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Pharmacognostical investigation of the endemic medicinal shrub *Capparis grandiflora* wall. EX Hook. F. & Thomson and its antibacterial activity

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Abstract

The objective of this perception is to build up a trustworthy pharmacognostic cram and to create valuable information with consider to its identification, characterization and standardization of an endemic shrub *Capparis grandiflora* Wall. ex Hook. f. and Thomson and also analyze antibacterial screening potential of the shrub. Macroscopic observations were made with naked eyes and centimeter scale was used to gauge the leaf size. The microscopic evaluation was carried over for selected medicinal plant (stem and leaf part) and powder microscopy of plant powder also observed. Stomatal index (%) may be calculated on both leaf surfaces. The organoleptic characters of plant powder and the plant successive extracts, extractive values of powder, preliminary phytochemical examination and further World Health Organization (WHO) recommended for standardization were analyzed. The antibacterial activity of this endemic medicinal plant is additionally breaking down. *Capparis grandiflora* belongs to the family Capparidaceae, commonly known as "Thorattimul" is one such endemic medicinal species of Manar beat. With the benefaction of veteran ethnic consortium traditional knowledge of this region, the species *Capparis grandiflora* was chosen for the pharmacognostical examination and also for antibacterial screening. Review of literature revealed that there were no previous pharmacognostical reports of this plant, explicitly to determine the anatomical and other physicochemical standards required for its quality control. The antibacterial screening of this species affirms the therapeutic potential. The results of this study could be helpful in setting some diagnostic records for identification, authentication and for future investigators in their pharmacological analysis of this species. However, these findings are still in its outset so future clinical examinations are required.

Keywords: *Capparis grandiflora*, pharmacognostic, morphoanatomy, phytochemicals, antibacterial activity

Introduction

Therapeutic plants have a long-standing history in many indigenous communities and persist to provide positive sign for treat a variety of diseases. Traditional system of medicine is so engrained in our culture; even now 75% of the Indian population depends on this indigenous system for relief. The act of customary medication depends on many long periods of conviction and perceptions, which originate before the turn of events and spread of present-day medication [1]. The world health organization is now actively cheering developing countries to use herbal medicine around the world which they have been traditionally used for centuries. They have recognized 3000 plants from the forests of India and other tropical countries which can be used as medicine [2]. However, a key obstacle which has hindered the acceptance of the alternative medicines in the urbanized countries is the lack of certification and stringent quality control. There is a requirement for documentation of research work completed on conventional prescriptions. With this backdrop, it becomes extremely important to make an effort to normalize the therapeutic plant material to be used as a drug.

Plant medley and Traditional knowledge:

With the patronage of veteran ethnic group traditional knowledge in Manar beat, the species *Capparis grandiflora* was selected for the complete anatomical examination which has a wider use for diverse ailments among the Irula tribes due to its augment medicinal value. The pharmacognostical profile give serving hand to the researcher in resolving the identity puzzle of the crude drug. In Indian traditional system of medicine, the study genus particularly the wood of the plant is used in biliousness, stomach ache and giddiness. The whole plant is used in Gas tralgia, vomiting and abdominal pain. The leaf juice is used as stomachic, diuretic, anti-rheumatic, shortness of breath and anti tumour. As there is no strong pharmacognostic work confirmed on this medicinally potent shrub earlier, the present work was undertaken to lay down the standards which could be helpful for establishing its authenticity. The findings of the current study can be useful to advancement and surge additional scientific investigation on the aerial parts of this species and also developing a standardized profile of aerial parts of *Capparis grandiflora* which would be of immense apply to identify and establish the authenticity of the study plant and also sustain the antibacterial screening potential of this plant species.

Distribution and Description:

Capparis grandiflora Wall. Ex Hook. f. & Thomson belongs to the family Capparidaceae, commonly known as "Thorattimul" is one such endemic species in Manar beat of

Karamadai range, Coimbatore, Western Ghats, Tamilnadu, India. The Capparidaceae family includes the genus *Capparis* which is the largest genus, comprising 250 species of endemic shrub widely distributed in tropical and subtropical regions; generally known as caper shrubs or caper bushes [3]. Among this, 29 species are reported in India [4]. *Capparis* is one of the genera that are highly endemic in India. This species mainly distributed in the forests up to 1000msl of Western Ghats in Tamil Nadu, Karnataka and Kerala. In Tamil Nadu, this species has been reported in the Districts of Coimbatore and Nilgiri of Western Ghats, Erode and Trichi in the Eastern Ghats. It occurs over a wide range of habitat in the subtropical and tropical zones. It is an endemic shrub, branched, stems straggling with stellately brown pubescent and spines hooked; Leaves are ovate – elliptic in shape, acute, obtuse, coriaceous, glabrous inside, tomentose outside, pubescent and petioles are 3-6mm long; Flowers are axillary in position, solitary on pedicles, 0.8 – 1.5cm long, white and yellow at centre; Stamens about 65 – 90 and filaments about 2.5 – 3cm long; Ovary ovoid-ellipsoid, beaked, 3-5 ridged; Berries also ovoid and ellipsoid in shape, 5 or 6 angled, red when ripe; seeds are many– orbicular and it is the important identification characters among the other genus [5].

Antibacterial Study

Infectious diseases are the severe world's leading cause of premature deaths and killing approximately 50,000 people every day. Various huge pandemics brought about by microorganisms have been reported over the earlier hundreds of years which include smallpox, cholera, flu etc. Many human diseases and infections are restored by plants or plant derivative products. The main cause for the proceeding with consideration and wide research on plants for antibacterial properties is the sign of testing strains of microscopic organisms. This study was also aimed on validating the traditional use of selected medicinal plant against human pathogenic gram positive and negative bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*, *Pseudomonas aeruginosa*) it causing several human infections including skin diseases, infections of wounds, intestinal worm infections etc.

