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RESEARCH ARTICLE

Ethnobotany and Antimicrobial Activity of Traditional Plant -*Holotelea integrifolia*

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Abstract

*Holoptelea integrifolia (*Ulmaceae) is widely used in traditional systems of medicine for healing ailments. The plant is preferred in the treatment and curing of diseases like leprosy, inflammation, rickets, leucoderma, scabies, rheumatism, eczema, malaria, and many more. The plants exhibit various pharmacological activities like antimicrobial, nociceptive, antiaging, inflammation, anthelmintic, antidiabetic, adaptogenic, anticancer, wound healing, hepatoprotective, and hypolipidemic. The study on morphology and microscopy was carried out on this plant as per the standards laid down by those who sought to find the genuine species. Physical constant values involving moisture content, ash, and extractives, as well as qualitative and quantitative estimation of various phytochemicals, have been studied. The presence of saponins, tannins, terpenoids, steroid, flavonoids and some other chemical constituents were recorded, followed by the antimicrobial activity of the plant against Gram-positive and Gram-negative bacteria. Looking forward, the potential aspects of the plant used in traditional system standardization parameters were to be considered for its validation and authentication.

Keywords: Antimicrobial, Ethnobotany, Phytoconstituents, Standardization

1. Introduction

Natural products are used as a source of biologically active compounds, which serve as a medium for efficacy and therapeutic action. The use of medicinal plants and herbs are increasing day by day for their potential uses in our daily life as a necessity to cure various disease. One such plant is *Holoptelea integrifolia*. This plant belongs to the Ulmaceae family, which consists of 15 genera and near about 200 species that are widespread and largely cover the tropical and temperate regions of India, Indochina and Srilanka. It is generally known as Begana (Hindi) and Waola (Gujarati). It is a potent pollen allergen plant found in India. Leaves are elliptic- Ovate, acuminate with a round base and subcordate in shape. Bears greenish-yellow flowers with suborbicular fruits on the membraneous wing. The plant parts namely bark and leaves serve as bitter, thermogenic, digestive, and astringent in the urinary part and rheumatism, The juice of the leaves is used in the regulation of fat metabolism, and eczema treatment is caused mainly by ringworm[1]. Moieties including racemic forms of Holoptelin friendly, epifriedlin, β-amyrin, 1,4-napthalenedione, betulin, betulinic acid, hexacosanol, and octacosanol were studied and isolated from the plant species[2]. The plant possesses numerous pharmacological activities like antioxidant, antimicrobial, anti-inflammatory[3,4], antitumour[5], adaptogenic[6], wound healing and many more to be used as a diverse plant for various diseases[7,8,9]. Hence the plant species were used for the study to explore its potential based on ethnobotany and ethnomedicine.

2. Materials and Methods

2.1 Plant Material

Holoptelea integrifolia leaves were collected from the wild sources of Gujarat in the Vadodara district, and they were identified and authenticated by Dr. P. Jayaraman, Director of the Plant Anatomy Research Centre, Chennai.

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The collected plant parts, namely the leaf, were cleaned properly and further pulverized using the grinder, passed through Sieve no. 40, and stored properly in a ploy plastic container.

2.2 Morphological Analysis

The organoleptic studies were carried out, which mainly depict the shape, size, color, odour, margin, and apex. The microscopy of the leaf and petiole was done as per the standard procedure.

2.3 Microscopical Studies

2.3.1 Collection of Specimens

The specimens were collected from the Vadodara region.

2.3.2 Sectioning

The procedure was followed as per Sass[10], Johansen[11] and O'Brien, *et al*[12].

2.3.3 Photomicrographs

Photographs of different magnifications were taken and indicated by scale bars.

2.4 Physico Chemical Parameters

The parameters were as per the Indian Pharmacopoeia.

2.5 Powder Analysis

Powder analysis of the drug was carried out to find out the various histo structures present in the leaf sample[13,14].

2.6 Quantitative Microscopy[15]

2.6.1 Determination of the Stomatal Number and Stomatal Index

The determination of leaf constants was carried out as per the standard procedure.

2.6.2 Determination of Vein-islet and Vein Termination Number

The determination of the vein islet and termination were carried out as per the standard procedure.

