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Original Article

Pharmacognostic Studies and Antibacterial Activity of *Corchorus olitorius* L. Leaf

Manvi, Mohammad Irfan Khan*, Badruddeen, Juber Akhtar, Mohammad Ahmad

ABSTRACT

Background: *Corchorus olitorius* L. (Family: Tiliaceae) or 'Tossa Jute or Nalta Jute is an important fiber crop in India and Bangladesh. Its leaves are used as folk medicine, food source and have demulcent, diuretic, lactagogue, purgative, antidiabetic, anticancer, antioxidant, anti-inflammatory and tonic effects. The aim of the research is to evaluate the pharmacognostic characteristics and antimicrobial activity of *Corchorus olitorius* L. (*C. olitorius*). **Materials and Methods:** The pharmacognostic studies were carried out in terms of organoleptic, macroscopic, microscopic, physico-chemical evaluation, phytochemical screening, fluorescence analysis, TLC fingerprinting by standard procedures. Antibacterial activity against gram-negative bacteria *E. coli* was determined by agar well diffusion method. **Results:** The leaf of *C. olitorius* showed serrate margin, stipules at the base, and acute to acuminate apex. Leaves surface microscopy showed numerous prismatic calcium oxalate crystals and covering trichomes. The moisture content 6.6% w/w, ethanol and water-soluble extractive value 4 and 17.6% w/w, total ash 12% w/w, while acid insoluble and water-soluble ash value 2.2 and 2.0% w/w respectively were determined. Phytochemical screening showed the presence of flavonoids, polyphenols, phytosterols/triterpenoids, alkaloids, carbohydrates, tannins, and proteins. Thin layer chromatographic profiling of ethanolic extract was carried out which showed different R*f* values. The antimicrobial test showed that aqueous extract has better antimicrobial activity around the tested bacteria (*E. coli*) than ethanolic extract. **Conclusion:** The results are helpful in the standardization of the leaf part of the plant *C. olitorius*. The aqueous leaf extract possessed antimicrobial properties. There is a need to isolate its constituents responsible for antimicrobial activity.

Keywords: *Corchorus olitorius*, Pharmacognostic, Fluorescence analysis, Standardization.

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INTRODUCTION

Bacteria are the main cause of urinary tract infection, commonly found in humans by *Escherichia coli* (*E. coli*) strains known as uropathogenic *E. coli* (UPEC). High resistance was observed against antibiotics limiting their clinical use.[1] Therefore, there is a dire need to investigate new antimicrobial drugs for the treatment of infectious bacterial diseases from medicinal plants.[2]

Corchorus olitorius L. commonly called Tossa Jute or Nalta Jute belongs to the family Tiliaceae. The largest producers of fibers are India and Bangladesh, but Jute leaves are used as a food source in the Middle East, South-East Asia, and different regions of Africa.[3] Synonyms of the plant are- (i) Sanskrit: Brihatchanchu (ii) Bengali: Pat, Banpat, (iv) English: Jew's Mallow (v) Gujrati: Chhunchho (vi) Hindi: Koshta, Pata (vii) Tamil: Peratti (viii) Marathi: Chhunchh (ix) Punjabi: Banphal (xi) Arabic: Molukhyia (xii) Africa: Ewedu (xiii) Philippines: Saluyot.[4] The plant belongs to the Spermatophyta phylum, Angiosperm subphylum, Dicotyledonae class and of Malvales order taxonomically. *C. olitorius* has been investigated with the various phytochemical constituents in different plant parts like seeds containing cardiac glycosides, triterpenes, etc., leaves containing triterpenoids, ionones, flavonoids, phenolic compounds, caffeic acid derivatives, polysaccharides, while the roots fatty acids and sterols.[5-7] Jute leaves are found to possess demulcent, diuretic, lactagogue, purgative, and tonic effects.[8] Different extracts showed potent antidiabetic, anticancer, antioxidant, anti-inflammatory, antimicrobial, cardioprotective, hepatoprotective, neuroprotective, analgesic, and wound healing effects.[9] The purpose of this research is to ascertain the pharmacognostic parameters and antibacterial activity of *C. olitorius* leaf against the *E. coli* bacterial strain.

MATERIALS AND METHODS

Chemicals

All the chemicals purchased from Thermo Fisher Scientific India Pvt. Limited, Rankem laboratory reagent, and Finar chemicals, India were of analytical grade.

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Procurement of plant materials

Fresh leaves were purchased from the local market of New Town, Kolkata, West Bengal in November 2021. The plant was authenticated at the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow (U.P.), and the specimen of whole plant has been deposited in the repository with CDR No. C078.

Organoleptic parameters

Parameters like color, odor, and taste were evaluated organoleptically.

Macroscopic evaluation

Different macroscopic characteristics of fresh leaves of *C. olitorius* were observed like characters of the lamina, presence of petiole, and leaf base. Lamina consists of distinguishing characters such as venation, shape, texture, apex, phyllotaxis, petiole, margin, and surface.[10]

Microscopic evaluation

Both qualitative and quantitative microscopical studies were conducted. The model of microscope used for studying transverse sections and observing characters was Labomed Vision 2000 (LED) Binocular microscope.

