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Original Research Article

Pharmacognostic, phytochemical, biological and spectroscopic analyses of *Capparis decidua* (Forsk.) Edgew root and stem bark

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Abstract

Purpose: *To investigate the pharmacognostic, phytochemical, biological and spectroscopic analyses of Capparis decidua (Forsk.) Edgew root and stem bark.*

Methods: *Plant material (root and stem bark) was collected, authenticated, shade-dried and extracted by maceration using methanol as a solvent separately. Powder microscopy was performed using a binocular microscope. Fluorescence, physico-chemical analysis and phytochemical screening for the presence of secondary metabolites were performed using standard methods. Brine shrimp lethality bioassay was carried out using Artemia salina bioassay, while enzymatic modulatory study was performed by α-amylase inhibition assay. Microscopic analysis was carried out with scanning electron microscopy. Spectroscopic analysis was performed by Fourier transform infrared spectroscopy (FTIR).*

Results: *Powder microscopy showed the presence of different cellular structures. Various colors were observed under ultraviolet (UV) and ordinary light when treated with different reagents. Phytochemical screening revealed the presence of alkaloids, tannins, saponins and flavonoids but phenol and cardiac glycosides were absent from both extracts. The root bark of the plant showed significant brine shrimp lethality activity.*

Conclusion: *Capparis decidua (Forsk.) Edgew root and stem bark contain a variety of bioactive compounds that have medicinal and therapeutic potentials. Therefore, further investigations are required to elucidate their pharmacological properties.*

Keywords: *Capparis decidua (Forsk.) Edgew, Phytochemical, Spectroscopy, α-Amylase*

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INTRODUCTION

Majority of the synthetic drugs used at the present time for analgesic and antinociceptive effect have many side and toxic effects. Utilization of plant-based products is increasing because 80-90% rural population of developing countries use herbal products for prevention,

diagnosis, treatment and cure of diseases. For these reasons, the use of plant-based products is increasing day by day as 80 – 90 % of the world's population using herbal products for diagnosis, prevention, treatment and cure of many problems. Plants still account for a large untapped resource of structurally novel active compounds that might serve as lead source for the development of growth of synthetic novel drugs [1].

The genus *Capparis* comprises 250 species which include shrubs, trees and woody climbers. *Capparis decidua* (Forsk.) Edgew commonly known as Karer, Karukaril, or Karel, is a densely branching shrub or a small tree of the Thar Desert of Pakistan, also found in Central America, South American countries, southeast United States and southeast China [2]. Alkaloids, terpenoids, glycosides, fatty acids β-sitosterol [3], capparidisine [4], capparasinine [5], isocodonocarpine, codonocarpine, [6], rutin and stachydrine are present in different parts of the plant [7]. The plant possesses anti-hypercholesterolemic [8], anti-inflammatory, antidiabetic [9], antiplaque, antihypertensive [7], antimicrobial, anthelmintic, analgesic and purgative properties [10]. Due to their versatile medicinal properties and pharmacological actions of *Capparis decidua* (Forsk.) Edgew*,* the present research work was planned to standardize the different parts of plant using spectroscopic.

EXPERIMENTAL

Collection and authentication

Capparis decidua (Forsk.) Edgew root and stem bark was collected from the vicinity of District Muzaffar Garh (Punjab) in 2018. The plant parts such as root and stem bark were cleaned, identified and authenticated by Dr Zafar Ullah Zaffar, Taxonomist, Department of Botany Bahauddin Zakariya University Multan with a voucher specimen (no. Stewart F. W. Pak. 293) and the specimen deposited in herbarium of Department of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan.

Preparation of extract

Powdered root bark and stem bark of the plant were made after shade drying for 30 days at room temperature separately by using mechanical grinder. These powders were preserved in the air tight container at cool place. Then 500 g of each part was soaked separately in 1.0 L of distilled ethanol for 7 days in amber-colored glass bottles. The solvent was filtered through Whatman's filter paper No 1. The filtrate obtained was concentrated using rotary vacuum evaporator (Rotavapor-R-200 Buchi) at 40 - 50 °C. The *Capparis decidua* (Forsk.) Edgew ethanolic extract of root bark (CDERB) and ethanolic extract of (CDESB), were weighed and stored inat 4˚ C in biomedical freezer (Sanyo Biomedical freezer, model MDF- U333, Japan).