2.6.3 Determination of the Palisade Ratio

The determination of the palisade ratio was carried out as per the standard procedure.

2.7 Preliminary Phytochemical Analysis[16]

Preliminary phytochemical analysis of the extract was carried out by accurately weighing 100gm of dried powdered plant material which was subjected to maceration with different solvents as per the polarity. The extract was dried and the residue was weighed for further use 1.

2.8 Microbial Activity

2.8.1 Test Organism and Inoculums

Gram-negative bacteria: *Escherichia coli;* and Gram-positive bacteria: *Staphylococcus aureus, Bacillus subtilis*, *Bacillus aureus* and microorganisms *Candida albicans* and *Cryptococcus neoformans* were procured from the Department of Microbiology, Noida Institute of Engineering and Technology, Pharmacy Institute, Greater Noida, Uttar Pradesh.

2.8.2 Standard

Anti-Bacterial Amoxycillin disc of the concentration of 30ìg/disc and Antifungal Voriconazole of the concentration of 30ìg/disc were obtained from Span Diagnostics, Mumbai.

2.8.3 Media and Organism Preparation

Dehydrated nutrient agar media were prepared according to the Indian Pharmacopoeia[17,18].

2.8.4 Experimental Methods

Cup and Plate Method[18]. The hydroalcoholic extract of the plant at a concentration of 10 mg/ml was made in 1% Dimethyl Sulfoxide (DMSO). Amoxycillin (Discs 30µg/disc) and Voriconazole (30µg/disc) were used as standards. Micropipette was used to deliver the solutions into holes. The volume of solution added to each hole was kept uniform (0.1 ml in each hole). One strip of amoxycillin and voriconazole (standard) was placed aseptically in the centre hole of each plate.

3. Results

3.1 Macroscopic Characters of Leaf

Simple alternatives, with dark green on the upper surface and light green on the lower surface leaves are seen. They are penninerved, 8 – 13 × 4 – 6 cm., with elliptic to acuminate and further glabrous with an entire margin

and round base or cordate to leathery. The petiole is 6 – 13 × 0.1 – 0.15 cm. The petiole is a thin, brown colored attached to the base of the stem of the plant, contained uneven fractures as shown in Figure 1.

3.2 Anatomy of Leaf

The cross-sectional view of the leaf exhibits a thick midrib and thin lamina. The midrib is thick on the abaxial side and slightly raised on the adaxial side. The midrib is 950 µm in the vertical plane and 900 µm in the horizontal plane, as shown, in Figure 2.

3.3 Midrib Region

The midrib consists of a thick layer of small, thick-walled and conical epidermal cells. The ground tissue in the adaxial region is collenchymatous. In the other region of the midrib, there is a continuous layer of three cells thick sub-epidermal zone. Apart from the thick sub-epidermal layers, the ground tissue is parenchymatous, circular and compact. The vascular cylinder is thick and wide and planoconvex in outline as shown in Figure 3.

Figure 1. Morphology of *Holotelea integrifolia.*

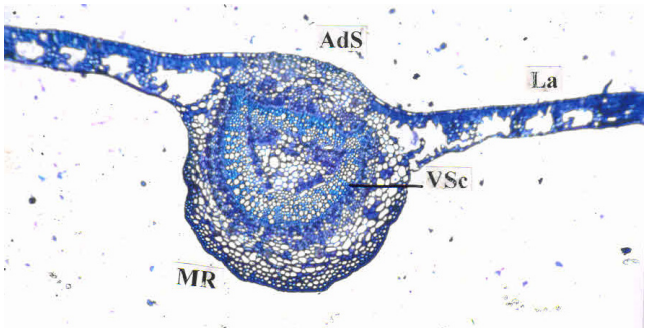

Figure 2. Transverse section of *Holotelea integrifolia* leaf.

Ads: Adaxial Side, La: Lamina Region, MR: Midrib Region, VSc: Vascular Cylinder.