Qualitative microscopy

The surface and transverse sections of the leaf under the microscope were observed. The section was stained using phloroglucinol and HCl (1:1) as per the standard method. Different microscopical structures were observed and photomicrographed.[11-14]

Surface view of leaf

The leaf was dipped in a 4% sodium hypochlorite solution for some hours until it lost its pigments and color.[15] All the characteristics of lamina, midrib, and petiole were thoroughly observed in bleached leaf under the microscope.

Transverse section (T.S.) of Leaf

The fresh leaf was inserted in water and freehand sections were cut transversely through the midrib. Fine sections were fixed on a glass slide using glycerine with no staining reagent and the slides were observed under the microscope. The sections were stained using freshly prepared phloroglucinol and conc. HCl. Different characteristic features, such as the trichomes, stomata, and cellular components were observed.[16,17]

Powder microscopy

Powdered dried leaves were observed under the microscope. For identification of lignified components staining reagent (phloroglucinol and HCl in the ratio of 1:1) was used. A little quantity of fine leaf powder was observed under the microscope. The powder was mounted in glycerol by adding 1-2 drops of phloroglucinol solution (0.1% w/v) and Conc. HCl and a coverslip was placed over the powder before examining under the microscope. The trichomes, presence of stomata with epidermal cells, xylem vessels, fiber, etc. were detected. The cell structures were observed and then photomicrographed.[18]

Quantitative microscopy

Estimation of the stomatal number and stomatal index

The stomatal number of the epidermis of leaf is defined as an average number of stomata per square millimeter. Stomatal index in a leaf is the percentage proportion of the total number of stomata to the number of epidermal cells in a given area. It can be calculated by using the equation: SI = (S / S +EP) x100, where, SI is the stomatal index, S is stomata/ mm^2, and EP is epidermal cells/ mm^2. By using forceps, a piece of leaf was peeled out for the upper and lower epidermis, separately. It was mounted in glycerol on the slide. The prepared slide was placed on the stage of a microscope and the epidermal cells and stomata were drawn with the help of camera lucida on a 4 mm^2 black paper divided into four equal fields. The stomatal number and the epidermal cells in each field were tallied. The average number of stomata was taken by counting in per square mm and the stomatal index was evaluated by the above formula separately for the upper as well as lower epidermis.[19]

Estimation of vein termination and vein-islet number

Leaf constants such as veinlet termination and vein-islets number were observed per mm^2 of leaf surface between the margin and the midrib. For the drawing camera lucida and black paper were arranged. A 4 mm square was constructed in the center of the field. The veins present within the square were traced, including overlapping islets between adjacent sides of the square.[20] The average vein islets number and veinlet termination of the four adjoining squares were recorded.[19]

Estimation of palisade ratio

A leaf fragment was used to observe the presence of palisade cells under each epidermal cell. Then, the palisade layer beneath the epidermal cells was traced off by using camera lucida on drawing paper. Four epidermal cells consisting of five groups each were calculated from different sections of the leaf and the average number was calculated as the palisade ratio.[20]

Determination of width and length of vessels and fibers

Xylem vessels were separated from other histological characters using Schultze's maceration fluid. Schultze's maceration fluid was prepared by adding sufficient potassium chlorate to the nitric acid solution (50%v/v) and heated in a water bath. A fragment of the leaf was placed in the above macerating fluid. For disintegration and softening of leaf tissues, potassium chlorate was added from time to time. The treated tissues of the leaf were taken on a slide, teased with help of a needle, and repeatedly washed with water to free the acid. The width and length of the vessels were recorded using a calibrated eyepiece micrometer. Dimensions of 50 xylem vessels and fibers were recorded.[21]

Determination of biocrystals of leaf

After bleaching Ca Ox crystals were separated and observed microscopically. The shape and sizes of crystals were photomicrographed. Various metabolic processes are responsible for the formation of crystals in leaves where they are mostly stored in cytoplasm and cell vacuoles. Usually, crystals are composed of calcium salts of calcium such as calcium carbonate and calcium oxalate.[22] According to Mazen, 2004 the leaf contains an abundant number of prismatic-shaped Ca oxalate crystals intracellularly and concluded that only aluminum was incorporated into these crystals.[23]

Physico-chemical evaluation

Moisture content determination by loss on drying method

Determination of moisture content was done by the loss on drying method under the thermogravimetric principle. 2 gm of fine leaf powder was taken into a thin china dish and kept in the oven at 100°C to 105°C for drying. Then allowed the dish to cool in a desiccator. Three consecutive weighings were noted down which do not differ more than 0.5 mg. The loss in weight was recorded referred to as moisture.[24]

Extractive value determination

The air-dried leaf powder of *C. olitorius* leaves (5 g) was taken in six closed flasks separately. One hundred ml of 95% v/v ethanol was added

to three flasks and distilled water (100 ml) was added to the remaining three flasks respectively. The flasks were occasionally shaken for the six hours and then placed for 18 hr without stirring. The contents were filtered. 25 ml each aqueous and ethanol filtrate was evaporated separately in tared flat china dishes and kept in the oven for drying to constant weight at 105°C. Table 2 shows the ethanol and water-soluble extractive values of *C. olitorius* leaves.[24]