Materials and Methods

Chemicals and instruments

A compound microscope, watch glass, glass slides, cover slips, petri dishes and other standard glasswares were used for this experiment. Photographs were taken with using Nikon labphoto 2 microscopic units. Petroleum ether, chloroform and ethanol solvents and reagents used for staining different sections such as toluidine blue, safranin, fast green and IKI-Lugol's iodine were procured from The Precision Scientific Co., Coimbatore, India. All the chemicals used for different studies were of laboratory grade.

Collection and authentication of plant material

The plant specimens for the proposed study were collected from Manar beat of Karamadai rang, Nilgiris, Western Ghats, Tamilnadu, India during the month of March 2018 to April 2018, before that we got proper permission from the Principal Chief Conservator of Forests, Chennai and the District Forest Officer, Coimbatore under Section 28 (i) of Wildlife Protection Act, 1972 in the month of December and the voucher herbarium specimen was identified and processed as per the standard methods [6]. The identification of the model specimens has been authenticated by Scientist E and Head, Botanical Survey of India, Southern Circle, TNAU Campus, Coimbatore, Tamil Nadu under the Voucher specimen number - BSI/SRC/5/23/2019/Tech./166. The dried samples were poisoned with 0.1% $HgCl_2$, dissolved in absolute alcohol and mounted with adhesive on a standard herbarium sheet (42 x 28 cm). The collected plants were identified with the help of existing floras [7, 8, 9]. The herbaria were deposited for future reference in the Department of Botany, Vellalar College for Woman, Thindal, Erode, Tamil Nadu, India (BHVCW 33). The photographs were also taken to additional supplement for the herbarium.

Macroscopic and microscopic observations:

The macroscopic and microscopic examinations of this plant were carried out according to the method [10,11]. Fresh and healthy leaves and stems have been separated from the plant and carefully washed with running water to remove adherent impurities for anatomical studies. At the time of collection care was taken to collect normal and healthy plants for sectioning. Some total leaves and aerial parts have been air dried, powdered and stored in airtight containers for further study. The necessary examples of various organs were cut and expelled from the plant and fixed in FAA (Formalin – 5 ml + Acetic acid – 5 ml + 70 % Ethanol – 90 ml). Following 24 hours of fixing, the specimens were dehydrated with reviewed arrangement of tertiary – butyl alcohol according to the schedule [12]. Infiltration of the specimens was carried out by progressive addition of paraffin (melting point 58 – 60 °C) until TBA solution reached super saturation. Specimens have been moulded in paraffin blocks.

The paraffin implanted specimens were separated with the help of Rotary Microtome. The sections had a thickness of 10-12 µm. Dewaxing of the sections was followed by a standard process [10]. The sections were stained with Toluidine blue according to the strategy distributed, since Toluidine blue is a polychromatic stain. The recoloring results were strikingly acceptable and some cytochemical reactions were also obtained. The colour was pink to the walls of the cellulose, blue on the lignified cells, dark green at the suberin, violet on the mucilage and blue at the protein bodies. At the end point, the necessary sections were also stained with safranin, fast green and IKIfor Starch [13].

Powder microscopy

For powder microscopy, the dried aerial plant parts of *Capparis grandiflora* were powdered. The powder was cleaned with 5% sodium hydroxide and mounted in a glycerol medium following staining. The staining reagents such as toluidine blue, safranin, fast green and iodine were used. Different cell components were studied and measured by photomicrography.

Stomatal number and stomatal index

Stomatal number or stomatal density is the number of stomata distributed in per square millimeter (mm) of epidermis on each surface of a leaf. Stomatal index is the percentage which is calculated from the number of stomata forms in the number of epidermal cells [14]. The epidermis on both sides of leaves has been peeled with the help of a pair

of forceps. The peel was mounted in dilute glycerin and observed under microscope for numerical data calculation i.e. number of epidermal cells and number of stomata per square millimeter. From this data stomatal index was calculated using the standard formula [15, 16].

$$I = \frac{S}{S + E} \times 100$$

Whereas, I= Stomatal Index; S= Number of stomata per unit area; E= No. of epidermal cells per unit area

Other features like, the size of epidermal cell and stomata were also calculated using calibrated Nikon microscope. Similarly, presence or absence of stomata was also recorded [16].

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs from various amplifications were taken with Nikon Labphoto 2 microscopic units. For normal observations bright field was used. For the investigation of crystals, starch grains and lignified cells, enraptured light was utilized. Since these structures have birefringent property under energized light, they show up splendid against dim foundation. Magnifications of the figures are indicated by the scale-bars. Expressive terms of the anatomical highlights are as given in the standard life structures books [17].

Shade drying and crushing of harvested plant material:

Freshly harvested aerial plant parts were cleaned to remove adhering dust and then dried in shade at 31˚C for 15 days. The plant materials dried in the shade were mechanically reduced to coarse powder and traversed a plant in Wiley to obtain a mesh size of 60 and used for physicochemical and fluorescence studies. Samples were stored in high-quality plastic containers that are kept at room temperature until analysis.

Physicochemical analysis

The organoleptic characters of plant powder and the plant successive extracts were carried out according to the method [18]. The behaviour of plant powder with different chemical reagents and Fluorescence behaviour of plant powder in different chemical reagents and the successive solvents were carried out as per the procedure [19].