Macroscopic analysis

Standard protocols were used for macroscopic studies to analyse the size, colour, taste, feature, texture and shape of *Capparis decidua* (Forsk.) Edgew*.* Root and stem bark [11].

Microscopic and histochemical studies

Microscopic studies of *Capparis decidua* (Forsk.) Edgew root and stem bark was performed according to the standard protocol established by World Health Organization [12].

Fluorescence analysis

Fine powder of root and stem bark (1 g each) was placed in a test tube and freshly prepared various reagents were added in different test tubes separately. It was shaken gently and placed for half an hour. Color of each test tube was observed in daylight, then placed these test tubes in UV lamp and observed the change in color at 254 nm and 365 nm wavelength [13].

Physicochemical analysis

Physico-chemical analysis was performed according to the protocol already established protocol.

Loss on drying

Powdered of root and stem bark (2.0 g) of each was weighed and placed in clean dried china dish. China dish with powdered material was weighed and dried in oven at 105 ^{o}C. Then heat it up to the constant weight until difference of not more than 0.5% is achieved between two successive readings on drying. The result was calculated by following formula:

% LOD = Weight of the sample after drying/ weight of the sample before drying × 100 [13].

Foaming index

Powdered root and stem bark (1.0 gm) each were taken in a separate conical flask with 100 mL of water and was heated for 30 minutes. The mixture was cooled, filtered and volume was

made up to 100 mL. After that 10 test tubes were taken and 1 to 10 mL of filtrate was added in each test tube in a gradually increasing sequence. The volume was adjusted up to 10 mL and the test tubes were shaken for 15 second and kept in a rack for 15 minutes without any disturbance after that height of froth was measured [13].

Swelling index

Powdered sample (2.0 g) of each plant part was taken in 25 mL stoppered flask and was shaken for 1.0 hr. The sample was kept for three hours and volume was measured occupied by the sample [13].

Phytochemical screening

Phytochemical screening was performed according to the standard described procedures [14].

The preliminary phytochemical screening of the methanol extracts of *Capparis decidua* (Forsk.) Edgew root and stem bark were performed by adopting the standard procedures.

Wagner's test

Methanolic extract of root and stem bark (2 mL) were taken in test tubes separately. 1 mL freshly prepared wagner's reagent was added in both the test tubes. The reddish-brown precipitates appeared which indicates the presence of alkaloid.

Dragendorff's test

Methanolic extract of root and stem bark (2 mL) were taken in a test tube separately. Add one 1 mL of freshly prepared Dragendorff's reagent in each test tube. Appearance of the white precipitates indicated the presence of alkaloid.

Mayer's test

Methanolic extract of root and stem bark (2 mL) were taken in a test tube separately. Add 1 mL of Mayer's reagent in both test tubes. The formation of creamy precipitates indicated the presence of alkaloid.

Hager's test

Methanolic extract of root and stem bark (2 mL) were taken in a test tube separately. Add 1 mL of Hager's reagent in both test tubes. The formation of yellow precipitates indicated the presence of alkaloid.

Tannic acid test

Methanolic extract of root and stem bark (2 mL) were taken in a test tube separately. Add 1 mL of tannic acid solution in both test tubes. The formation of buff color precipitates indicated the presence of alkaloid.

Killar Killani test

Methanolic extract of root and stem bark (2 mL) were taken in a test tube separately. 2 mL glacial acetic acid and 2 drops of $FeCl_3$ solution were added followed by the addition of conc. H_2SO_4 along the walls of the test tube.

A brown colored ring appearing at the interface of the test tube confirms the presence of cardiac glycoside.

Legal test

Methanolic extract of root and stem bark (2 mL) were taken in the test tube separately and dissolved in pyridine. Sodium nitroprusside solution was added to it to make it alkaline. Appearance of pink or red color indicates the presence of cardiac glycoside.

Water test

Methanolic extract of root and stem bark (3 mL) were taken in the test tube separately. Add 5 mL distilled water in both the test tubes and shake them vigorously. Froth formation confirms the presence of saponins.

Blood test

Methanolic extract of root and stem bark (1 mL) were taken in the test tube separately, to which were added 2 - 3 drops of blood. Hemolysis of blood confirmed the presence of saponins.

Ferric chloride test

Methanolic extract of root and stem bark (1 mL) were taken in the test tube separately, to which were added 4 - 5 drops of $FeCl_3$ solution. Appearance of a blackish precipitate confirmed the presence of tannins.