Ash value determination

Ash value is an important parameter of standardization to evaluate the purity and quality of raw plant material. Total ash contains remaining residues after incineration, which represent inorganic salts like silicates, carbonates, and phosphates of calcium, potassium, sodium, magnesium, etc. For determination of total ash accurately weighed 2 g air-dried *C. olitorius* leaves were incinerated (450°C) separately to constant weight in six tared silica crucibles. The ash in the first three crucibles was boiled with water (25 ml) separately. Ashless filter paper was used to gather water-insoluble ash which was ignited at 450°C after washing with hot water to constant weight. Ash in the other three silica crucibles was boiled separately with 25 ml 2M HCl. Similarly, the ashless filter paper was used to collect the acid insoluble ash which was ignited at 450°C after washing with warm water to constant weight. Table 2 shows the mean value of total ash, acid insoluble ash, and water-soluble ash of *C. olitorius*.[25]

Foaming index determination

According to WHO, the foaming index is measured by determining the ability of an aqueous decoction of the herbal drug to form foam. Accurately weighed 1 g leaf powder was taken in a 100 ml conical flask, added boiling water to it then cooled down. It is then filtered in a volumetric flask, and made the volume up to 100 ml. 10 test tubes were taken containing sequential quantities of 1 to 10 ml of decoction. The remaining volume was adjusted with water up to 10 ml and closed the tubes were closed with a cork. The test tubes were shaken vigorously and kept aside for 15 min. The height of the foam was measured in each test tube. Calculated the foaming index after foam measurement.[25]

Swelling index determination

According to WHO, the swelling index is a measure of volume (ml) of the swelling of 1 g of the crude drug. Transferred 1 g of leaf powder into a 25 ml stoppered measuring cylinder. Then add 25 ml of water to it. Agitated gently the mixture occasionally for 24 hr. This mixture was placed at room temperature without disturbing it. The volume occupied by the powdered drug along with sticky mucilage was measured in ml.[25]

Phytochemical screening

Secondary metabolites are usually responsible for the pharmacological activities of the crude drug. Ethanolic and aqueous extracts of the powdered drug were screened for the presence or absence of flavonoids, polyphenols, phytosterols/ triterpenoids, alkaloids, carbohydrates, tannins, proteins etc.[26,27]

Fluorescence analysis

The standard procedure of fluorescence analysis was followed for the leaf powder.[28] For the analysis of leaf powder in little quantities was treated with different types of solvents. The powdered samples were noticed by their color change in an Ultraviolet chamber in the visible, short wave, and long wavelengths respectively after treating with reagents.[29,30] Fluorescence is important for the qualitative assessment in pharmacognostical evaluation because some chemical compounds show fluorescence in UV light while some are in the visible range in daylight. The characteristic color changes in appearance were observed and recorded in the data.[31]

Thin-layer chromatographic analysis

Thin-layer chromatography also known as fingerprint profiling is used to separate non-volatile compounds. The samples using capillary were applied to TLC-precoated plates of Silica gel 60-F_{254} (Merck KGaA, Darmstadt, Germany) in the form of bands. The chromatographic plates were run in a TLC chamber presaturated with solvent system. After the separation of compounds towards the mobile and stationary phase, the TLC plates were removed. The solvent was evaporated at room temperature. The TLC plates were observed in daylight and in a chamber under UV 254 and 365 nm. The spots were quantified by the retention factor (Rf). R*f* value changes and depends on the polarity of the mobile and stationary phase.[32]

Antibacterial activity in *C. olitorius* leaves extracts

Bacterial strains

The antibacterial potency of ethanolic and aqueous extracts (EE and WE) of *C. olitorius* leaves was evaluated against Gram-negative *Escherichia coli* (ATCC25923) bacteria causing urinary tract infection. The bacterial strains were collected from the Department of Biosciences, Integral University, Lucknow, India.

Agar well diffusion technique

The antibacterial activity of plant extracts was done firstly by spreading 100 µL of *E. coli* culture suspension in a Petri dish via a metal spreader on already casted EMB agar (HIMEDIA). After inoculation of the agar plate, 60 µL of EE and WE at 40 and 80 mg/ml respectively were filled in the agar wells. Finally, the Petri plates were placed in an incubator for 24 hr at 37°C. The evaluation of antibacterial property was performed by calculating the zone of inhibition (diameter expressed in millimeters) of the plant extracts, compared with Gentamycin (standard).[33]

Statistical Analysis

For each treatment triplicate plates were prepared and the average zone of inhibition excluding well was recorded. All the measured data were given as mean ± standard deviation.

RESULTS

Organoleptic evaluation of leaf

The organoleptic evaluation of the leaves showed dark green color on its upper part and a lighter green shade on the lower part. The powdered leaf also emerged as green in appearance, bitter in taste, and distinct but characteristic in odor.

Macroscopic evaluation of the leaf

The study of morphological characters and taxonomy was done for macroscopic evaluation. The characteristics of leaves were observed morphologically which showed the presence of stipules at the leaf base and long petiole with opposite phyllotaxis. The structure of the lamina was found to be simple, 3 veined from the base with reticulate venation, finely serrate margin, acute to acuminate apex, smooth (glabrous) surface, and slippery texture with lanceolate shape. The average size of leaves was found as 6.5 cm in length and 1.8 cm in width. The average length of the petiole and stipule was found 2.2 cm and 6.6 mm respectively (Figure 1).

Microscopic evaluation of the leaf

Leaf microscopy

Surface view

The photomicrograph of the ventral leaf surface shows 3 veins including the midrib (Figure 2 a). The petiole showed numerous multicellular non-glandular trichomes. The leaf surface showed Ca Ox crystals in abundance along the vein islets (Figures 2 b-d and 3 c). Sharply conical and uniseriate one to bicellular to multicellular non-glandular (Figure 3 d) and few capitate glandular trichomes with the long multicellular stalk were present on the surface (Figure 3 e), margin (Figure 2 c), and on the petiole (Figure 2 a). The epidermis showed paracytic stomata with kidney-shaped guard cells. These are surrounded by two subsidiary cells whose longitudinal axes lie parallel to the aperture (Figure 3 b).