Soxhlet extraction

Dried and powdered aerial plant powder (20 g) of *Capparis grandiflora* was filled in the thimble and extracted successively with petroleum ether, chloroform and ethanol (20 g / 200 ml) using a Soxhlet extractor for 5-6 hrs to yield the crude extract. Extracts thus obtained will be concentrated in rotary evaporator, separated in glass vials and finally stored at 4 ˚C in refrigerator for further use. Before that the yield percentage was calculated for the individual extract later used for phytochemical screening and antibacterial studies.

$$\text{Yield Percentage (\%)} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant material}}$$

Preliminary phytochemical screening:

Phytochemical screening of different successive solvent extracts was carried out using the standard procedure [20].

Antibacterial screening

Preparation of inoculums: Stock cultures were maintained at 4 ˚C on slopes of nutrient agar medium. Active cultures for experiments were prepared by transferring a full loop of mother culture cells into samples of liquid nutrient medium and were incubated without agitation for 24 hrs at 37˚C. The cultures were diluted with fresh liquid nutrient broth to achieve optical densities corresponding to 2-106 colony forming units/ml for bacteria.

Bacterial strains selected: The antibacterial activity was examined from the aerial plant part (*Capparis grandiflora*) extracts of the species against four different human pathogenic bacterial strains which include Gram-positive strains such as *Staphylococcus aureus* (MTCC3160); *Bacillus cereus* (MTCC 430) and Gram-negative organisms such as *Escherichia coli* (MTCC 1698); *Pseudomonas aeruginosa* (MTCC 424). These pathogenic micro-organisms were obtained from IMTECH, Chandigarh were swabbed on the surface of the agar plates.

Determination of antibacterial activity: Antibacterial activity was determined using well diffusion method [21]. Further, the bacterial culture was streaked over the surface of the Muller-Hinton agar plate. Using Cork borer wells were made on the plates and these wells were filled with 100µl leaf extract at different concentrations (50 and 100 mg/ml). The crude extract was dissolved in 1% DMSO solution, to obtain the different concentrations. Kanamycin disk was used as positive control and these plates were kept for incubation. After the incubation period, the zone of inhibition was measured [22]. All experiments were carried out in triplicate and the average was taken into consideration as the end result.

Results

Macroscopical observations

The morphology of the study plant *Capparis grandiflora* Wall ex Hook. f. & Thomson is given in Plate 1. The macroscopical and organoleptic characters of stem, leaf, flower and fruit were observed and the results are tabulated in Table 1.

Table 1: Macroscopical characters of *Capparis grandiflora*Wall ex Hook. f. & Thomson

Organoleptic characters of stem		**Organoleptic characters of leaf**	
Characters noted	Observation	Characters noted	Observation
Colour	Green	Colour	Green
Odour	Characteristic	Odour	Characteristic
Taste	Bitter	Taste	Bitter
Shape	Cylindrical	Size	2 – 4 × 0.8 – 1.8 cm
Texture	Stellately brown pubescent hairs	Shape	Ovate – elliptic / Suborbicular
Fracture	Straggling	Texture	Variable
Spine	Hooked	Base	Truncate – cuneate / Subcordate
Size	2 – 3 mm long	Apex	Retuse / mucronate
Organoleptic characters of flower		Margin	Entire
Characters noted	Observation	Phyllotaxy	Alternate
Colour	White, Yellow at centre	Petiole	Long, pubescent
Inflorescence	Axillary, solitary	Organoleptic characters of fruit	
Size	5 – 6 cm	Characters noted	Observation
Pedicel	0.8 – 1.5 cm long, pubescent	Colour	Red when ripe
Apex	Acute	Type	Berry
Stamens	65 - 90	Apex	Beaked
Filaments	2.5 – 3 cm long	Size	4 – 7 × 1.5 – 2.5 cm long
Ovary	Ovoid - ellipsoid		

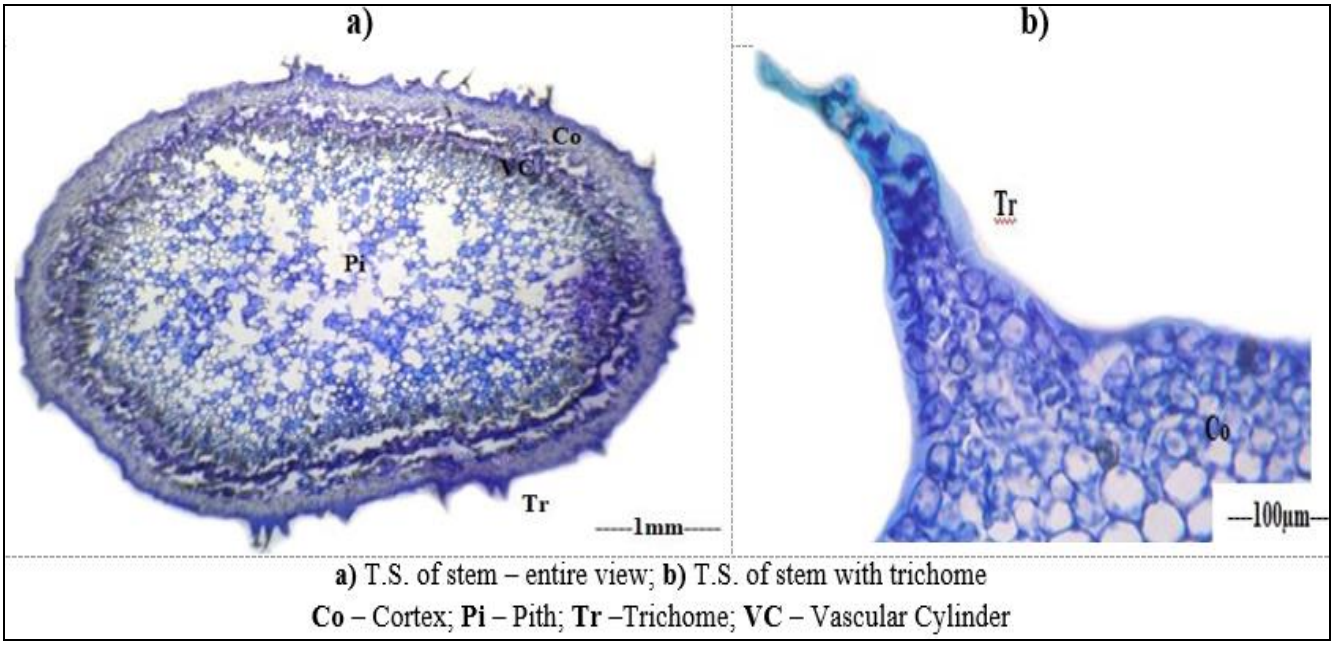
Plate 1: Habit of *Capparis grandiflora*

