger printing of *Capparis divaricata*

The chromatographic separation profile of leaf methanol extract scanned at 254 nm reveals thirteen spots (Figure-5), with spot 13 having the highest composition with Rf at 0.90. While the densitogram scanned at 366 nm revealed 12 spots, spot 12 had the highest composition at Rf 0.90 (Figure-7). It is evident from the data that these are characteristics of the studied drug, which will help in the identification and authentication of the drug. This can be considered a valuable standard in the pharmacopeia. At 254 nm, thirteen spots appear at Rf 0.12, 0.18, 0.23, 0.26, 0.32, 0.41, 0.46, 0.53, 0.58, 0.66, 0.67, 0.75, and 0.90 (all brown) (Figure-6) with various concentrations. while at 366 nm, 12 spots appear at Rf: 0.18 (blue), 0.23 (blue), 0.29 (yellow), 0.32 (blue), 0.35 (blue), 0.40 (yellow), 0.46 (yellow), 0.49 (blue), 0.58 (blue), 0.60 (blue), 0.75 (red), and 0.90 (red) (Figure-8). This is a vital fingerprint parameter to ensure the reliability and reproducibility of drugs.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.12	159.3	0.12	3.8	1.15	0.14	149.3	69.0	0.37
2	0.17	146.0	0.18	9.2	2.78	0.19	149.3	128.3	0.69
3	0.19	153.2	0.23	46.2	13.99	0.25	160.1	2746.1	14.82
4	0.25	160.4	0.26	29.2	8.85	0.28	145.2	937.8	5.06
5	0.31	133.7	0.32	11.9	3.60	0.34	122.7	279.8	1.51
6	0.39	123.9	0.41	9.4	2.85	0.41	133.3	171.8	0.93
7	0.42	137.9	0.46	43.1	13.04	0.48	129.7	2164.9	11.68
8	0.49	127.9	0.53	20.7	6.26	0.54	150.0	784.2	4.23
9	0.58	143.5	0.58	13.4	4.05	0.60	138.5	310.1	1.67
10	0.65	136.8	0.66	3.5	1.05	0.66	139.1	44.9	0.24
11	0.67	141.9	0.67	5.7	1.71	0.68	143.3	100.2	0.54
12	0.72	154.6	0.75	33.0	9.98	0.79	151.1	2169.7	11.71
13	0.80	151.4	0.90	101.4	30.70	0.94	210.5	8620.7	46.53

Fig. 5: HPTLC densitogram of Methanolic leaf extract of *C. divaricata* scanned at 254 nm by using chloroform: methanol: butanol (7: 2: 1 v/v)

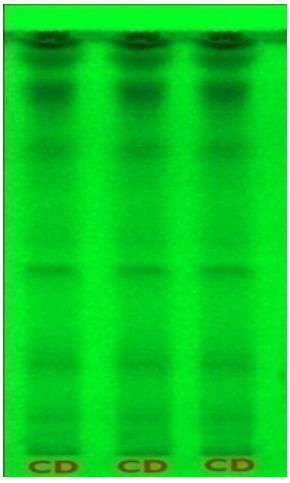
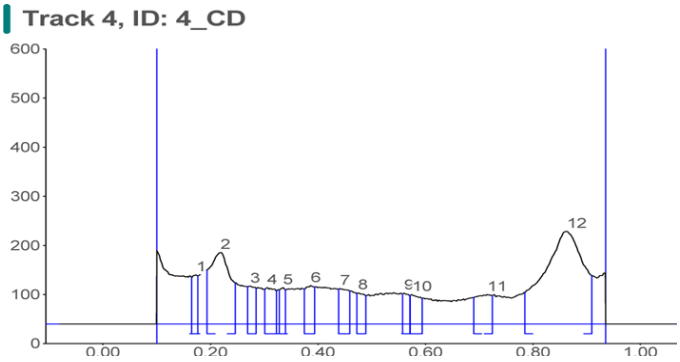


Figure-6: High performance thin layer chromatography (HPTLC) image of *C. divaricata* at 254 nm in chloroform: methanol: butanol (7: 2: 1 v/v)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.17	95.3	0.18	8.9	3.38	0.18	98.1	124.4	0.84
2	0.20	110.2	0.23	61.8	23.53	0.26	84.6	2862.2	19.36
3	0.28	76.5	0.29	2.7	1.03	0.30	72.8	40.3	0.27
4	0.31	71.1	0.32	4.7	1.78	0.34	67.5	96.5	0.65
5	0.34	69.4	0.35	7.7	2.91	0.35	70.0	98.9	0.67
6	0.39	71.8	0.40	15.4	5.85	0.41	75.5	372.2	2.52
7	0.46	71.3	0.46	13.4	5.11	0.48	67.3	371.3	2.51
8	0.49	62.1	0.49	6.6	2.53	0.51	58.2	124.7	0.84
9	0.58	61.7	0.58	12.1	4.61	0.60	58.6	216.7	1.47
10	0.60	58.7	0.60	10.1	3.84	0.62	52.5	250.0	1.69
11	0.72	54.3	0.75	10.0	3.80	0.76	58.2	435.8	2.95
12	0.82	64.5	0.90	109.5	41.64	0.95	98.7	9792.9	66.23