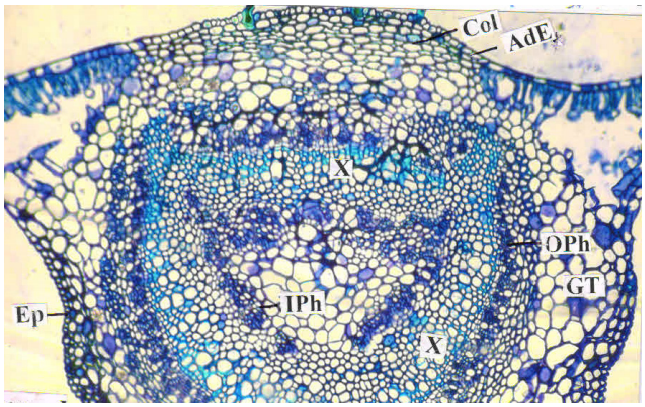

Figure 3. Transverse section of the enlarged midrib.

Ep: Epidermal Layer, IPh: Inner Phloem, OPh: Outer Phloem, AdE: Adxaial Epidermis, X: Xylem, GT: Ground Tissue, Col: Collenchyma Cells.

The vascular cylinder of the midrib consists of compact radical lines of angular xylem elements. On the outer part of the xylem, a cylinder occurs thick masses of phloem elements, the phloem elements are small and thick-walled. Along the inner part of the vascular cylinder occur elongated, thick segments of the inner phloem. The inner phloem elements are thick-walled and darkly stained. The central core of the midrib consists of wide, polyhedral, thick-walled parenchyma cells, as shown in Figure 4.

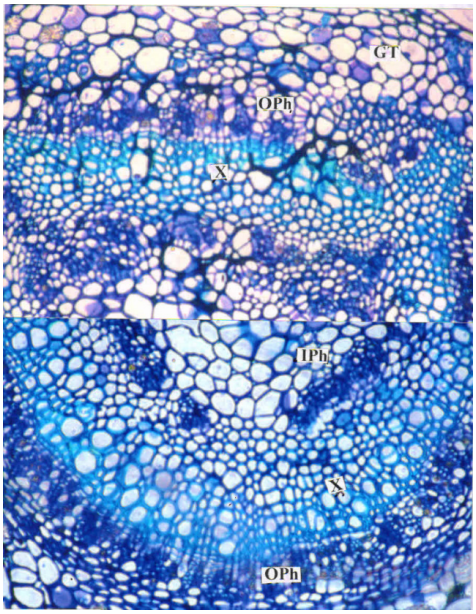

Figure 4. Arrangement of xylem and phloem.

GT: Ground Tissue, IPh: Inner Phloem, OPh: Outer Phloem, X: Xylem.

3.4 Lamina

The lamina consists of thick and thin regions the alternate in the horizontal layer. The lamina is dorsiventral (Figure 3). The epidermal layer on the adaxial side is rectangular and thick-walled. It consists of a thick article. The abaxial epidermis has slightly smaller, thick walled epidermal cells (Figures 2 and 3). The midrib of the leaf is 650 µm in thickness. It consists of thick, triangular xylem elements that are angular and thick-walled. The protoxylem is adaxial in position. Phloem occurs in a small, thin layer on the adaxial part of the xylem strand. The palisade zone consists of a single horizontal layer of cylindrical cells. In between the vascular bundles of the lateral vein, there are largely empty chambers as shown in Figures 5 and 6.

3.5 Leaf Margin

The leaf margin is straight and semi-circular. It consists of small epidermal cells with a thick article. The sub-epidermal region has a compact mass of thick-walled cells. There is a wide horizontal layer of parenchymatous mesophyll cells. Tannin containing long horizontal cells occurs on the lower part of the leaf margin. The marginal part of the leaf is 60 µm thick as shown in Figure 7.

3.6 Petiole

The petiole is more or less circular in cross-sections view with a straight, flat adaxial region (Figure 4). The epidermal cells of the petiole are small and darkly stained. The ground tissue is comprised circular thin-walled cells and sparsely distributed, thick-walled angular sclerenchyma cells as shown in Figure 8.

The vascular system is crude, prominent and planoconvex. It consists of short parallel lines of xylem elements that are 3 or 4 cells in length. The xylem elements are circular in outline. Along the outer region of the xylem cylinder occur several long radical lines of phloem elements. The phloem elements are two cells with thick and thin walls. Along the inner part of the vascular cylinder occur as shown in Figure 9.

