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Phytochemical Screening by, UV and FTIR, HPLC Analysis of *Capparis zeylanica* Leaf Extracts

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Abstract

Here, we determined in qualitative and quantitative study of phytochemicals by using UV, FTIR, HPLC, of *Capparis zeylanica*. The leaves of medicinal plant were extracted with 70% methanol. The extracts were scanned in the wavelength ranging from 260-900 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm^{-1} and their functional groups. The peak values of the UV and FT-IR were recorded. Each and every analysis was repeated twice for the spectrum confirmation. The phytochemical character of the *Capparis zeylanica*, leaves were investigated. Among the various extracts, methanol extract contains higher concentration of saponin, flavonoids, steroids, terpenoids, tannin and polyphenols as compare to aqueous, petroleum ether and hexane extract. Quantitative analysis showed that *Capparis zeylanica* leaves contain significant amount of total phenol, alkaloids, flavonoids and terpenoids. HPLC profiles of *Capparis zeylanica* leaves extract showed the presence five compounds namely Quercetin (5.467), myricetin (25.350) and Kaempferol (267.243) and confirmed based on the previous literature studied. Based on UV, FTIR, HPLC, spectrum analysis showed the evidences has been identified as flavonoids in the *Capparis zeylanica* leaves. These findings introduce *Capparis zeylanica* as a potentially useful for anti-oxidant, antidiabetic and anticancer.

***Key words*:** Quantitative study UV, FTIR, HPLC, *Capparis zeylanica*

Since ancient times, medicinal plants have been utilized as therapeutic agents to manage health and treat illnesses due to their capacity to promote health and contain bioactive components [1]. According to the prevailing reports, it is estimated that about 70–80% of world population, particularly in developing countries, depend on herbal medicine prevent and cure diseases [2]. The capacity to use active substances derived from plants or their synthetic equivalents in medicine has improved with the development of phytochemistry and pharmaceutical chemistry [3]. Plants have developed an array of defence strategies to manage oxidative stress. In these systems, there is a wide variety of antioxidants such as ascorbic acid, gluthione, uric acid, tocopherol, carotenoids, and phenols [4]. Secondary metabolites such as alkaloids, glycosides, flavonoids, saponins, tannins, steroids which have been extensively used in the drug and pharmaceutical industries. High Performance Liquid Chromatography (HPLC) analysis is most widely used methodology and easily adapted for the flavonoids quantification during last 20 years [5-6]. The flavonoids, which are the largest and most studied polyphenols, are gaining interest as antioxidants because of their high capacity to scavenge free radicals. Flavonoids prevent hydroxy radical induced damage 10 by donating an electron to neutralize the species [7]. *Capparis zeylanica* Linn. (Capparidaceae), commonly known as Indian caper, is a climbing shrub found throughout India and has been used as a Rasayanaí drug in the traditional Ayurvedic system of medicine [8]. *Capparis zeylanica* leaves have been used as folk medicine and an ingredient in various Ayurvedic preparations [9]. An attempt was made to evaluate the Phytochemical Screening by HPLC, UV and FTIR Spectroscopy of *Capparis zeylanica* leaf extracts.

MATERIALS AND METHODS

Collection and preparation of plant extracts

New leave of *Capparis zeylanica* were collected and dried in room temperature. The leaf powder was subjected to successive Soxhlet extraction by various solvents namely methanol, ethanol, aqueous, petroleum ether and hexane. The concentrated extract was separated through Whatman No.1 paper and stored at 4 °C until further use [10].

Preliminary phytochemicals screening

Phytochemical screening was carried out on the plant extracts using different solvents to identify the secondary metabolites. Harborne [11-12], Kumar *at al*. [13].

UV and FTIR spectroscopic analysis

In this proximate analysis, the extracts were examined in both visible and UV light. The extracts were centrifuged at 3000

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rpm for 10 minutes in order to filter them through Whatman No. 1 filter paper using a high-pressure vacuum pump in preparation for UV and FTIR spectrophotometer examination. Using the same solvent, dilute the sample to a ratio of 1:10. Using a Perkin Elmer Spectrophotometer, the extracts were scanned in the wavelength range of 260-900 nm, and the distinctive peaks were found. The Perkin Elmer Spectrophotometer equipment was utilized for FTIR analysis in order to identify the distinctive peaks and their functional groups in the 400–4000 cm^{-1} range. Both the FTIR and UV peak values were noted. For the purpose of confirming the spectrum, every analysis was conducted twice [14].