Anatomy of stem

The stem is slightly elliptical in cross sectional, 3.5 mm in diameter and it bears thick conical trichomes all around. It consists of epidermal layer, thick cortex, thin continuous vascular cylinder and wide pith. The epidermal layer is thin with small circular cells. Inner to the epidermis is the cortical zone which consists of small angular and compact thin-walled cells. Cortical zone is followed by thick uneven layer of fibres (sclerenchyma cells) and are wide, thick walled and lignified. The vascular cylinder is thin and continuous. It consists of outer thick cylinder of secondary phloem and secondary xylem. Secondary phloem consists of long radial compact lines of sieve elements, phloem parenchyma and phloem rays. The sieve elements are wide, less thick walled, square shaped, thin walled and they occur in continuous radial lines. The companion cells are small and are located with sieve elements along the corners. Xylem consists of vessels, fibres and xylem rays (Plate 2a). The xylem fibres are vertically elongated, narrowed, highly thick walled and lignified. Xylem rays are narrow thin radial lines comprising with thin-walled cells.

Trichome

Thick, multicellular trichomes are frequently occurring on the stem and are 60 µm thick at the base and 10 µm at the tip. It is gradually tapering towards the tip (Plate 2b). The cells of the trichomes are thin walled, densely staining and organized secretory function.

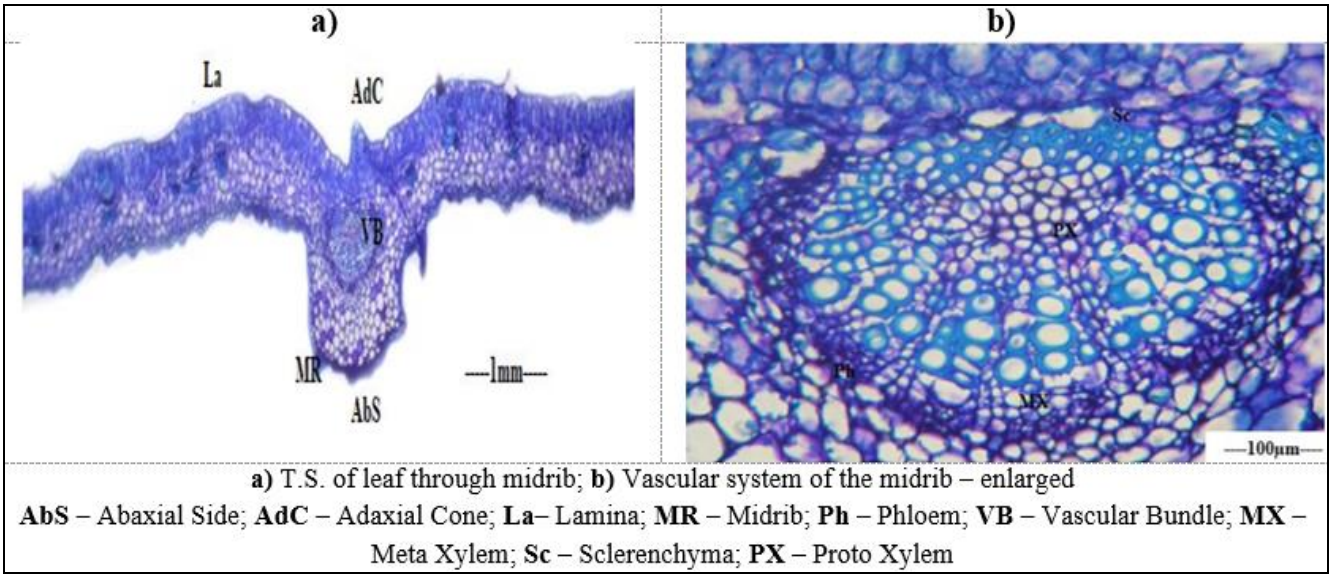


a) T.S. of stem – entire view; **b)** T.S. of stem with trichome
Co – Cortex; **Pi** – Pith; **Tr** –Trichome; **VC** – Vascular Cylinder