Figure 1: Leaf of *Corchorus olitorius* (ventral and dorsal view).

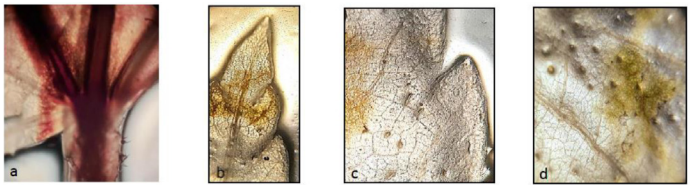

Figure 2: Photomicrograph of ventral leaf surface showed, (a-d) midrib, numerous crystals along the veins, vein islets, and non-glandular trichomes on margin and over the surface (x100).

Transverse Section of leaf

In Figures 4 and 5, the transverse section (T. S.) of the leaf of *C. olitorius* through the midrib showed projection adaxially and abaxially.

Epidermis: Uniseriate epidermis of both lower and upper surfaces located beneath the cuticle and interrupted by stomata.

Mesophyll- Palisade parenchyma: Palisade layer of the mesophyll in the leaf just below the upper epidermis, regularly arranged 2 layers, cells oblong in shape, densely packed, and in contact with each other, continuous over the vascular bundle but absent on the underside where the midrib projects.

Spongy parenchyma: Spongy parenchymatous cells small and in varying shape and sizes, loosely arranged, enclose smaller air spaces.

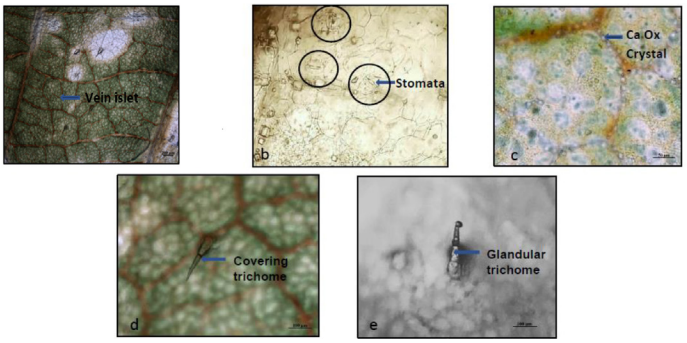

Figure 3: Photomicrograph of *C. olitorius* leaf surface showed, (a) palisade mesophyll cells with vein islets (Bar= 200 µm), (b) stomata with epidermal cells (x200), (c) Palisade cells, calcium oxalate crystals along the veins of lamina (Bar= 50 µm), (d) Bicellular covering trichome (x100), and (e) glandular trichome (x100).

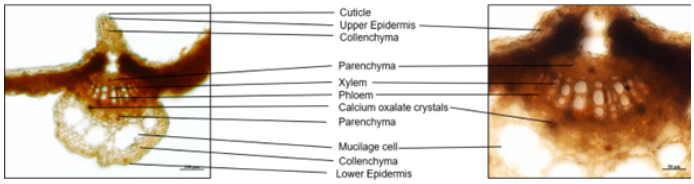

Figure 4: Photomicrograph of T.S. of *C. olitorius* leaf showed vascular bundles (x100, x200)

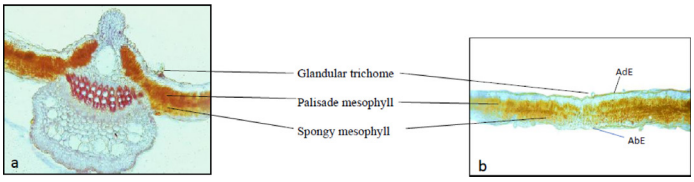

Figure 5: Photomicrograph of T.S. of *C. olitorius* leaf with staining (x100), and T.S. of Lamina showed adaxial epidermis (AdE), Abaxial epidermis (AbE), palisade cells (x100).

Vascular region: A large vascular bundle (found in the midrib region) surrounded by parenchymatous bundle sheath, collenchyma cells which connected the upper and lower epidermal cells by many layers of hypodermal collenchyma cells, lignified xylem vessels and non- lignified phloem, presence of large mucilage cells.

Powder microscopy

The color of the powdered leaf was dark green with a specific peculiar smell and bitter taste. The powder showed the following fragments:

1- Paracytic stomata with epidermal cells (Figure 6)

2- Uniseriate to multicellular covering trichome and capitate glandular trichome (Figure 7)

3- Palisade cells with mesophyll (Figure 8)

4- Xylem vessels, fibers, and epidermal cells with stomata (Figure 9)

5- Lignified thickening in annular vessels (Figure 10)

6- Phloem fiber (Figure 11)

Quantitative microscopy

The various parameters evaluated for leaf surface constants were observed like stomatal number (upper and lower), stomatal index (upper and lower), vein islet number, veinlet termination number, palisade