Lead acetate test

Methanolic extract of root and stem bark (2 mL) were taken in the test tube separately. Add 2 - 3 drops of 1 % lead acetate. Yellowish precipitate confirms the presence of tannins.

Gelatin test

Methanolic extract of root and stem bark (2 mL) were taken in the test tube separately and added 1 mL of 1% gelatin solution. White precipitate indicated the presence of tannins.

Lead acetate test

Methanolic extract of root and stem bark (2 mL) were taken in the test tube separately and added 1 mL of basic lead acetate. Reddish-brown precipitate indicated the presence of flavonoids.

$FeCl_3$ test for flavonoids

Methanolic extract of root and stem bark (2 mL) were taken in the test tube separately and mixed few drops of $FeCl_3$. Blackish precipitate indicated the presence of flavonoids.

Alkaline solution test

Methanolic extract of root and stem bark (2 mL) were taken in the test tube separately and added 1 mL of NaOH. Yellow to red precipitate indicated the presence of flavonoids.

H_2SO_4 test

Methanolic extract of root and stem bark (2 mL) were taken in the test tube separately and added 2 mL of concentrated H_2SO_4. Appearance of yellow to orange color indicated the presence of flavonoids.

Stain test

Methanolic extract of root and stem bark (100 mg) were pressed between two folds of filter paper. Stains left on paper indicated the presence of fixed oils.

Saponification test

Methanolic extract of root and stem bark (2 mL) were taken in the test tube separately and added 2 mL of NaOH. Saponification indicated the presence of lipids.

Ferric chloride test for phenols

The methanol extract of root and stem bark (2 mL) were taken in the test tube separately and added 1 mL of $FeCl_3$ solution.

Appearance of intense blue color indicated presence of phenol in test solution.

Hydrochloric acid (HCl) test

Methanolic extract of root and stem bark (2 mL) were taken in the test tube separately and added 1 mL HCl. Appearance of pink color indicated the presence of resins.

Ferric chloride test for resin

Methanolic extract of root and stem bark (2 mL) were taken in the test tube separately and added 1 mL $FeCl_3$ solution. Appearance of intense green color indicated the presence of phenol in test solution.

Test for quinones

Methanolic extract of root and stem bark (2 mL) were taken in test tube separately to which was added 2 mL HCl. Appearance of yellow color indicated the presence of quinones.

Brine shrimp lethality assay

The sample (ethanolic extract of root and stem bark, 20 mg of each) was dissolved separately in 2 mL of solvent and concentration was made 10, 100 and 1000 µg/mL respectively and kept them overnight for drying, then hatched 10 larvae/vials was placed using pipette.

Final volume was made up to 5 mL with sea water and incubated at 25 - 27 ^oC for one day under light, after that number of survivors in each vial was calculated. The experiment was performed in triplicate. Etoposide was used as a standard drug and tested as a positive control. Finney computer program was used to analyze the data for the determination of LD_{50} with 95 % confidence intervals [15,16].

Enzymatic activity

Plant extract (root and stem bark) of different concentrations was incubated along with 50 µg/mL of porcine pancreatic alpha amylase at 37 ^oC for 10 min. After incubation, substrate solution (1 % starch) was added in each test tube. Alpha amylase without extracts was used as control for starch. The absorbance was then estimated using DNSA assay at 540 nm. The inhibitory activity was calculated using Eq 1 [17].

$$\text{Inhibition (\%)} = \{(Ac - At)/Ac\}100 \quad \ldots\ldots\ldots\ldots (1)$$

Where Ac and At are the absorbance of control and test samples, respectively.

Microscopic analysis

Scanning electron microscope was used to carry out surface morphology of root and stem bark powder. Sample powder was placed in the sample chamber of SEM and scaaning was performed at a different magnification ranging from 1000 to 4000 at 10, 20 and 40 µm.

Spectroscopic analysis

Spectroscopic analysis was performed by using Fourier transform infrared spectroscopy (FTIR) (Bruker Tensor series 27, Germany) with ATR technology. The infrared spectra of extract was recorded from 500 to 4000 cm^{-1} wavenumber.

RESULTS

Macroscopic analysis shows that root bark was light brown in colour, with smooth texture and tough fracture, while the stem bark was whitish grey in colour, with rough texture and fibrous fracture as shown in Table 1.

