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PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION OF FICUS AMPLISSIMA SM. STEM BARK

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Abstract: -
The present study investigates various Pharmacognostic parameters of the stem bark of *Ficus amplissima* Sm. (family: Moraceae). Macroscopic and microscopic evaluations, physicochemical analyses, and the behaviour of the powdered drug with different chemical reagents were systematically examined. Successive extraction of the stem bark using solvents of increasing polarity was performed, and the resulting extracts were subjected to phytochemical screening. The analysis revealed the presence of key bioactive constituents such as alkaloids, glycosides, flavonoids, and phenolic compounds. These preliminary findings contribute to establishing standardization parameters for the stem bark of *Ficus amplissima* Sm. Furthermore, High-Resolution Liquid Chromatography–Mass Spectrometry (HR-LCMS) was employed as a robust analytical tool to identify and quantify secondary metabolites, highlighting its significance in botanical and Pharmacognostic studies.

Keywords: Microscopy, Physiochemical, Phytochemistry, *Balanites aegyptiaca*, secondary metabolites, phenolic compounds, pharmacological activities, phytochemical profile, HRLCMS.

INTRODUCTION: -

Ficus amplissima Sm., a member of the family Moraceae, is a large evergreen tree distributed widely across tropical and subtropical regions of India, Sri Lanka, and Southeast Asia (Singh et al., 2011). In India, it is commonly found in Karnataka, Kerala, Tamil Nadu, and Maharashtra. The species is well recognized in Ayurvedic literature under the name ‘Plaksha’ and in English as the Indian Fig Tree. Locally, it is also known as ‘Atti’ in Kannada and ‘Plaksha’ in Sanskrit. The tree, which can reach heights of up to 25 meters, is known for its ecological significance and resilience to varying climatic conditions (Rao et al., 2014).

Traditionally, various parts of *F. amplissima* are employed in folk and classical medicine for treating a range of ailments including diabetes, diarrhoea, ulcers, and skin disorders (Joshi et al., 2012). The stem bark, in particular, is valued for its anti-inflammatory, wound-healing, and antimicrobial properties. Phytochemical investigations have revealed the presence of bioactive compounds such as flavonoids, glycosides, tannins, and sterols (Kumari et al., 2016). Methanolic extracts of the bark have demonstrated antioxidant and antidiabetic activities in experimental studies, supporting its ethnomedicinal claims (Patil et al., 2018).

Phytoconstituents isolated from *F. amplissima* include lupeol, β-sitosterol, and various phenolic compounds, which may contribute to its pharmacological effects (Chopra et al., 2017). Recent chromatographic and spectroscopic analyses have further identified triterpenoids and flavonoid glycosides in the bark and leaves (Sharma et al., 2020). Given its diverse therapeutic applications and rich phytochemical profile, *F. amplissima* holds promise as a source of novel bioactive compounds for pharmaceutical development.

Distribution: It comes from Central and Southern Peninsular India, Sri Lanka, and the Maldives. It is widely found in the Western Ghats of India. (India Biodiversity Portal, 2018) It is most commonly planted to provide shade in coffee plantations due to its dense and wide foliage. The ripened figs attract many birds, especially during the spring. (India Biodiversity Portal, 2018).

Description: Large trees with spreading branches and without aerial roots; all parts glabrous. Leaves alternate, coriaceous, broadly ovate to elliptic-lanceolate, 7-15 x 3-8 cm, narrowed or rounded at base, entire, acute or obtusely cuspidate; petioles 3-5 cm long; stipules ovate, 1.5-2.5 cm long, acuminate. Receptacles axillary, solitary, crowded at the ends of branches, globose, 1-1.5 cm in diam., smooth, purple; basal bracts 3, minute, broadly ovate, scarious. Male flowers sessile; tepals 3, ovate, acuminate; stamen one, shorter than the tepals. Gall and fertile female flowers shortly stalked; tepals 3, ovate. Achenes ovoid-reniform. (Naik, 1998).

MATERIAL AND METHODS

2.1) Plant material collection

The stem bark of *Ficus amplissima* was collected by self in the month of July Latitude N19o,49',07.5" Longitude E075o,37',73.5" Altitude 447.5 m, from Palthi Nagari, Paithan, Aurangabad. Bark was pulverized in the mechanical grinder to a fine powder to carry out different Pharmacognostic and phytochemical evaluation and was stored in a well closed airtight vessel for further analysis (Table No: - 1).

2.2) Behaviour of bark powder towards some chemical reagents.

The powder of *Ficus amplissima* bark was treated with different chemical reagents. The mixture of the powdered drug and chemicals were allowed to warm and cold down for two hours. Changed colour of powdered drug was noted (Table No: - 2).

2.3) Physico-chemical Evaluations.

Physico-chemical parameters such as water-soluble ash, water insoluble ash, acid insoluble ash, acid soluble ash, total ash, loss of weight on drying 105^0C was determined. Considering the diversity of chemical nature and properties of contents of drugs, different solvents benzene, petroleum ether, chloroform, methanol, water, alcohol, chloroform water of extractive values was determined as per reported methods (Mukherjee PK 2002, Kakate CK 1994, Khandelwal KR 2005) (Table No: - 3).