Figure-7: HPTLC densitogram of methanolic leaf extract of *C. divaricata* scanned at 366 nm by using chloroform: methanol: butanol (7: 2: 1 v/v)

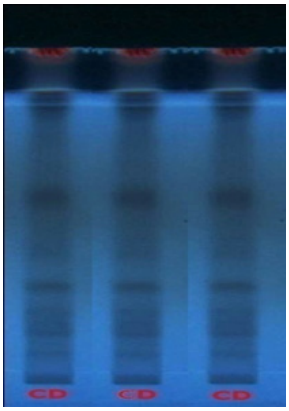


Figure-8: High performance thin layer chromatography (HPTLC) image of leaf extract of *C. divaricata* at 366 nm in chloroform: methanol: butanol (7: 2: 1 v/v)

DISCUSSION

The results of the phytochemical analysis revealed the presence of various compounds in the leaf extracts, including alkaloids, carbohydrates, flavonoids, tannins, saponins, phenols, steroids, terpenoids, glycosides, and resins, in the hexane, chloroform, ethyl acetate, acetone, and methanol extracts. However, proteins, amino acids, leuco-anthocyanins, anthraquinones, quinones, and gums were not found in any of the extracts. The highest yield of methanol crude extract was observed at 31.7%, while the lowest yield was observed in the chloroform extract at 11.1%. Additionally, FTIR data indicated the presence of phenolic compounds, hydroxyl groups, alkanes, alkenes, amines, aromatic amines, and aliphatic groups in *C. divaricata.* HPTLC analysis of the methanolic extract of the leaves showed the presence of 13 spots at 254 nm and 12 spots at 366 nm, with a spot at Rf 0.90 and a maximum concentration of 66.23% at 366 nm, which may serve as biomarkers and fingerprint parameters for identification of the plant.

CONCLUSIONS

The phytochemical components of the leaves attest that they are essential for good health and can be a source of new medications in the future. The results of the analyses will undoubtedly make it easier for them to quantify their estimation and isolation of chemical compounds that are pharmacologically active. The Fourier transform infrared spectroscopy, is a relatively quick and affordable approach for describing a functional group. It is a crucial instrument for figuring out the composition and structure of organic compounds as well as profiling biological substances that are present in herbal extraction. Herbal remedies are quite variable because they are made up of numerous different ingredients. Therefore, it is crucial to establish trustworthy chromatographic fingerprints that represent the chemically unique and pharmacologically active constituents of herbal medicine. For the accurate identification of medicinal plants, the HPTLC fingerprinting profile is a crucial criterion of herbal medication standardization. In future investigations, the *C. divaricata* quality and purity can be assessed using the current HPTLC fingerprinting profile as a diagnostic tool.

ACKNOWLEDGEMENT

The authors are thankful to Prof. P. Kamalakar, Department of Botany, Osmania University, Hyderabad, for providing facilities. The authors are thankful to Prof. Santhosh Kanade, School of Life Sciences, Department of Plant Sciences, University of Hyderabad, for providing FTIR analysis facilities.

REFERENCES

[1.] Mahomoodally, M. F. (2013). Traditional medicines in Africa: An appraisal of ten

potent African medicinal plants. Evidence-Based Complementary and Alternative Medicine, 2013, 1-14.

[2.] F. Anwar et al. Moringa oleifera: a food plant with multiple medicinal uses Phytother. Res. (2007)

[3.] Sahib, N.G., et al. (2013) Coriander (*Coriandrum sativum* L.): A Potential Source of High-Value Components for Functional Foods and Nutraceuticals: A Review. Phytotherapy Research, 27, 1439-1456.

[4.] WHO, 2022. https://www.who.int/news/item/25-03-2022-who-establishes-the-global-centre-for-traditional-medicine-in-india.

[5.] Ephraim P.L. and Helena N.P. Caper: The genus Capparis. Boca Raton; CRC press. 2014.