Plate 2: Anatomy of *Capparis grandiflora* stem

Anatomy of the leaf

In sectional view, the leaf shows thick midrib and smooth thick lamina (Plate 3a). The midrib has sharp conical adaxial outgrowth, which are wide and also has somewhat squarish abaxial midrib. The midrib is 480 µm thick in vertical plane and 430 µm wide in horizontal plane. The vascular system of the midrib consists of deep and is shaped with xylem and phloem. The xylem is consisting of several long, vertical parallel lines of xylem elements which are circular, highly thick walled with wide lumen. The cell walls are highly lignified. The phloem elements occur along the outer lower part of the xylem arc. Phloem elements are occurring in continuous thick layer. The sieve elements of the phloem are small with thick walls and darkly stained (Plate 3b). A layer of isodiametric shaped, thick lignified walls of sclereids are located on the upper end of the vascular arc.



a) T.S. of leaf through midrib; **b)** Vascular system of the midrib – enlarged
AbS – Abaxial Side; **AdC** – Adaxial Cone; **La**– Lamina; **MR** – Midrib; **Ph** – Phloem; **VB** – Vascular Bundle; **MX** – Meta Xylem; **Sc** – Sclerenchyma; **PX** – Proto Xylem

Plate 3: Anatomy of *Capparis grandiflora* leaf

Lamina

The lateral lamina is 250 µm thick. The abaxial part of the lamina is undulatewith wide furrows and ridges. The adaxial epidermis has wide rectangular cells with thick cuticle. The abaxial epidermal cells are smaller, but the cuticle is thick. The mesophyll tissue consists of dark and made up of compact two horizontal layers of cylindrical palisade cells. The spongy mesophyll cells are six layered and the cells are angular, thin walled and compact. The region of the lamina where there is the lateral vein, the lamina becomes thick. The lateral vein is circular and has a large mass of xylem elements and phloem form a deep arc on the lower part. Thick units of sclerenchyma cells are located on the adaxial part of the vein.

Leaf margin

The marginal part of the lamina is 250 µm thick and is slightly bent down. The adaxial epidermal cells are dilated and the cuticle is thicker, especially along the tip of the lamina. The mesophyll tissues are remaining unchanged and

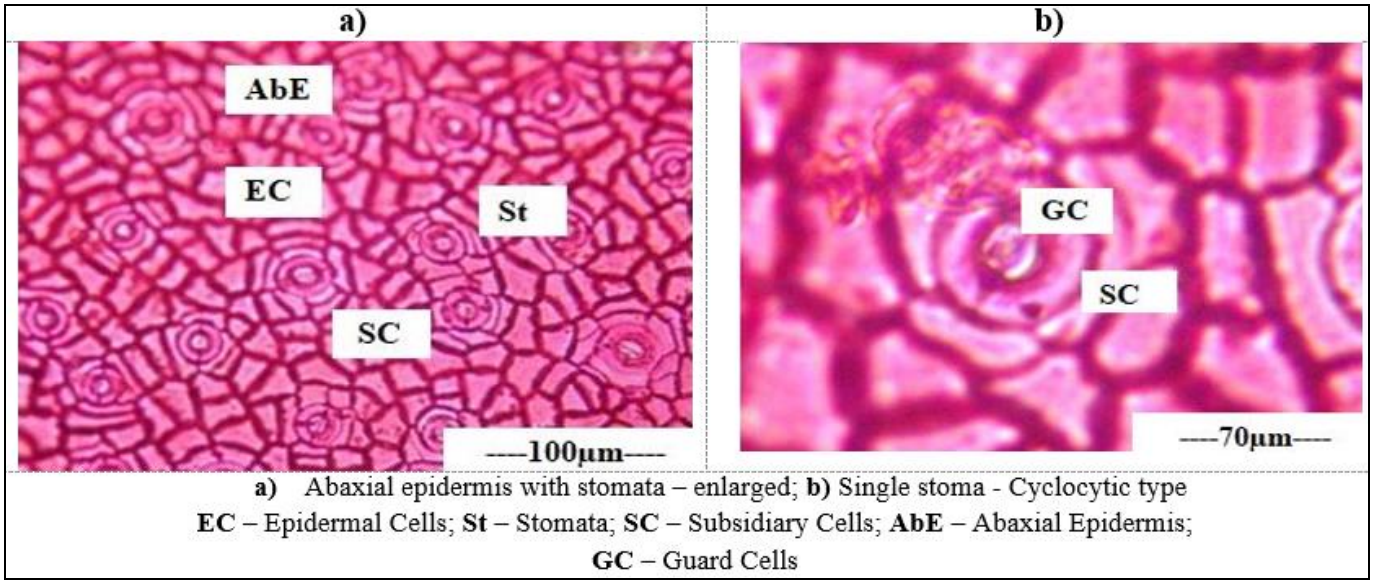
it also consists of adaxial layer of palisade cells, adaxial zones of spongy mesophyll and a small vascular strand.

Powder microscopic Studies

Leaf Powder

The leaf powder shows both adaxial and abaxial epidermal peelings. The adaxial epidermal cells are polyhedral, highly thick walled and the cells are random in orientation. The cells have thick straight anticlinal walls.