High performance liquid chromatography

The chromatographic analysis of HPLC was performed using the Shimadzu HPLC system's chromatographic equipment, which included the Supelco C18 (RP) column (25 cm × 4.6 mm; 5 µm) regulated at 30°C, the diode array detector (SPD-M10AVP), and the LC - 10ATVP pump. Software from the Shimadzu Class VP Series was used for data collection and peak integration. Results were compared to standards at UV detection wavelengths of 278 nm. The sample was prepared according to the procedure. The extraction was carried out using 2 ml of fermented broth with 50 mL of 95% ethanol under 80 KHz, 45°C in ultrasonic extraction device for 30 min, repeated twice. Methanol and 0.1% v/v acetic acid made up the gradient system of mobile phase used in the investigation. 0–15 min, 5% methanol; 15–40 min, 80% methanol; 40–42 min, 5% methanol; and 42–50 min, 5% methanol were the intervals employed in the gradient. A 20 µl injection volume and a flow rate of 1.0 ml/min were used Samee *et al*. [15].

RESULTS AND DISCUSSION

On the basis of therapeutic potential of secondary metabolites, the phytochemical characters of the *Capparis zeylanica* leaves were investigated and represented in (Table 1). The phytochemical character of the *Capparis zeylanica* leaves were investigated. Among the various extracts, methanol extract contains higher concentration of saponin, flavonoids, steroids, terpenoids, tannin and polyphenols as compare to ethanol aqueous, petroleum ether and hexane extract. These qualitative phytochemical screening results were similar to those reported by Nour and El-imam Y [16].

Table 1 Preliminary phytochemical analysis in *Capparis zeylanica* leaf extracts

S. No.	Phytochemicals	Methanol extract	Ethanol extract	Aqueous extract	Petroleum ether extract	Hexane extract
1	Tannin	++	++	+	-	-
2	Phlobatannins	-	-	-	-	-
3	Saponin	+	+	++	-	-
4	Flavonoids	++	++	++	-	-
5	Steroids	++	++	-	+	+
6	Terpenoids	++	++	+	+	+
7	Triterpenoids	+	+	-	-	++
8	Alkaloids	++	++	-	-	-
9	Anthroquinone	++	++	++	-	+
10	Polyphenol	++	++	+	+	+
11	Glycoside	++	++	+	+	+
12	Coumarins	++	-	++	-	-
13	Emodins	-	-	++	-	-
14	Anthocyanins	+	+	-	-	-

"+" indicates presence of the compounds; "-" indicates absence of the compounds, "++" indicates the high concentration