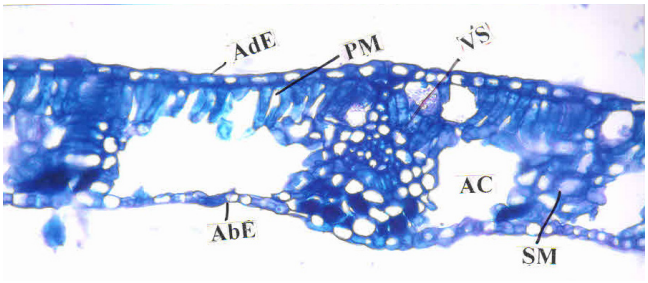

Figure 5. Section of lamina enlarged.

AdE: Adaxial Epidermis, AbE: Abaxial Epidermis, PM: Palisade Mesophyll, AC: Air Chamber, Vs: Vascular Strand, SM: Spongy Mesophyll.

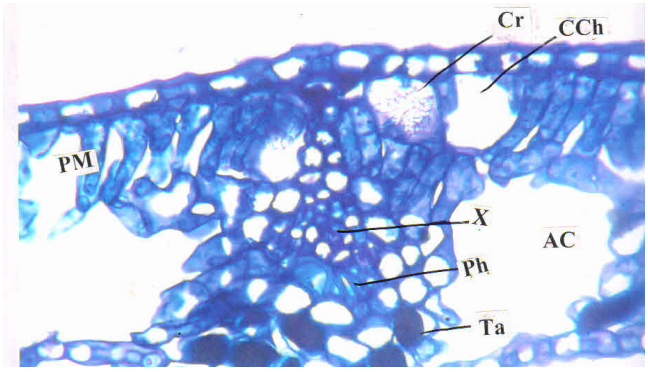

Figure 6. Arrangement of cells in lamina region.

Cr: Crystal, CCh: Crystal Chamber, PM: Palisade Mesophyll, X: Xylem, Ph: Phloem, AC: Air Chamber, Ta: Tannin.

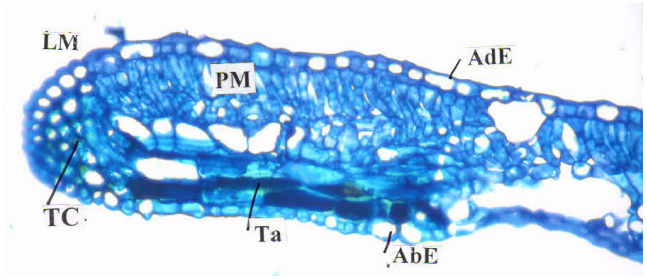

Figure 7. Arrangement patter of cells in the leaf margin.

LM: Leaf Margin, PM: Palisade Mesophyll, AdE: Adaxial Epidermis, AbE: Abaxial Epidermis, Ta: Tannin, TC: Terminal Cells.

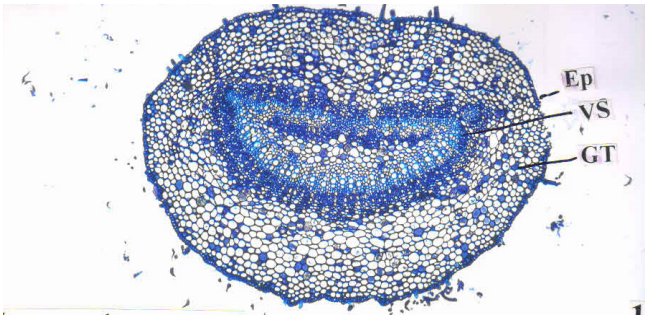

Figure 8. Transverse section of petiole part.

Ep: Epidermis, GT: Ground Tissue, Vs: Vascular Strands.

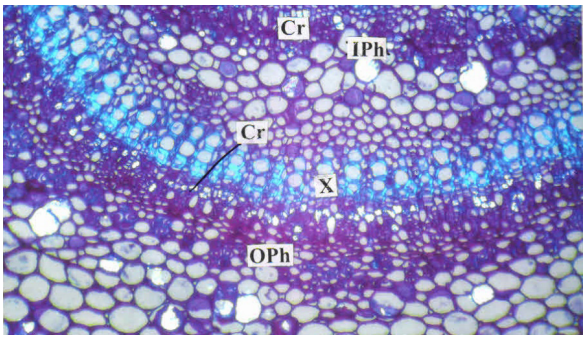

Figure 9. Arrangement of crystals with xylem and phloem cells in the petiole region.