Epidermal cells and stomata: The epidermis is apostomatic (lacking stomata) on adaxial side. From the epidermal cells, often arises non-glandular epitrichomes. Around the epidermal cell there are three or more circles of epidermal cells are seen. When the trichome withers, the epidermal cells of the trichome dry and form dead cells. This dry dead circle of cells is called cicatrix. The abaxial epidermal cells are also having thick straight walls. The abaxial epidermis is densely stomatiferous. The stomata are random in orientation (Plate 4a). The guard cells are circular with slitlike stomatal pores. The stomata are cyclocytic type (Plate 4b). Each stoma has two or three circles of rectangular subsidiary cells.



a) Abaxial epidermis with stomata – enlarged; **b)** Single stoma - Cyclocytic type
EC – Epidermal Cells; **St** – Stomata; **SC** – Subsidiary Cells; **AbE** – Abaxial Epidermis; **GC** – Guard Cells

Plate 4: Epidermal tissue and stomata

Stem powder

Stem powder consists of rectangular thick-walled cells which are arranged in parallel lines. The cell walls are thick and straight stomata are also occasionally seen in the epidermis.

Epidermal trichomes: Epidermal trichomes are seen frequently in the powder and it is non-glandular type. It consists of a thin short stalk cell from which about six long, thick, cylindrical conical branches spread in all sides. The cells are thin walled and contain granular contend. **Fibres:** Long, thin needle shaped fibres are common in the powder. These narrow fibres are 2 mm long and 10 µm thick (Plate 5a). A second type of fibres called wide, short fibres are also common. These fibres have thick walls, wide lumen and tapering ends (Plate 5b). These wide fibres are 450 µm long and 15 µm thick.

Vessel elements: Vessel elements of different sizes are common in the powder. Some vessel elements are long with wide of end wall slightly oblique perforations (Plate 5c). Thus, vessel elements are 230 µm long and 30 µm wide. Fairly short cylindrical vessel elements measuring 120 µm long and 55 µm wide are also met with in the powder (Plate 5d). A third type vessel element 110 µm long and 40 µm wide is also seen. All these vessel elements have wide, horizontal perforations at the end walls (Plate 5e).

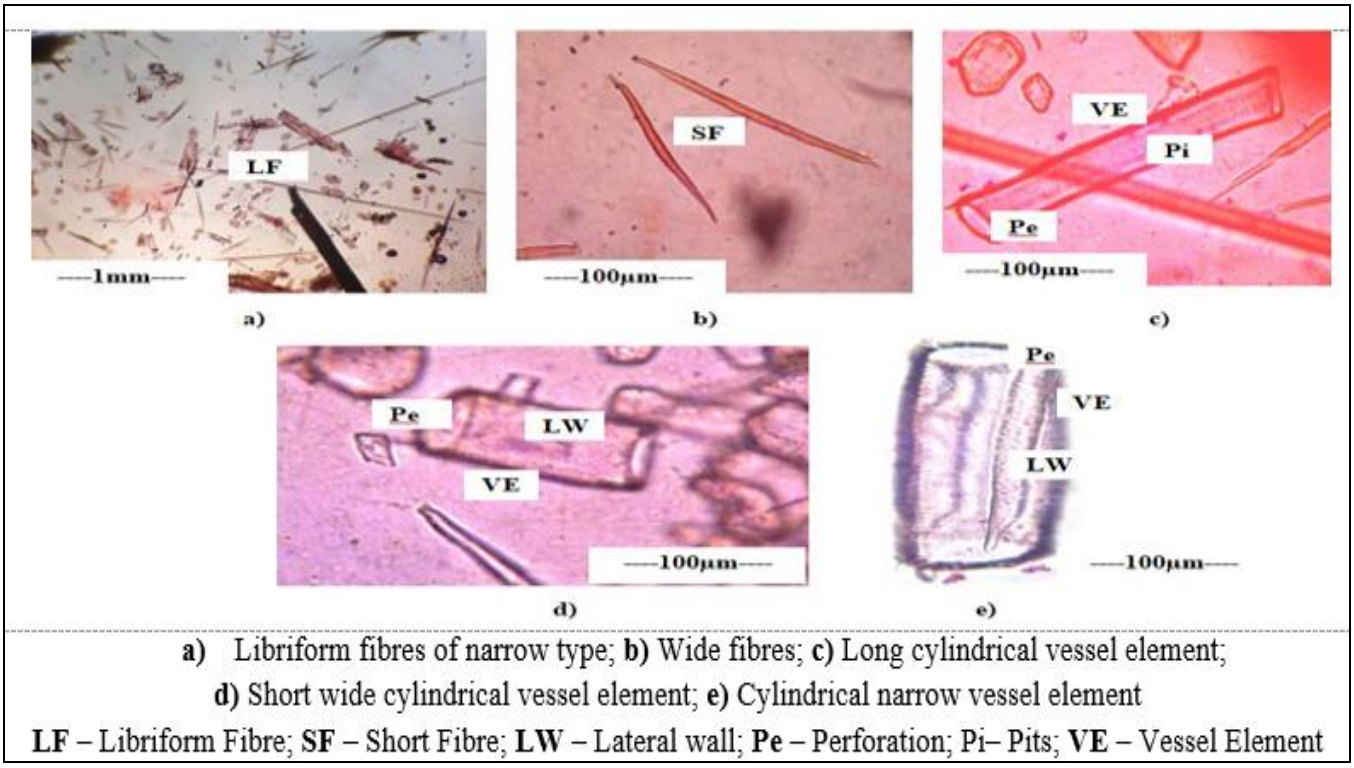


Plate 5: Powder Microscopic observations of *Capparis grandiflora*

Determination of stomatal Index

The stomatal index (%) can be calculated from the total number of stomata scattered on both the epidermal surfaces of leaves. The data of stomatal index for *C. Grandiflora* are tabulated and exhibited in Table 2. The results depicted that when compare to the leaf upper epidermis the average number of stomatal index was higher in lower epidermis. The stomata are completely absent in adaxial surface.