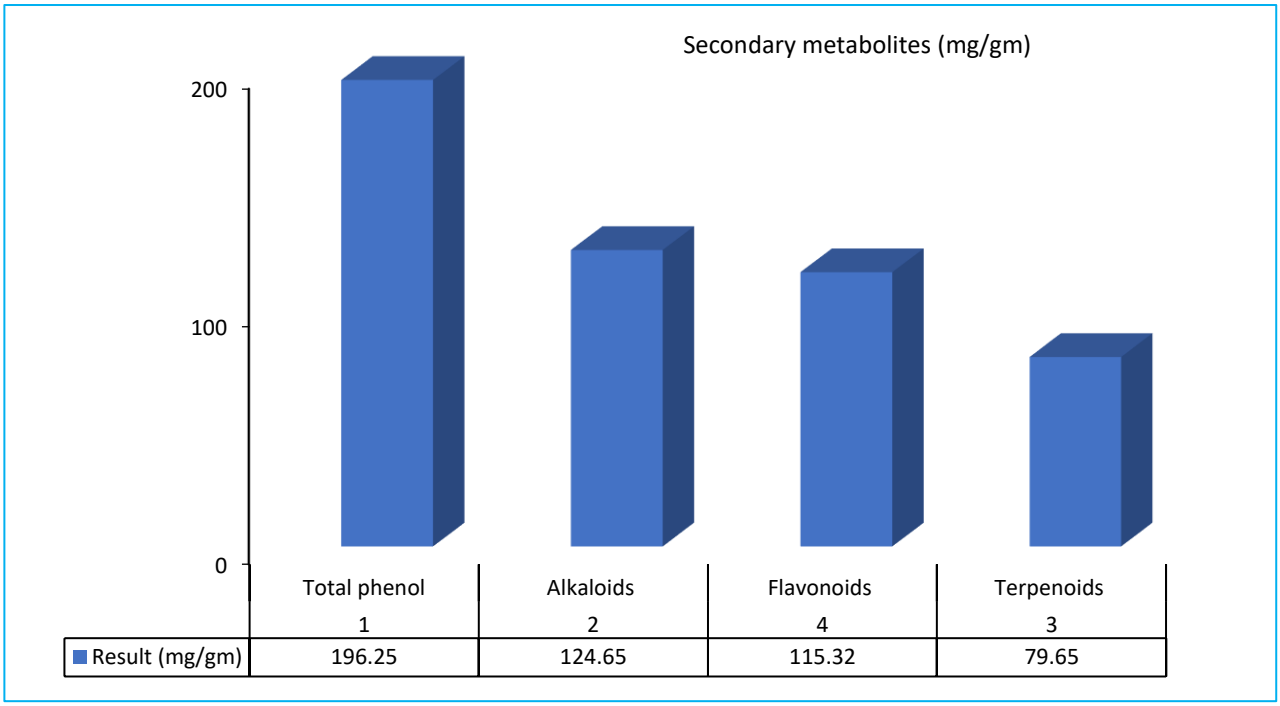

Fig 1 Quantitative analysis of methanolic extract of *Capparis zeylanica*

Table 2 Quantitative analysis of methanolic extract of *Capparis zeylanica* leaves

S. No.	Secondary metabolites	Result (mg/gm)
1	Total phenol	196.25 ± 18.65
2	Alkaloids	124.65 ± 13.25
3	Flavonoids	115.32 ± 12.44
4	Terpenoids	79.65 ± 9.02

Values are expressed as Mean ± SD for triplicates

Quantitative phytochemical analysis

Quantitative analysis of *Capparis zeylanica* leaves was investigated and represented on (Table 2, Fig 1). Significant amount of Total phenol (196.25 ±18.65), Alkaloids (124.65 ± 13.25), Terpenoids (79.65 ± 9.02) and Flavonoids (115.32 ± 12.44) were present. The above phytoconstituents were tested as per the standard methods. Among the various phytoconstituents, phenol was found to be present in highest concentration. The similar report revealed the presence of bioactive compound reported in fruits of C. decidua are rich in phenolics and flavonoids, which are the compounds primarily responsible for antioxidant, antibacterial and antidiabetic potential of kair fruits [17]. Tlili *et al*. [18] studied phenolic content and Ghafoor *et al.* [19] evaluated total phenolic content and total flavonoid content values in the *C. spinosa* fruits, the values of which were somewhat similar to the values of the current study.

UV spectrum analysis

The result of UV-VIS spectroscopic analysis confirms the presence of flavonoids in the *Capparis zeylanica* leaves extract and the peak values represent in (Table 3, Fig 2). There were six peaks were recorded in leaf extract.

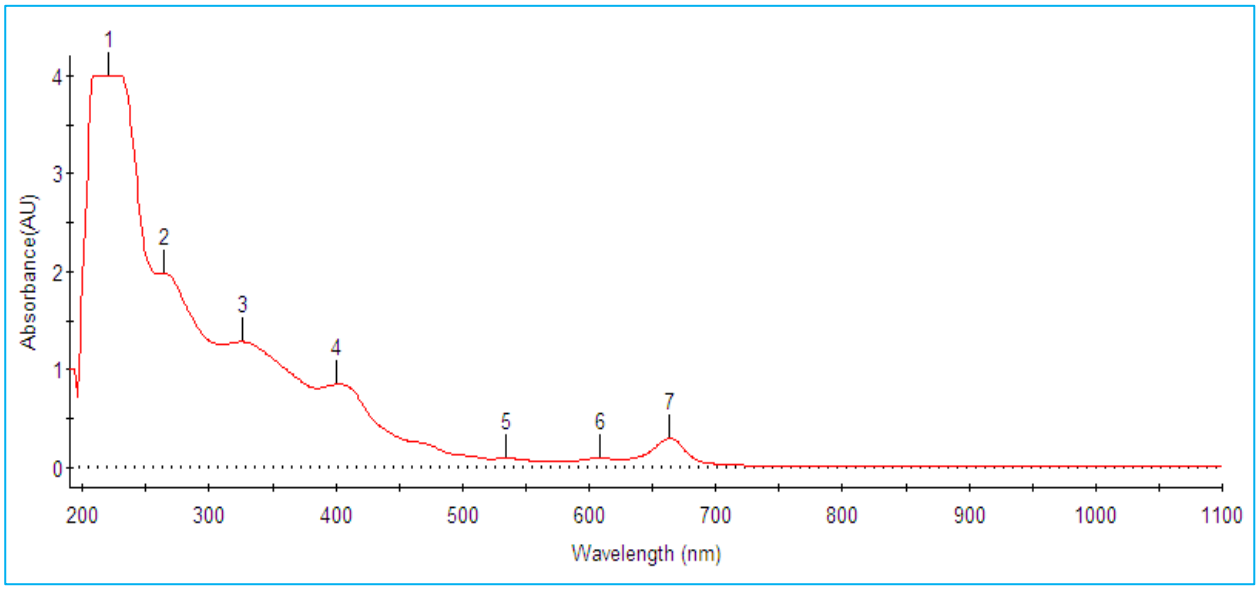

Fig 2 UV spectrum of *Capparis zeylanica*

Table 3 Peak area of sample under UV visible spectral analysis

S. No.	Peak (nm)	Peak (AU)
1	219.90	4.0000
2	263.80	1.9826
3	325.70	1.2835
4	401.30	0.8479
5	534.20	0.0947
6	608.70	0.0951
7	663.25	0.0297