Cr Crystal, OPh: Outer Phloem, X: Xylem, IPh: Inner Phloem.

3.7 Inner Phloem

The inner phloem is thick in the upper horizontal region and it is in small circular units along the lower horizontal region. Druses type of crystals is sparsely distributed in the ground tissue. The petiole is 1.1 mm in the vertical plane and 1.6 mm in the horizontal plane. The xylem elements are circular and they are scattered in small radial clusters among the xylem fibres (Figure 5). Phloem tissues occurs both on the outer phloem and include a thick darkly staining continuous layer as shown in Figure 10.

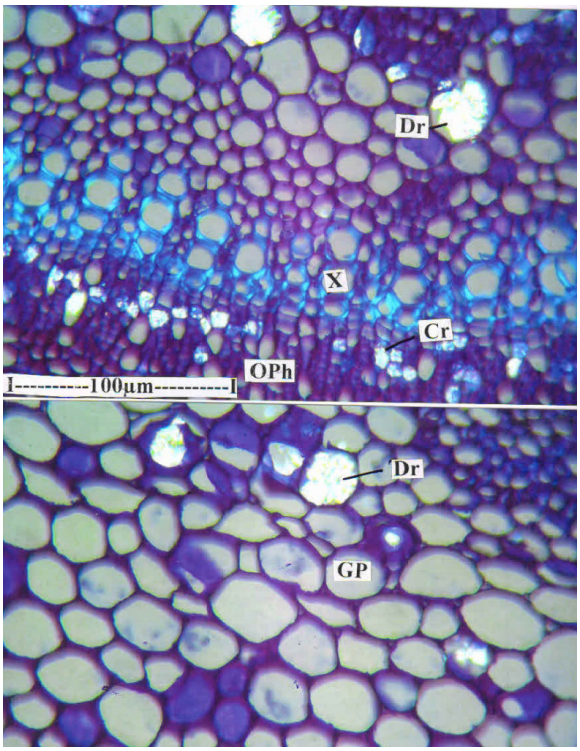

Figure 10. Arrangement of phloem cells.

Dr: Druses, GP: Ground Parenchyma, Cr: Crystal, X: Xylem, OPh: Outer Phloem.

3.8 Stem

The stem is circular and smooth in outline. It is 1.5 mm in diameter. The stem consists of a thin epidermal layer of small cells. The cortical zone includes several layers of circular compact parenchyma cells. Some of the cortical cells are densely filled with mucilage. The vascular cylinder is amphiphloic comprising outer and inner phloem tissue. The xylem elements are circular and they are scattered in small radial clusters among the xylem fibres. Phloem tissues occurs both on the outer and inner regions of the xylem cylinder. The outer phloem includes a thick, darkly stained continuous layer. The outer phloem is thicker and consists of several compact radial lines. The inner phloem cells are wide angular and thin-walled. The phloem includes sieve elements and phloem parenchyma as shown in Figures 11 and 12.

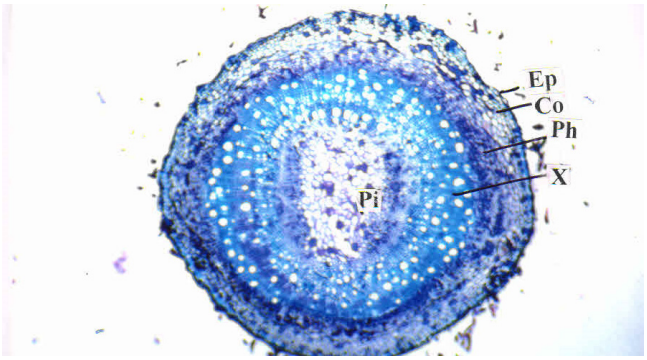

Figure 11. Gross section of the stem part of *Holotelea integrifolia.*

Ep: Epidermis, Co: Collenchyma Cells, Ph: Phloem, X: Xylem, Pi: Pith.