Table 2: Stomatal index for the leaves (Lower epidermis) of *Capparis grandiflora*

S. No.	Number of stomata	Number of epidermal cells	Stomatal index (%)
1.	90	460	16.4
2.	65	540	10.7
3.	75	520	12.6
4.	80	410	16.3
5.	65	550	10.6

Average stomatal index = 13.32

Physico-chemical analysis of the test plant:

Organoleptic characters of plant powder and the plant Successive extracts: The plant powder showed granular texture, characteristic odour and bitter taste. After drying and powdering of the sample, the colour of the powder changed from green to greenish yellow as shown in Table 3. The organoleptic characters such as colour, consistency and odour of successive aerial plant extracts of *Capparis grandiflora* were noted and tabulated in Table 4.

Table 3: Organoleptic characters of plant powder of *C. grandiflora*

S. No.	Characters	Observations
1.	Colour	Greenish yellow
2.	Texture	Granules
3.	Taste	Bitter
4.	Odour	Characteristic

Table 4: Organoleptic characters of plant successive extracts of *C. grandiflora*

S. No.	Extraction Medium	Colour	Consistency	Odour
1.	Petroleum ether	Greenish yellow	Semi solid	Characteristic smell
2.	Chloroform	Green	Semi solid	Characteristic smell
3.	Ethanol	Light green	Semi solid	Characteristic smell
4.	Aqueous	Dark brown	Semi liquid	Characteristic smell

Successive solvent extraction- Percentage yield

The plant powder was extracted with different solvents in the order of non-polar to polar solvents. The yield of different solvent extracts using successive solvent extraction was calculated and presented in Table5. The percentage of yield was higher in ethanol extract (78.5%) followed by petroleum ether (71.95%) and chloroform (64.35%). The lowest percentage of yield was noted in aqueous extract (49.6 %). The extractive values will be useful in identification, authentication and valuable for the determination of the exhausted or adulterated drug.

Table 5: Successive extractive values of *C. grandiflora* powder with various solvents

Method of extraction	Solvents used	Yield (%)
Continuous hot percolation using Soxhlet apparatus	Petroleum ether	71.95
	Chloroform	64.35
	Ethanol	78.5
Hot and cold percolation	Aqueous	49.6

Phytochemical screening - Qualitative analysis

To investigate the chemical constituents of *Capparis grandiflora* plant powder, the successive solvent extracts were subjected to qualitative phytochemical screening. The

results of the preliminary phytochemical screening of *C. grandiflora* powder with different solvents of Petroleum ether, Chloroform, Ethanol and Aqueous extracts showed the presence of various phytochemicals (Table 6). The non-polar to polar solvents revealed the presence of alkaloids, flavonoids, phenols, tannins, proteins, amino acids, steroids, anthraquinones, glycosides and gum, which all are noted in high, medium and low concentrations. All the extracts exhibited high concentration of alkaloids and flavonoids. Also, the extracts showed negative response for quinines and terpenoids in petroleum ether extract, terpenoids in chloroform extract and saponins, coumarin and fixed oil in aqueous extract, finally which indicated in not detectable range. Among all the extracts treated, the ethanol extraction was more efficient which contains a greater number of phytoconstituents than other solvents used.

Table 6: Preliminary phytochemical analysis of *Capparis grandiflora* successive extracts

S. No.	Phyto constituents	Petroleum ether	Chloroform	Ethanol	Aqueous
1.	Alkaloids	+++	+++	+++	+++
2.	Flavonoids	+++	+++	+++	+++
3.	Phenols	++	+	++	+
4.	Tannins	+++	++	+++	++
5.	Quinones	-	+	+++	+
6.	Saponins	+++	++	++	-
7.	Protein	+++	+++	+++	++
8.	Amino acids	+++	+++	+++	++
9.	Steroids	++	++	+++	++
10.	Anthraquinones	+	++	++	+
11.	Terpenoids	-	-	+++	++
12.	Coumarin	++	++	+++	-
13.	Glycosides	++	++	+++	+
14.	Gum	++	+	++	+
15.	Fixed oil	+	+	++	-

Note: +++ = High concentration; ++ = Medium concentration; += Low concentration; − = Not detectable.

Antibacterial Screening

The results of this study indicated that the crude solvent extracts obtained from the leaves of *C. grandiflora* showed antagonism against the studied bacterial pathogens. At 10 mg/100 µl, the ethanol extract had a slightly higher antibacterial activity against gram-positive and gram-negative bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*) respectively. The average antibacterial activity against bacterial strains ranged from 8 to 18 mm (the zone of inhibition). Among these, *E. coli* was highly susceptible to the ethanolic extract with the zone of inhibition of 18 mm. The ethanol extract showed zone of inhibition in *Bacillus sps*. (50µl-8.8 ± 0.46 and 100µl-15.3 ± 0.56), in *Staphylococcus sps*. (50µl-10.8 ± 0.70 and 100µl-15.97 ± 0.51), in *E. coli* (50µl-10.73 ± 0.65 and 100µl-18.83 ± 0.60) and in *Pseudomonas sps*. (50µl-11.13 ± 0.80 and 100µl-15.4 ± 0.62) (Table 7). Observed zone of inhibition against bacterial organisms are shown in Fig. 1.