Table 1: Macroscopic characters of *Capparis decidua* (Forsk.) Edgew

Characteristic	Root bark	Stem bark
Color	Brown	Whitish-grey
Odor	Odorless	Odorless
Taste	Slightly bitter	Tasteless
Fracture	Tough	Fibrous
Texture	Smooth	Rough

Physicochemical analysis results are presented in Table 2 which indicate that total ash of root bark was 0.45 g, acid insoluble ash was 0.025 g and water-soluble ash was 0.045 g whereas stem bark total ash value was 0.045, acid insoluble ash was 0.005 g and water-soluble ash was 0.01 g.

Table 2: Physicochemical parameters of *Capparis decidua* (Forsk.) Edgew root and stem bark

Parameter	Root bark	Stem bark
Total ash (g)	0.45	0.045
Acid insoluble ash (g)	0.025	0.005
Water soluble ash (g)	0.045	0.01
Swelling index (mL)	7.5	5.5
Foaming index	<100	<100
Loss on drying (%)	0.50 ± 0.01	0.7 ± 0.01

The results of fluorescence analysis of root and stem bark powder showed characteristic coloration under visible and UV light upon treatment with different chemical reagents (Table 3).

Powder microscopy of root bark showed the presence of different structures like calcium oxalate crystal, fibers, parenchyma and starch granules, while stem bark showed the parenchyma, starch, sclereoids and non-glandular trichome, as indicated in Figure 1 and Figure 2, respectively.

The phytochemical investigation of the methanolic extract of root and stem bark of *Capparis decidua* (Forsk.) Edgew showed the presence of alkaloids, resin, tannins, flavonoids, saponins and fats while cardiac glycosides and phenols were absent in both extracts (Table 4).

The result of the Brine Shrimp lethality of the methanolic extract of *Capparis decidua* is given in Table 5. The extracts did not show any enxymatic activity (Table 6)

Table 3: Fluorescence analysis of *Capparis decidua* (Forsk. Edgew) root bark and stem bark

Powdered drug	Visible/daylight		UV Light	
	Root bark	***Stem bark***	***Root bark***	***Stem Bark***
Powder + water	Brown	Light brown	White	Turquoise
Powder+ conc. HNO_3	Light orange	Orange	Blackish brown	Brown
Powder + chloroform	Light brown	Light brown	Light brown	White
Powder+ acetic acid	Brown	Light brown	Light green	Turquoise
Powder+ conc.H_2SO_4	Black	Green	Light green	Green
Powder + methanol	Brown	Light green	White	white
Powder + Iodine	Light brown	Light brown	Light green	Turquoise
Powder + glycerin	Brown	Light brown	White green	Turquoise
Powder + 5% $FeCl_3$	Yellowish green	Blood red	Blackish green	Black
Powder + 1% NaOH	Dark brown	Brown	Light Cream	Light green
Powder+glacial acetic acid	Light brown	Light brown	Cream	Turquoise
Powder + Picric acid	Brown	Yellow	Brown	Brown
Powder + NH_4OH	Brown	Dark brown	Turquoise	White
Powder + acetonitryl	Mud Brown	White	White	light pink
Powder + Diethyl ether	Light brown	Light brown	White	Turquoise

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The scan of the electron microscopy of the plant root and stem bark is provided in Figure 3 while the FTIRscan are shown in Figure 4

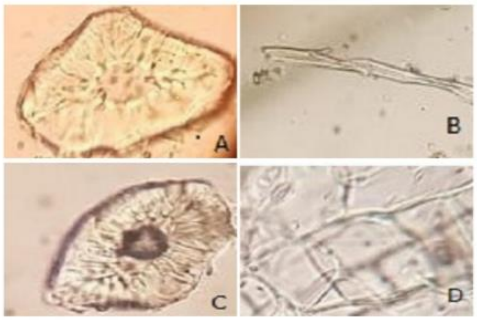

Figure 1: Powder microscopy of root bark *Capparis decidua* (A) calcium oxalate crystal (B) Fibers (C) Parenchyma (D) Starch

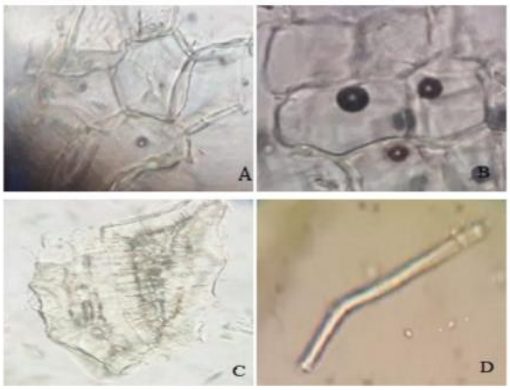

Figure 2: Powder microscopy of Capparis decidua (A) parenchyma, (B) starch (C) Sclereids (D) non-glandular trichome