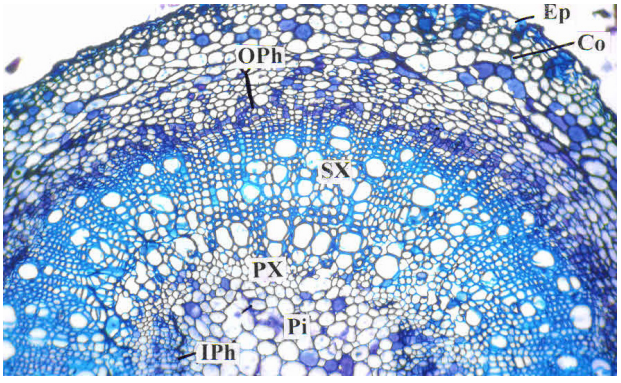

Figure 12. Section of stem part enlarged.

Ep: Epidermis, Co: Collenchyma Cells, OPh: Outer Phloem, SX: Secondary Xylem, PX: Primary Xylem, IPh: Inner Phloem, Pi: Pith.

3.9 Crystal Distribution

There is a large number of druses type of crystals in the outer phloem region and sparsely distributed druses in the cortical tissues. The druses are solitary in each cell and they occupy the entire lumen of the parenchyma cells as shown in Figure 13.

3.10 Stomata

The stomata occur in the abaxial epidermis of the leaf. They are sparsely distributed with wide space in between the stomata. The epidermal cells are thick and with wavy anticlinal walls (Figure 9). The stomata are diacytic type. The guard cells are widely elliptical with wide elliptical, stomata dove. The guard cells are 15 x 25 µm in size as shown in Figure 14.

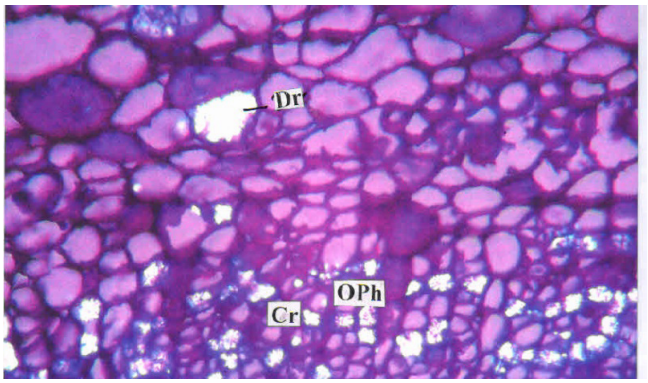

Figure 13. Arrangement of crystals.

Dr: Druses, Cr: Crystal, OPh: Outer Phloem.

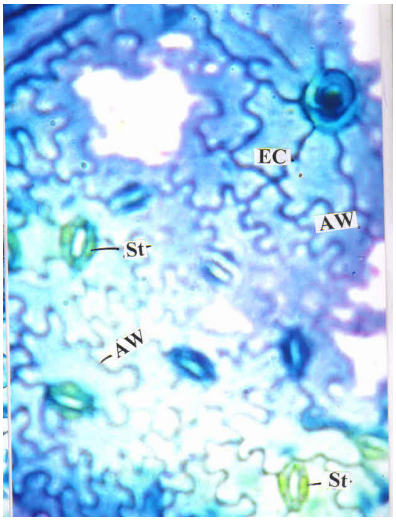

Figure 14. Position and structure of stomata.

EC: Epithelial Cells, AW: Anticlinal Wall, St: Stomata.

3.11 Venation Pattern

The venation pattern system is densely reticulated. The veins are thick and profusely branched. The vein islets are wide and irregular in outline. Some of the vein terminations are simple and unbranched. There are also well-branched vein terminations that are thick and short. The vein has a dense branching system with distinct vein islets and vein terminations.

Long thick gradually tapering epidermal trichrome is sparsely seen on the epidermis as shown in Figure 15.

3.12 Powder Microcopy

The green-colored powder of the leaf drug was used for the study. The powder was stained with phloroglucinol and concentrated hydrochloric acid. It was mounted in glycerin and examined under 10 X and then magnified with 40 X. On microscopic examination, it showed prism-shaped calcium oxalate crystals. Trichomes were unicellular and uniseriately covered, vessels were spiral, starch grains were spherical, and fibres were long, slender and non-lignified in nature as shown in Figure 16.