FTIR spectrum analysis

The FT-IR is proved to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plant extracts. The FTIR spectrum of the *Capparis zeylanica* is given in (Fig 3, Table 4). The FTIR analysis of *Capparis zeylanica* gave broad peak at 3369.62cm^{-1} which indicated the presence of phenolic C-OH stretching. It showed strong peaks at 1652.04cm^{-1} (–C=C– stretch) which indicated the presence of alkenes whereas the peaks at 1407.34 cm^{-1} attributed to presence of C–C stretch (aromatic ring). The results of the study supported that the presence of quercetin and phenolic groups. These functional groups represent the primary, secondary and tertiary hydroxyl groups, and on the basis of these chemical bonds, a compound can be determined [20].

Table 4 FT-IR peak values and functional groups identified in the extract of *Capparis zeylanica* leaves

Peak	Bond	Functional group
3369.62	O–H Stretch, H–Bonded	Alcohols, Phenols
2975.58	C-H stretch	Alkanes
2927.61	C-H stretch	Alkanes
2900.95	C-H stretch	Alkanes
2133.53	-C≡C- stretch	Alkynes
1652.04	-C=C- stretch	Alkenes
1453.34	C-H bend	Alkanes
1407.34	C-C stretch (in-ring)	Aromatics
880.81	C-H “oop”	Aromatics

HPLC analysis of leaves extract of Capparis zeylanica

The HPLC chromatogram showed presence of 3 peaks in the methanolic extracts (Fig 4, Table 5). Each compound was analyzed individually using the mobile gradient phase consisting of ethanol and 1% acetic acid in water during 30 minutes run time. The peak numbers, retention time (min) and peak height is presented in (Table 3). HPLC profiles of *Capparis zeylanica* leaves extract showed the presence five compounds namely Quercetin (5.467), myricetin (25.350) and Kaempferol (267.243) and confirmed based on the previous literature studied. The retention time were more or less similar to that of previous studies [13]. These similar studies were reported in *Capparis grandis* by Khandare [21].