Table 4: Phytochemical profile of *Capparis decidua* (Forsk.) Edgew root and stem bark

Test	Reagent	Root bark	Stem bark
	Dragendorff	+ + +	+ + +
Alkaloids	Wagner	+ + +	+ + +
	Mayer	+ +	+ +
	Hager	+ +	+ +
Resin	$CuSO_4$	+ +	+ +
Cardiac glycoside	Killer Killani	-	-
	Lead acetate	+ +	+ +
Tannins	$FeCl_3$	+ +	+ +
	Gelatin	+ +	+ +
	Lead acetate	+ +	+ +
Flavonoids	$FeCl_3$	+ +	+ +
	NaOH	+ +	+ +
Phenols	$FeCl_3$	-	-
Fats	Saponification	+	+
Quinones	KOH	-	-
Saponins	Dist. water	+ +	+ +

Table 5: Brine Shrimp lethality of the methanolic extract of *Capparis decidua* (Forsk.) Edgew root and stem bark

Dose (µg/mL)	No. of shrimps	No. of survivors		Mortality (%)		Mortality (%)
		Root bark	Stem bark	Root bark	Stem bark	
10	30	27	21	10	30	
100	30	13	20	56.66	33.33	46.66
1000	30	04	19	86.66	36.66	

Table 6: Enzymatic activity of methanolic extract of Capparis decidua (Forsk.) Edgew root and stem bark

Part used	Activity	Standard drug	Standard enzyme
Root bark	No activity	Acarbose	α-Amylase
Stem bark	No activity		

DISCUSSION

Standardization of medicinal plants and their extract have great importance because quality control plays a major role in the safety and efficacy of drug. It starts at the initial stages from the production of quality materials because according to World Health Organization (WHO), before testing any herbs for the corresponding pharmacological activity, it needs to be standardized by set of guidelines for establishing identity and purity of the drug materials [18]. Ash

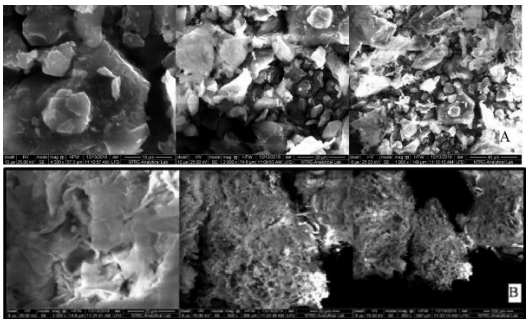

Figure 3: Scanning electron microscopy of *Capparis decidua* (Forsk.) Edgew (A) root bark (B) stem bark

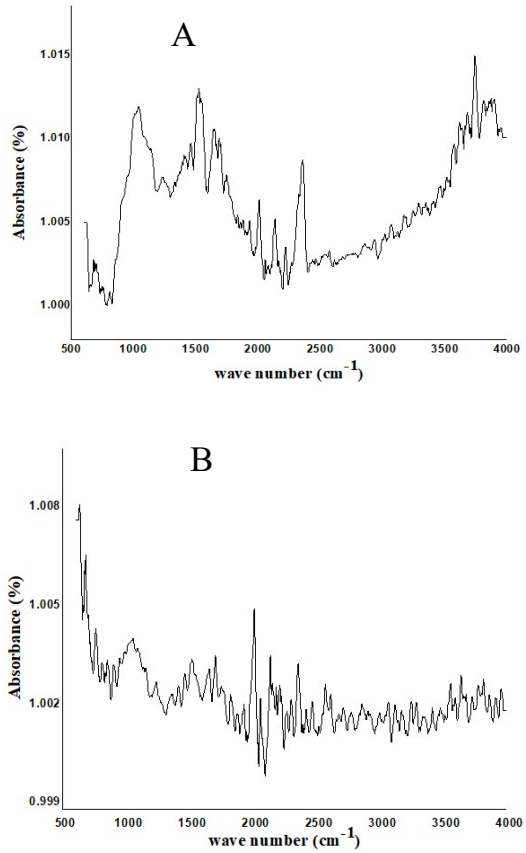

Figure 4: FTIR of *Capparis decidua* (Forsk.) Edgew root bark (A) and stem bark (B)

value is important parameter for the determination of quality and purity of crude drugs because it is helpful for the identification of different impurities like silicate, carbonate and oxalate. The presence of inorganic compounds is determined by water soluble ash, while acid insoluble ash is used for the determination of contamination with earthy material [19].