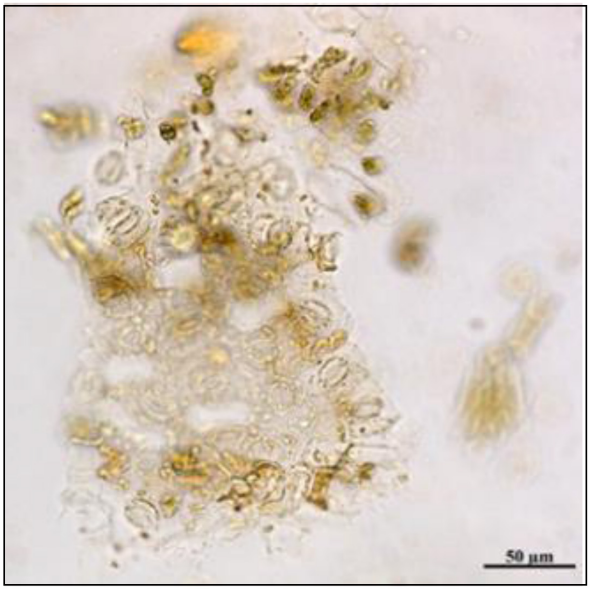

Figure 6: Paracytic stomata with epidermal cells.

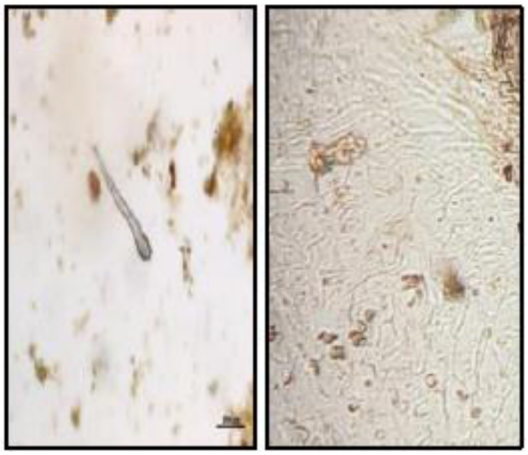

Figure 7: Multicellular covering trichome & Capitate glandular trichome.

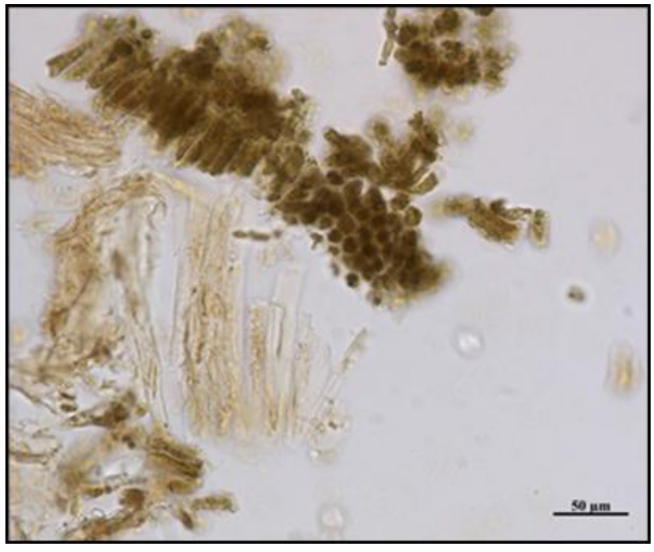

Figure 8: Palisade cells.

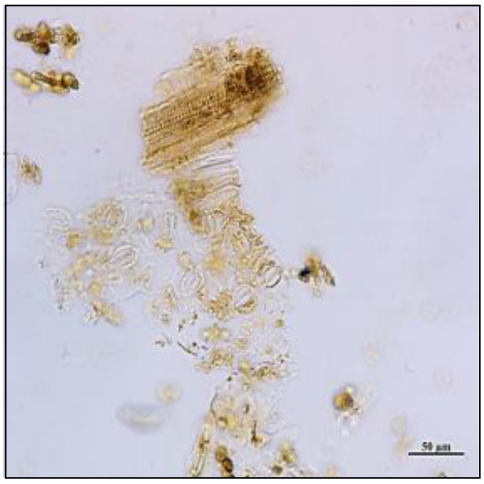

Figure 9: Xylem vessels, fibers, epidermal cells with stomata.

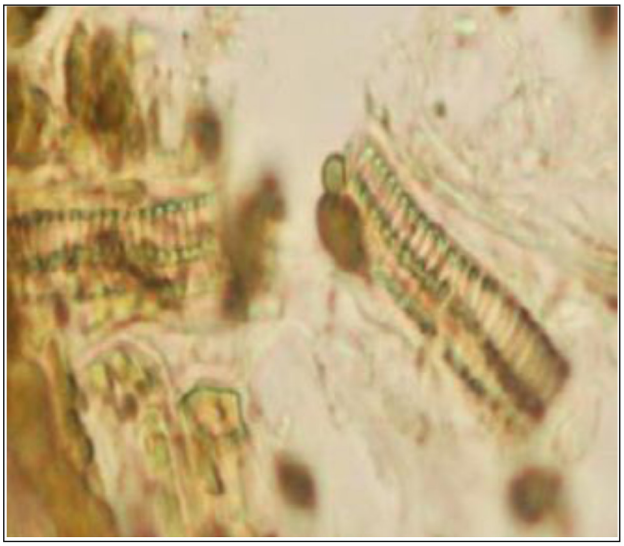

Figure 10: Lignified thickening in annular vessels.