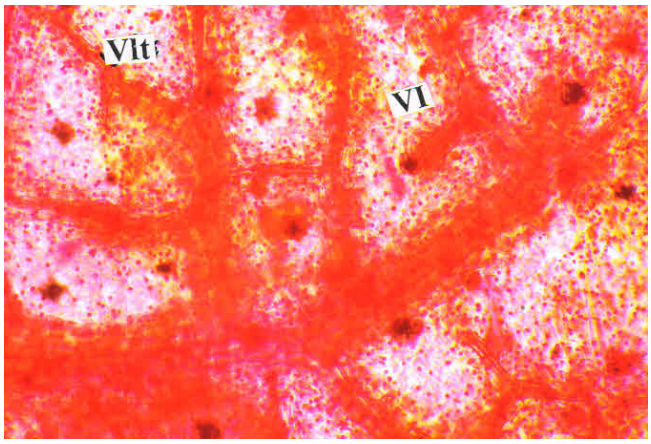

Figure 15. Pattern of vennations.

VI: Vien Islets, VIt: Veinlets.

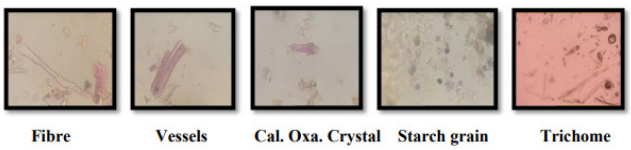

Figure 16. Powder microscopy of leaf drug of *Holotelea integrifolia.*

3.13 Quantitative Microscopy

Quantitative microscopy was performed and the results are tabulated in Table 1.

3.14 Fluorescence Analysis

The powder drug and extracts were subjected to fluorescence analysis as per the standard procedure in Tables 2, 3 and 4.

Table 1. Quantitative values of leaf constants

S. No.	Leaf Constants	Values
1	Stomatal Number	12
2	Stomatal Index	37.5
3	Palisade Ratio	4.8
4	Vein Islet Number	11
5	Vein Termination	15

Table 2. Fluorescence analysis of an ethanolic extract of *Holotelea integrifolia*

Drug	Visible Range (400 nm)	Narrow Ultraviolet (256 nm)	Long Ultraviolet (365 nm)
Sample	Greenish Black	Dark Green	Dark Green
Sample + Water	Powdery Green	Brownish Green Tint	Lightest Green
Sample + Conc. Mineral Acid	Green	Dark Green	Light Green
Sample + Conc. Sulphuric Acid	Black	Black	Brick Red
Sample + Conc. Nitric Acid	Green	Earthy Green	Light Green
Sample + NaOH	Light Green	Muddy Green	Light Green
Sample + Acetone	Light Green	Light Green	Yellowish Green
Sample + Alcoholic Part	Light Green	Dark Green	Light Green
Sample + Acetic Anhydride	Pale Green	Light Green	Yellowish Green
Powder + (2M) HCl	Green	Dark brown Green	Brownish Green

Table 3. Fluorescence analysis of a hydro-alcoholic extract of *Holotelea integrifolia*

Drug	Visible Range (400 nm)	Narrow Ultraviolet (256 nm)	Long Ultraviolet (365 nm)
Sample	Yellow	Light Green	Black
Sample + Water	Light brown	Light Green	Black
Sample + Conc. Mineral Acid	Brown	Light Green	Black
Sample + Conc. Sulphuric Acid	Reddish Black	Dark Green	Black
Sample + Conc. Nitric Acid	Yellow	Light pale green	Black Brown
Sample + Sodium Hydroxide	Dark Yellow	Pale Green	Black tint
Sample + Acetone	Yellow	Basil Green	Reddish Black
Sample+ Alcoholic Part	Dark Yellow	Light Green	Black
Sample + Acetic Anhydride	Yellow	Greenish Black	Black
Sample + (2M) HCl	Yellow	Light Green	Black