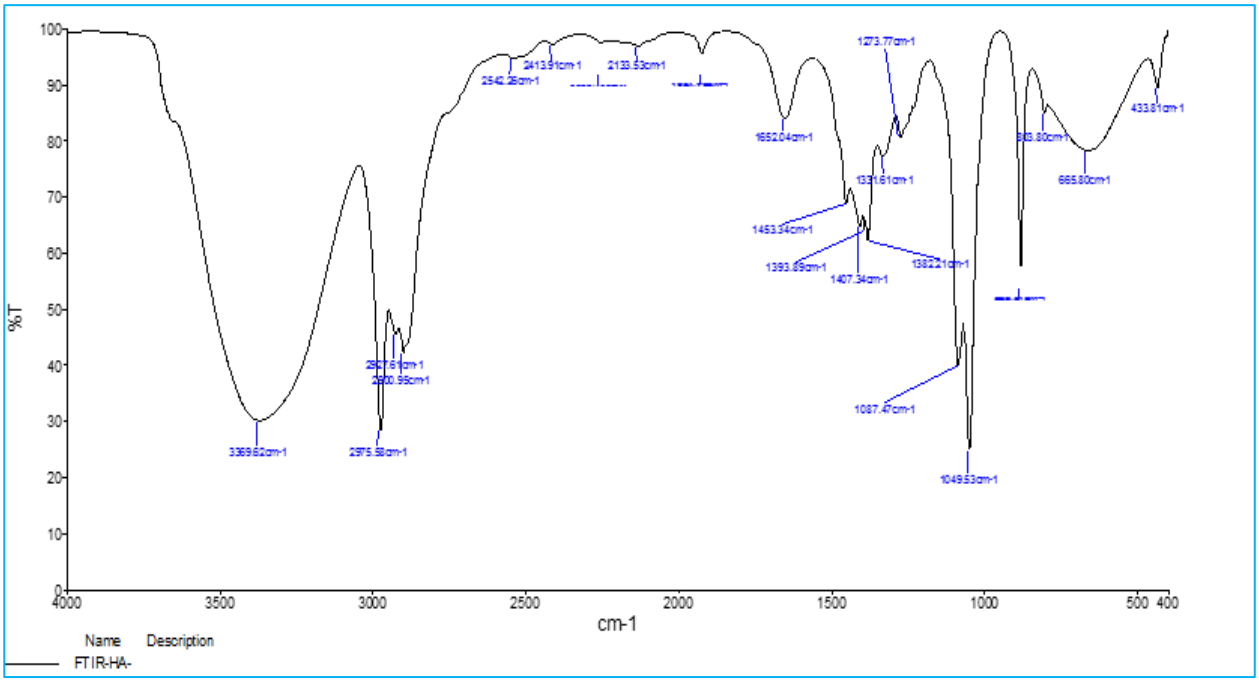

Fig 3 FTIR spectrum of *Capparis zeylanica*

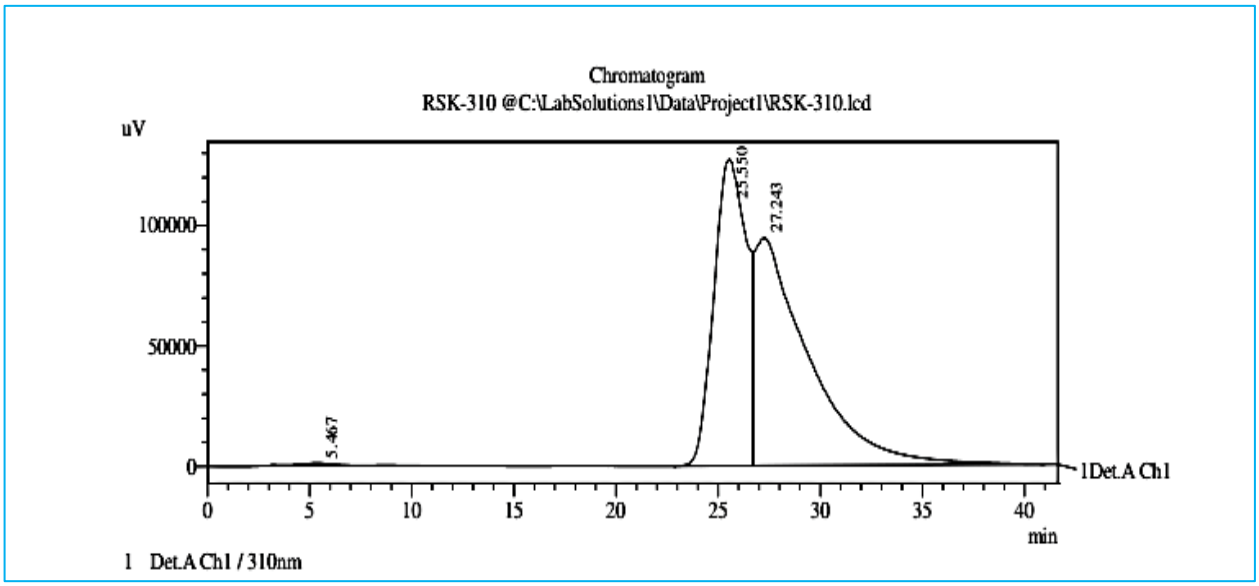

Fig 4 Chromatogram of HPLC analysis of *Capparis zeylanica* leaves extract

Table 5 HPLC analysis of *Capparis zeylanica* leaves extract

Peak	Retention time	Area %	Height	Height %	Literature (RT)	Name of the compound
1	2.802	77.054	62705	2.700	2.879	Ascorbic acid [22]
2	5.467	20.158	10775	14.210	5.970	Quercetin [23]
3	25.350	0.364	677	0.893	25.567	Myricetin [24]
4	27.243	0.065	152	0.201	27.463	Kaempferol [24]

CONCLUSION

In this study the methanolic extract of the *Capparis zeylanica* leaf extracts were analyzed for their active components of medicinal values by UV-VIS, FTIR, and HPLC analysis. The major constituents belong to the alkaloids and fatty acids. These identified components are used in curing various diseases as reported by the researchers. Overall, the comprehensive phytochemical profiling of Capparis zeylanica underscores its potential for pharmacological applications, aligning with its traditional use in treating various ailments and supporting the need for further detailed studies on its medicinal properties. *Capparis zeylanica* contain variety of bioactive compounds that have medicinal potentials and therefore need to be fully investigated for their pharmacological activities.

Conflict of interest

We declare that we have no conflict of interest.

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