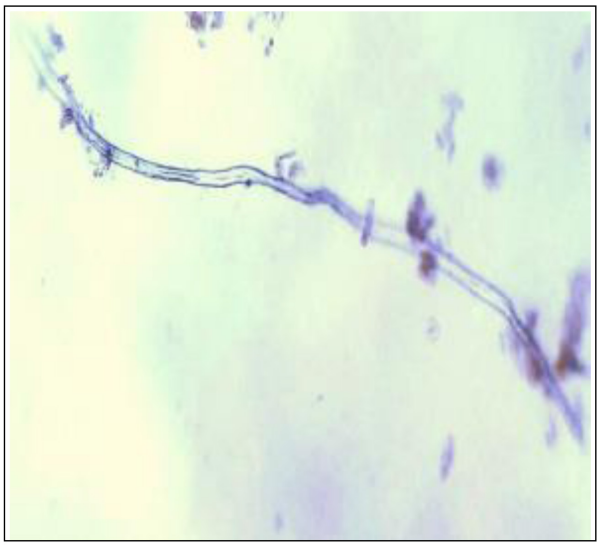

Figure 11: Phloem fiber.

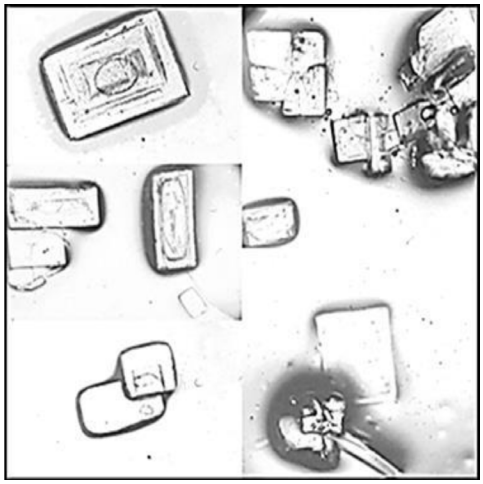

Figure 12: Photomicrograph showed prismatic crystals of calcium oxalate (x100).

Table 1: Leaf surface constants of *C. olitorius* leaf (Quantitative microscopy).

Parameters	Results
Stomatal number (upper)	12-(15)-17
Stomatal index (upper)	10.8-(12.7)-14.5 (%)
Stomatal number (lower)	16.0-(18)-20
Stomatal index (lower)	15.5- (17) – 19.3 (%)
Vein islet number	12-(14)-18
Veinlet termination number	18-(20)-22
Palisade ratio	5-(6)-8
Mean length of xylem vessels*	337.5 µm
Mean width of xylem vessels*	22.5 µm
Mean length of fibres*	465.8 µm
Mean width of fibres*	24.5 µm

* mean of 50 vessels and fibers

ratio, mean length of xylem vessels and fibers, and mean width of xylem vessels and fibers. The results are shown in Table 1.

Biocrystals of leaf

Figure 12 shows the presence of abundant calcium oxalate crystals of prismatic shape in various sizes. Some crystals show flat rectangular and square shapes in varying sizes.

Physico-chemical parameters

The various parameters that were evaluated to determine the purity of the *C. olitorius* leaves were moisture content, ethanol and water-soluble extractive values, total ash, acid insoluble and water-soluble ash, foaming index, and swelling index as shown in Table 2.

Phytochemical screening

Preliminary phytochemical screening of ethanolic and aqueous extracts showed the presence of flavonoids, polyphenols, phytosterols/ triterpenoids, alkaloids, carbohydrates, tannins, and proteins. The results are shown in Table 3.

Fluorescence analysis

The powdered drug was treated with various reagents like HCl, picric acid, iodine, etc., and the color of the drug after treatment with these

Table 2: Physico-chemical parameters.

Parameters	Results
Moisture content*	6.6 % w/w
Ethanol soluble extractive value*	4 % w/w
Water soluble extractive value*	17.6 % w/w
Total ash*	12 % w/w
Acid insoluble ash*	2.2 % w/w
Water soluble ash*	2.0 % w/w
Foaming index	>100
Swelling index	up to 10 ml

n=3: *dry weight basis

Table 3: Phytochemical screening of various extracts of *C. olitorius* leaf

Class of phytoconstituents	Ethanolic extract	Aqueous extract
Flavonoids	+++	+++
Polyphenols	++	+++
Tannins	++	++
Alkaloids	+	+
Proteins	+	+
Carbohydrates	+	+
Triterpenoid	+	+
Phytosterol	+	+

reagents was observed in an Ultraviolet chamber under visible, UV light (254 nm and 366 nm). The results are shown in Table 4.

Thin-layer chromatographic analysis

A standard solution of ethanolic extract was prepared and loaded quantitatively on precoated silica gel TLC plates. The plates were developed in 0.5% anisaldehyde- H_2SO_4 and also observed under UV lamp in the chamber at 254 nm and 365 nm respectively. Using the mobile phase Hexane: Ethyl Acetate: Acetone (8.5: 0.5: 1.5) showed the presence of nine spots as shown in Figure 13 while TLC of ethanol extract using mobile phase Benzene: Methanol (8.9: 1.1) showed the presence of 14 bands as shown in Figure 14. The R*f* values of the bands in both solvent systems are given in Table 5.