[6.] Hirave R.V., Kondawar M.S. Anti-Cancer Activity of *Capparis divaricata* Leaves Extract, International Research Journal of Pharmacy., 2016. 7 (8): 13-15

[7.] Jacintha Tamil Malar, Sarvana Gandhi and Uma Maheshwari.2017 phytochemical screening of the leaf extract of *Capparis divaricata* lam. By GC-MS analysis. International Journal of Applied and Pure Science and Agriculture (IJAPSA) Volume 03, Issue 1, e-ISSN: 2394-5532, p-ISSN: 2394-823X.

[8.] Trupti M. Rajamanya, Manish. Kondawar and Nitin N. Mali. 2012. Analgesic activity of Capparis divaricata Lam. and evaluation of synergistic activity of Capparis divaricata Lam. and Caesalpinia bonducella Linn. J. Pharm. Res., 5(8): 4259-4262.

[9.] R.V. Hirave and M. S. Kondawar.2017. Development & evaluation of herbal fast dissolving tablet of *Capparis divaricata* Lam International Journal of Advances in Pharmaceutics 2017; 06(01): 24-30.

[10.] Gunasekaran, M., and Balasubramanian, P., 2012.*Capparis divaricata* Lam. (Capparaceae) Ethnomedicinal uses of Sthalavrikshas (temple trees) in Tamil Nadu, southern India. A journal of plants, people, and applied research Ethnobotany Research and Applications.10:253-268.

[11.] Ramachandran, VS., 2007. Wild edible plants of the anamalais, Coimbatore district, Western Ghats, Tamilnadu. Indian journal of traditional Knowledge vol 6 (1) pp173-176.

[12.] Quattrocchi, U. (2012). CRC World Dictionary of Medicinal and Poisonous Plants: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology (5 Volume Set) (1st ed.). CRC Press. https://doi.org/10.1201/b16504.

[13.] Raman, N., 2006. Phytochemical Techniques. New India publishing Agency, New Delhi.

[14.] Anonymous., 2015b. Quality Standards of Indian Medicinal Plants, Medicinal Plants Division, Indian Council of Medicinal Research, New Delhi. Vol, 13,329-330.

[15.] Trease, G.E., and Evans, W.C., 1982. Pharmacognosy. Baillene Tindal, London. P -149.

[16.] Wagner, H., Bladt, X.S., Gain, Z., and Suie, E.M., 1996. Plant drug analysis. Springer Veralag, Berlin, Germany.360.

[17.] Fisher DD, 1968. Protein staining of ribboned epon section for light Microscopy. Histochem. 16:81-96.

[18.] Sasikumar R and Balasubramanian P, Govindaraj P and Krishnaveni T,2014. Preliminary Studies on Phytochemicals and antimicrobial activity of solvent Extract of Coriandrum sativum L. roots, Journal of Pharmacognosy and Phytochemistry. 2, 6: 74-78.

[19.] Ramakrishnan S, Prasannan KG and Rajan R, 1994. Textbook of medicinal biochemistry orient Longman, New Delhi, India.

[20.] Kokate CK, 1999. Practical Pharmacognosy. Vallabh Prakashan Publication, New Delhi, India. 4th Edition.

[21.] Yasuma A and Ichikawa, 1953. Ninhydrin-schiff and alloxan- Schiff staining. A new histochemical staining method for proteins

J.Lab clin Med.41: 296-299.

[22.] Ruthmann AC, 1970. Methods in cell research, Cornell University Press, New York, U.S.A.

[23.] Gahan PB, 1984. Plant Histochemistry and Cytochemistry: An Introduction Academic press, Florida, U.S.A.

[24.] Pakkirisamy M, Kalakandan SK and Ravichandran K. Phytochemical Screening, GC-MS, FT-IR Analysis of Methanolic Extract of *Curcuma caesia* Roxb (Black Turmeric). Pharmacog J. 2017;9(6):952-6.

[25.] Sravan Kumar S, Manoj P, Giridhar P. Fourier transform infrared spectroscopy (FTIR) analysis, chlorophyll content and antioxidant properties of native and defatted foliage of green leafy vegetables. J Food Sci Technol. 2015 Dec;52(12):8131-9.doi: 10.1007/s13197-015-1959-0.

[26.] Sethi, P.D., 1996. High-Performance Thin Layer Chromatography, CBS Publishers and Distributors, New Delhi.

[27.] Hook.F., 1872. Fl. Brit. India.1: 171,174.

[28.] Pullaiah, T., 2015a. Flora of Telangana The 29thState of India 1: 72, 81,373.

[29.] Reddy, K.N., & Sudhakar Reddy, C., 2016. Flora of Telangana State, India. 55.

[30.] Gamble, J.S., 1915. Flora of the Presidency of Madras, vol.1, 42, 45.