Fluorescence is an important phenomenon displayed by various phytoconstituents of plant materials. Some show fluorescence in the visible range in daylight. The ultraviolet light produces fluorescence in many natural products, which do not visibly fluorescence in daylight. Some non-fluorescent substances may be converted into fluorescent compounds after reacting with different chemical and reagents and it is helpful in qualitative assessment of crude drugs [20]. Higher plants produce secondary metabolites in large quantity which serves as important source for the botanical pesticides, natural drugs as well as precursors for many synthetic drugs [21].

Brine shrimp (*Artemia salina*) lethality assay is a preliminary toxicity screening test commonly used to check the cytotoxic effect of bioactive chemicals [22]. In the present research work, methanolic root bark extract showed significant activity at higher concentration, while methanolic stem bark shows no activity. Globally, more than 415 million people are affected by diabetes mellitus and number is increasing on regular bases despite of sufficient knowledge about the management of diabetes mellitus. [23]. Blood glucose level increases in type 2 diabetes due to starch hydrolysis by pancreatic α- amylase and reuptake of glucose by α-glucosidase in small intestine [24]. Enzymatic modulatory activity of methanolic extract using α-amylase inhibition assay both root and stem bark extract showed no activity.

Scanning electron microscopy is an important technique for the determination of morphological parameters. In present study scanning electron microscopy of root bark powder shows irregular shape which are not uniformly attached having many spaces. Molecules are present in the form of clumps having continuous arrangements of the molecules and have sharp edges.

Scanning electron microscopy of stem bark powder shows irregular shape having many spaces. It shows collision attachment and have net-like structures, and molecules are present in the form of clumps, having irregular shape with many spaces which indicates the large capacity of drug entrapment. Similarly, at 20 µm molecules, present in round globules shape with less spaces and also showed infrared spectroscopy is useful tool for obtaining structural information about different organic compounds. Different functional groups show different peaks of absorption, so different functional groups can be readily identified [25].

Infrared spectroscopy of root bark powder showed different stretching of peaks which indicates the presence of different compounds in root bark. Stretching is observed at wavenumber

3269 cm^{-1} (-H stretching), which shows that compounds may carboxylic acid. Stretching observed at wavenumber 2117 cm^{-1} (N=C=S) stretching, which shows that compound may belongs to isothiocyanate class. Similarly, stretching is observed at wave number 703 cm^{-1} which indicates C-H bending that shows compounds o monosubstituted benzene derivative.

Infrared spectroscopy of stem bark powder shows 1st stretching at wave number of 3659 cm^{-1} (O-H) stretching, which indicates that compounds may be belongs to free alcoholic class. Stretching. is observed at 3352 cm^{-1} wave numbers (N-H) stretching, which indicates the compounds may be belongs to aliphatic primary amines. Strong stretching is observed at 2189 cm^{-1} wavenumber (CΞ C) stretching, which indicates the compounds are of alkyne class. 4th stretching is observed at wave number 2018 cm^{-1} of (N=C=S stretching) which shows that compounds o isothiocyanate class. stretching is observed at 866 cm^{-1} wave number (C-H) bending, which indicates the compounds have 1,3-disubstituted molecules.

CONCLUSION

Capparis decidua (Forsk.) Edgew root and stem bark contain variety of bioactive compounds that have medicinal potentials and therefore need to be fully investigated for their pharmacological activities.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. M Asif Wazir contributed to the collection of plant materials, extraction and microscopic analysis. Talal Shaheer performed the physicochemical analysis, while Muhammad Iqbal Azhar originated the idea and supervised the work. Zafar Alam Mehmood helped in fluorescence analysis. Faheem Ahmad Siddique helped with FTIR, while Khizar Abbas collected, analyzed the data and performed the brine shrimp lethality assay and M. Younis Khan performed enzymatic analysis. Waqar Hussain performed scanning electron microscopy. Muhammad Imran Qadir prepared the manuscript and provided technical support throughout the project.

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