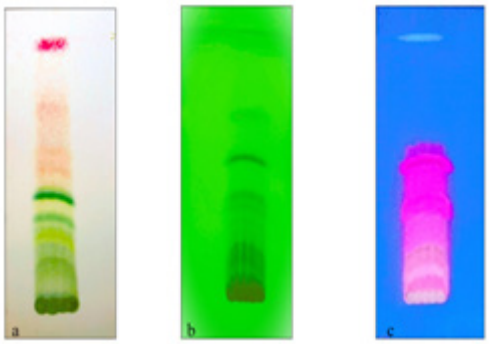

Figure 13: (a) Thin-layer chromatography of leaf ethanolic extract in visible light after spraying with anisaldehyde-sulphuric acid reagent, (b) TLC of leaf ethanolic extract in UV-long wavelength, (c) TLC of ethanolic extract in UV-short wavelength.

Table 4: Fluorescence analysis of *C. olitorius* leaf powder

Treatment	Visible light	Under UV light	
		Short wavelength (254 nm)	Long wavelength (366 nm)
Powder as such	Dark green	Dark green	Black
Powder + Water	Dark green	Dark green	Black
Powder + HCl	Dark green	Greenish black	Black
Powder + HNO_3	Leafy green	Dark green	Greenish black
Powder + H_2SO_4	Dark green	Blackish green	Black
Powder + 1N NaOH in Methanol	Dark green	Dark green	Blackish green
Powder + Picric Acid	Yellowish green	Fluorescence green	Black
Powder + Iodine	Dull green	Dull green	Blackish brown
Powder + $FeCl_3$	Dark green	Greenish yellow	Blackish brown
Powder + Lead acetate	Light green	Light green	Blackish brown

Table 5: Thin layer chromatograms of ethanol extract of *C. olitorius* leaves.

S. No.	Mobile system	Ratio	R_f values (After derivetization with 0.5% anisaldehyde- H_2SO_4)
1.	Hexane:Ethyl Acetate:Acetone	8.5:0.5:1.5	0.12, 0.21, 0.25, 0.29, 0.39, 0.49, 0.56, 0.72, 0.9
2.	Benzene:Methanol	8.9:1.1	0.10, 0.14, 0.19, 0.23, 0.33, 0.39, 0.54, 0.58, 0.63, 0.69, 0.73,0.82, 0.94, 0.98

Table 6: Results of observation of antimicrobial activity against *E. coli* bacterial strai.

Microbial strain	Diameter of zone of inhibition (mm)				
	EE		AE		Gentamycin
E. coli	40 mg/ml	80 mg/ml	40 mg/ml	80 mg/ml	(30 µg)
	7.3±1.2	8.23±0.7	6.23±0.02	16.3±1.7	19.0±1.87

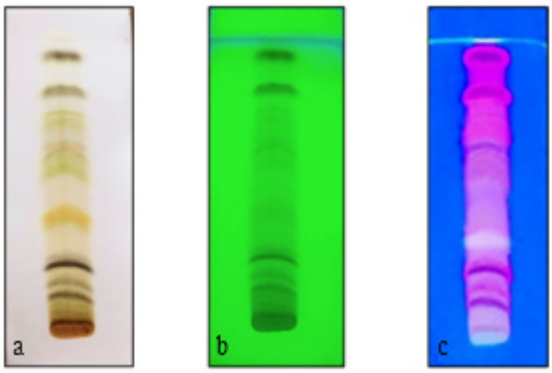

Figure 14: (a) Thin-layer chromatography of leaf ethanolic extract in visible light after spraying with anisaldehyde-sulphuric acid reagent, (b) TLC of leaf ethanolic extract in UV-long wavelength, (c) TLC of ethanolic extract in UV-short wavelength.

Antibacterial activity in *C. olitorius* leaves extracts

The zone of inhibition of ethanolic extract (40 and 80 mg/ml) and aqueous extract (40 and 80 mg/ml) against *E. coli* was compared with standard Gentamycin as shown in Table 6.

DISCUSSION

According to WHO, the first step for plant standardization is macroscopical and microscopical evaluation of a medicinal plant. A detailed pharmacognostic study of the leaf of *C. olitorius* was performed by macroscopy, microscopy, physicochemical analysis, preliminary phytochemical testing, and TLC was done and the antibacterial activity against *E. coli* was done by the agar well diffusion method.

The macroscopy of the leaf revealed the presence of a specialized feature of stipule at the base which helps in providing energy to the leaf.

In earlier findings done by Ghosh *et al.*, (2004), described the paracytic type of stomata complexes in *C. olitorius* leaf.[34] The abundance of prismatic calcium oxalate crystals is the characteristic of the leaf. It may provide structural support to the leaf tissues.

In the present study of microscopy (both qualitative and quantitative), the transverse section of *C. olitorius* leaf revealed some prominent features like paracytic stomata, mucilage cells, and collenchyma below an upper and lower layer of the epidermis and an abundance of calcium oxalate crystals. The presence of calcium oxalate crystals is responsible for calcium regulation, and homeostasis and it also plays a vital role in heavy metal detoxification.[35]

The physicochemical parameters were evaluated for moisture content for the presence of water because the greater value in any sample would promote the growth of microorganisms, insects, or fungi, and probably the constituents become hydrolyzed which may lead to a deterioration of the drug.[36] The moisture content was found to be 6.6% w/w. The lower value of moisture content is responsible for the protection of the drug from further degradation.