Table 4. Fluorescence analysis of an aqueous extract of *Holotelea integrifolia*

Drug	Visible Range (400 nm)	Narrow Ultraviolet (256 nm)	Long Ultraviolet (365 nm)
Sample	Reddish Brown	Green	Dark Green
Sample + Water	Light Brown	Green	Dark Green
Sample + Conc. Mineral Acid	Light Green	Brown	Dark Green

(*Continued*)

Table 4. (Continued)

Drug	Visible Range (400 nm)	Narrow Ultraviolet (256 nm)	Long Ultraviolet (365 nm)
Sample + Conc. Sulphuric Acid	Dark Brown	Light Green	Blackish Brown
Sample + Conc. Nitric Acid	Reddish Brown	Green	Blackish Red
Sample + NaOH	Dark Reddish Brown	Pine Green	Blackish Brown
Sample + Acetone	Greenish Brown	Light greenish black	Black
Sample+ Alcoholic Part	Light Brown	Greenish brown	Black
Sample + Acetic Anhydride	Light Brown	Light green	Blackish green
Sample + (2M) HCl	Dark Brownish Black	Emerald Green	Blackish Brown

3.15 Physicochemical Parameters

The parameters are listed in Table 5. In addition to it, extractive values of the extracts were carried out like petroleum ether (1.2% W/V) with yellowish-grey, ethyl acetate (1.9% W/V) with dark brown, chloroform (2.1% W/V) blackish brown, ethanolic (3.8% W/V) with brown, hydro alcoholic (50:50) (4.4% W/V) with brownish green and finally with an aqueous extract (4.6% W/V) with light greenish-brown colour was observed and tabulated in Table 5.

Table 5. Physicochemical analysis of *Holotelea integrifolia*

S. No.	Standardization Parameters	Results
1	Total Ash	11%[a]
2	Acid Insoluble Ash	6.27%[b]
3	Acid Soluble Ash	5.1%[b]
4	Insoluble Ash	2.8%[b]
5	Soluble Ash	3.4%[b]
6	Loss on Drying	1.52%[a]
7	Water Soluble Extractive Value	68.5%[b]
8	Alcohol Soluble Extractive Value	14.58%[b]

a:W/W, b: W/V

3.16 Preliminary Phytochemical Analysis

The analysis is listed in Table 6.

Table 6. Preliminary phytoconstituents analysis of different extracts of *Holotelea integrifolia*

Phytoconstituents	Ethanolic Extract	Hydroalcoholic Extract	Aqueous Extract
Alkaloids	++	+	-
Amino Acid	++	-	+
Carbohydrates	-	+	+
Flavonoids	+	-	-
Glycosides	+	-	+
Phenols	+	-	+
Proteins	-	+	-
Quinines	+	-	-
Saponins	+	-	++
Tannins	+	+	
Sterols	+	-	++
Terpenoids	+	++	-

+ indicates the presence in slight quantity, ++ indicates the high Content, - indicates the absence of the phytoconstituents.

3.17 Antimicrobial Studies

The results are in Table 7.

Table 7. Antibacterial and antifungal activity of *Holotelea integrifolia*

Samples	Zone of Inhibition (mm)					
	Staphylococcus aureus	*Bacillus subtulis*	*Bacillus aureus*	*Escherchia Coli*	*Candida albicans*	*Crytococcus neoformans*
Ethanolic Extract of HI (100mg/ml)	24	22	26	22	19	11
Hydro-Alcoholic Extract of HI (100mg/ml)	22	24	25	24	18	14
Aqueous Extract of HI (100mg/ml)	24	20	25	22	22	16
Amoxycillin (30µg/disc)	21	21	21	26	-	-
Voriconazole (30µg/disc)	-	-	-	-	20	18

4. Discussion

Every plant in nature has a specific structure, appearance, morphological characters, geographical features, chemical constituents and potency of effects. Hence, it becomes necessary to understand these properties as they exert various pharmacological actions or therapeutic effects. It is also important to understand the various quality control parameters involved in the identification of crude drugs as recommended by the World Health Organization for medicinal plants.

5. Conclusion

The plant *H. integrifolia* can be used as an effective therapeutic remedy in the prevention and treatment of various ailments. However, further studies can be carried out on its chemical entity.

6. Acknowledgements

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