Table 7: Antibacterial activities of selected plant using ethanol extract

Test Organisms	Zone of inhibition of *C. grandiflora* (mm in diameter)			
	Control (50µl)	Concentration (50µl)	Concentration (100µl)	Kanamycin (PC) 30mcg/disc
Bacillus cereus	-	8.8 ± 0.46	15.3 ± 0.56	22.4 ± 0.85
Staphylococcus aureus	-	10.8 ± 0.70	15.97 ± 0.51	26.8 ± 1.25
Escherichia coli	-	10.73 ± 0.65	18.83 ± 0.60	22.4 ± 0.44
Pseudomonas aeruginosa	-	11.13 ± 0.80	15.4 ± 0.62	25.8 ± 1.01

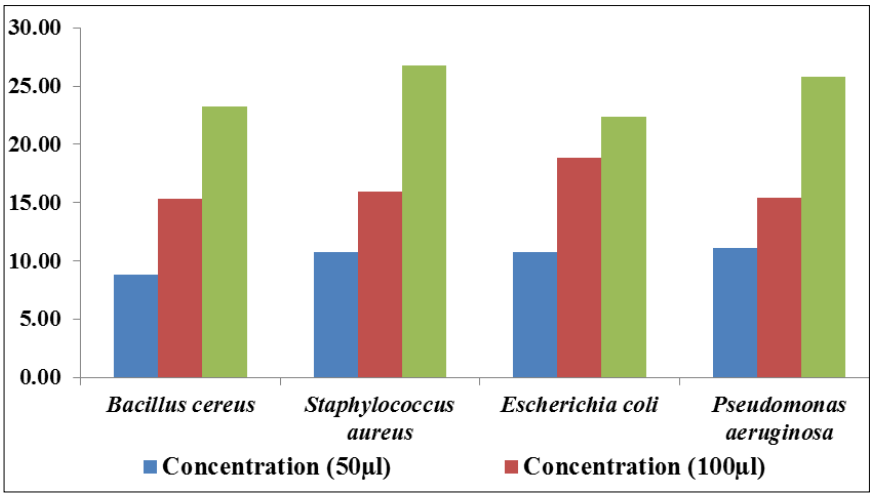


Fig 1: Antibacterial activity of *Capparis grandiflora* Ethanol Extract against Microorganisms (in mm)

Discussion

The present investigation was carried out to undertake the quality profiling of the aerial plant parts of *C. grandiflora* and also characterize their economically important raw drug material. Nowadays the characterization is the very important step to spread out pharmacognostic determination of medicinal plant used in different medications and drug discovery. Also, the identification and authentication of natural medicines by pharmacognostical studies is more reliable and economic than the modern strategies. According to WHO the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity of the herbal drug [23, 24]. The macroscopical investigations of study plant highlighted it is an endemic shrub, branched and having hooked spines. The stem is straggling and leaves are alternate, ovate – elliptic in shape. In addition, the stem and leaves showed characteristic odour and bitter in taste. A few earlier workers have received macroscopical highlights as one of the successful parameters for the pharmacognostical identification of several plant determined crude drugs [25, 26]. Natural authentication of a phytodrug includes identification of the plant by its botanical characters and the diagnosis of the plant with its infinitesimal characters. The present anatomical study provides a set of characters specific to the identity of the plant in fragmentary form.

Microscopic evaluation revealed the presence of cyclocytic stomata surrounded by two or three circles of rectangular subsidiary cells, multicellular and non-glandular trichomes. The adaxial epidermis of *C. grandiflora* leaf is apostomatic type whereas the abaxial epidermis is stomatiferous which is of cyclocytic type. The epidermal cells of the trichome form dry dead circle called cicatrix. The presence of narrow and wide fibres and vessel elements are also seen in powder microscopy. Thus, generally this study provides some referential botanical information for correct identification of the plant. Additionally, the higheramount of stomatal index was calculated from the fresh leaves particularly from lower epidermis. The anatomical characters, epidermal and stomatal cells [27], pharmacognostical evaluation [28] of *Capparis diversifolia* and *Capparis divaricata* were extensively studied in earlier. The similar anatomical explorations were carried out in the same genera *Capparis* by one researcher [29]. Overall, the total sum of macroscopic characteristics combined with microscopic parameters will provide the protocol for clinical or pharmaceutical research [30]. Organoleptic profile was one of the diagnostic parameters used for the proper identification of raw materials [31]. The macroscopic organoleptic characters of the study plant indicate good condition of species. Extractive values are mainly useful for determining drug exhaustion or adulteration [32]. In the present study the extractive values are found higher in polar solvent like ethanol. The phytochemical evaluation is one of the tools for the quality assessment of phytoconstituents produced by plant species. The preliminary phytochemical analysis of different solvent extracts of *C. grandiflora* reveals the presence of various pharmacologically important phytoconstituents like alkaloids, phenols, flavonoids, steroids, proteins, amino acids, tannins and glycosides. The preliminary screening test may be useful in the detection of the bioactive phytocomponents which may lead to the drug discovery and development [33]. A number of studies on antibacterial activity of medicinal plant extracts have appeared in the literature [34, 35, 36]. The current study focuses on ethanol extract of *C. grandiflora* showed significant activities against some bacterial strains. It exhibits highly significant results against the bacteria *Escherichia coli*.

Conclusion

This present cram will helpful in the authentication and standardization of the plant *Capparis grandiflora* Wall. ex Hook. f. & Thomson and further used as traditional medicine for the treatment of some ailments and finally used to standardize drug formulations. In conclusion the study plant holds biologically active compounds that may serve as applicant for the discovery of new drugs in the treatment of antibacterial activities. In addition, the evaluation of the antibacterial properties of these extracts and isolation of the compounds responsible for the antibacterial activity is required.

Conflict of Interest Statement

Authors proclaim that they have no conflict of interest.

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