The determination of ethanol and water-soluble extractive values is used to estimate the phytochemicals present in the herbal plant. It may become a change if any exhausted material is added to the crude drug.[37]

The ethanol soluble extractive value was found to be 4% and the water-soluble extractive value was found to be 17%. The higher extractive value in water indicates the compounds present in the leaves are more water-soluble.

Ash value is also an important parameter because it is used to establish the purity and quality of the herbal plant. Total ash contains remaining residues after incineration at a temperature of 450˚C while carbon is totally removed. Total ash contains mainly carbonates, phosphates, silicates, and silica.[38]

The maximum limit for total ash value in the powdered medicinal plant is 14%.[39] Total ash was found to be 12% w/w which was, within the limit which signifies the purity of the crude drug.

Further results showed that the foaming index was observed to be >100 which shows the presence of saponins in the leaf and the swelling index was up to 10 ml which shows the mucilaginous content of the leaf.

In the identification of crude drugs, the presence of certain phytoconstituents plays a crucial role because they cause a distinct physiological effect on humans.[40] A preliminary qualitative test was done to detect the bioactive compounds. The leaves indicated the existence of flavonoids, polyphenols, phytosterols/ triterpenoids, alkaloids, carbohydrates, tannins, and proteins, which signifies the presence of various phytoconstituents.

One of the methods for qualitative analysis is fluorescence analysis for the authentification of the plant material. The fluorescence study signifies the specific color for a specific compound. The fluorescence of the non-fluorescent compounds is done by adding certain reagents which leads to the conversion to fluorescent derivatives or decomposition compounds.[41] The colors and the characteristic fluorescent properties of

leaf powder of *C. olitorius* recorded in this study (Table 4) could be used for identification purposes.

Thin-layer chromatography (TLC) is an important technique that is invaluable for the preliminary fingerprinting of various phyto-constituents.[42] TLC of ethanolic extract was done and a total of 14 R*f* values were found which signifies the presence of 14 phytochemicals in the extract.

The microbiological assay was done in order to determine the antibacterial activity of the ethanolic and aqueous extract. The standard drug gentamycin showed a zone of inhibition of 19.0±1.87 mm at the concentration of 30 µg whereas the ethanolic extract showed a zone of inhibition of 7.3±1.2 mm at a concentration of 40 mg/ml and 8.23±0.7 mm at 80 mg/ ml.

The aqueous extract showed a zone of inhibition of 6.23±0.02 mm at 40 mg/ml and 16.3±1.7 mm at 80 mg/ ml. This result concludes the efficacy of aqueous and ethanolic extract at the concentration of 40 mg/ml was almost equivalent but the efficacy of aqueous extract significantly raised at 80 mg/ml as compared to ethanolic extract.

The efficacy of aqueous extract was nearby the standard value at the concentration of 80 mg/ml.

The phenolic and flavonoidal compounds present in the plant may be responsible for the antibacterial activity, and further investigation is required for the specificity of the compounds.

CONCLUSION

The pharmacognostic study of the leaf will provide certain standards for correct identification for future works. The antibacterial study confirms that the aqueous extract of the leaf has potent antimicrobial activity against the *E. coli* strain.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

UPEC: Uropathogenic *E. coli* strain; **HCl:** Hydrochloric acid; **TLC:** Thin Layer Chromatography; **UV:** Ultra Violet; **EMB agar:** Eosin-methylene blue agar; **MIC:** Minimum Inhibitory Concentration.

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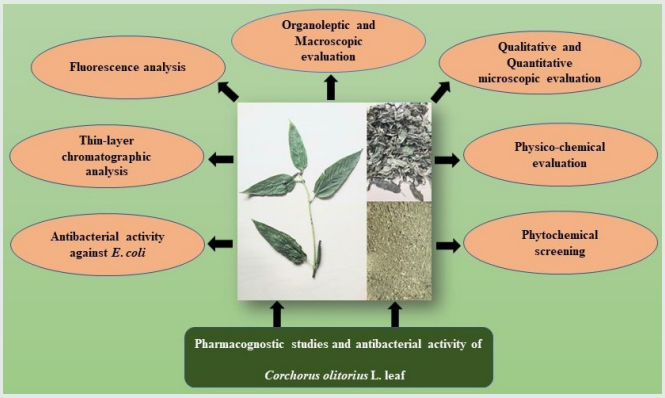
GRAPHICAL ABSTRACT



SUMMARY

Corchorus olitorius is an important green leafy vegetable in many places and is also used in folk medicine. In the present study, the pharmacognostic evaluation of *C. olitorius* was performed, which could be helpful for the correct identification and authentication of the species for future studies. Macroscopically the fresh leaves were examined. Anatomically the leaf was characterized by paracytic stomata, dorsiventral mesophyll, numerous prismatic calcium oxalate crystals, and covering trichomes. Both transverse section and powder were studied thoroughly. In physico-chemical evaluation parameters like moisture content, ash values and extractive values were determined. Leaf powder was also identified by using different reagents in fluorescence analysis. The phytochemical analysis of both the aqueous and ethanolic extracts of plant leaves and their antibacterial activities against *Escherichia coli* was investigated. The phytochemical analysis revealed the presence of flavonoids, polyphenols, phytosterols/ triterpenoids, alkaloids, carbohydrates, tannins, and proteins. TLC fingerprinting of ethanolic extract was done for the separation of phytoconstituents for qualitative analysis. According to the above antimicrobial activity results, at a concentration of 80 mg/ ml, it was observed that the aqueous extract exhibited good antibacterial activity.

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