2.4) Phytochemical screening

Qualitative examination of *Ficus amplissima* bark inorganic matters and determination of heavy metals was done as per reported methods. The dried powdered bark was subjected to preliminary phytochemical screening for qualitative detection of phytoconstituents. The dried powdered bark (100g) was extracted successively hexane, petroleum ether, benzene, benzene, chloroform, acetone, methanol, water in Soxhlet Extractor by continuous hot percolation. Each time before extracting with the next solvent of higher polarity the powdered material was dried in hot air oven below 50^0C for 10 minutes. Each extract was concentrated in vacuum on a Rote Evaporator and finally dried in hot air oven. The dried extracts were dissolved in respective solvents, with it was extracted, and were subjected to various qualitative phytochemical tests for the identification of chemical constituents present in the plant material (Harborne JB 2005) (Table No: - 4 and 5).

2.5) Morphology, Anatomy and Maceration: -

The morphological characters of the trees were studied in detail and their herbarium sheets were prepared which were preserved in the Herbarium of Department of Botany, Pratishthan Mahavidyalaya, Paithan. Fresh and dried bark samples were studied morphologically in the field as well as in the laboratory regarding their colour and texture of inner and outer surfaces, splitting, quelling etc.

2.6) High-Resolution Liquid Chromatography–Mass Spectrometry (HR-LCMS) Analysis

For advanced phytochemical profiling, the methanolic extract of *Ficus amplissima* stem bark was subjected to HR-LCMS analysis. The prepared sample was sent to **Sophisticated Analytical Instrumentation Facility (SAIF), Indian Institute of Technology (IIT), Bombay** for analysis. HR-LCMS was carried out using a [mention the model if known, e.g., "Thermo Scientific Q Exactive Orbitrap"] system equipped with an electrospray ionization (ESI) source operating in positive and negative ionization modes. The chromatographic separation was achieved on a [mention column type if known, e.g., C18 reverse-phase] column using a gradient of water and acetonitrile containing 0.1% formic acid. The obtained spectra were

analyzed to identify phytoconstituents based on their mass-to-charge ratio (m/z) and compared with known databases for compound identification.

The anatomical characters of the barks were taken by free hand sections with the help of blades. Sections were dehydrated with different alcohol grades and stained with safranin and light green. From each bark some sections were unstained while others were double stained. Both unstained and stained sections were permanently preserved. These permanent preparations were observed under microscope (Khandelwal, 2006) and photographed by microphotographic techniques.

The barks were also studied by maceration techniques. The pieces of barks were boiled in Jeffery's fluid (Chromic acid 10% and Nitric acid 10% in 1:1 proportion) the macerated cells were studied in detail (Johanson, 1940; Choudhary et al. 1992 and Khandelwal, 2006). Their figures were drawn with the help of camera lucida and inked by rotring pens. Their photographs were taken by microphotographic techniques. The dimensions of the cells were measured with help of microscope and by micrometry.

2.7) Qualitative and Quantitative Analysis: -

Physical evaluation: - Dry matter (DM), Bulk density

Chemical analysis

a. Qualitative: - Tannins, Saponins, Alkaloids, Phenolic acids and flavonoids.

b. Quantitative: - Nitrogen (N), Water soluble nitrogen (WSN), Crude proteins (CP), Crude fats (CFat), Crude fibres (CF), Total ash (TA), Acid insoluble ash (AIA), Acid soluble ash (ASA), Calcium (Ca), Phosphorus (P), Potassium (K), Total carbohydrates (TC), Cellulose, Hemicellulose, Lignins, reducing sugar, non-reducing sugar, Total sugar, Gross energy (GE) and Extractive values.

Results: -

Organoleptic Evaluation: - The organoleptic characters such as touch, colour, taste, and Odor are discussed in (Table No: - 1).

Macroscopic Evaluation: -

In Pharmacognosy the term "bark" is used to describe all the tissue found external to the cambium in the branch, stem or root. Barks consist following tissues: - Rhytidoma (dead tissues), cork, Phellogen (meristematic), Phelloderm, cortex and secondary phloem.

Shape and size: - Curved or channelled very hard, varies in length, 40-62 cm in width and thickness of fresh bark is 17-22 mm and thickness of dried bark is 9-14 mm.

Outer Surface: - . Outer bark is rough, light greenish to ash in colour, outer stem bark split into rectangle to irregular pieces, fissures deep, longitudinally as well as transverse.

Inner surface: - Inner surface of bark smooth, reddish in colour; longitudinally striated and fibrous; fracture difficult, fibrous, fracture irregular,

Fracture: - Hard, outer is granular, inner is splintery.

Taste: - astringent.

Odour: - Odourless.

Behaviour of Bark Powder towards some Chemical Reagents

The observations are reported in the table 2.

Physico-Chemical Evaluation

The physicochemical studies and successive extractive values of stem of *Ficus amplissima* are summarized in table 3 and 4.

Phytochemical Screening

Inorganic substances (Ca, Fe, Mn, P, S, and K) were present. The results demonstrated presence of saponin, flavonoids, tannins, alkaloids mainly in the stem bark of *Ficus amplissima*. The presences of various Phyto constituents in various extracts are summarized in Table 5..

Results and Discussion: -

Table: 1 organoleptic characteristic of stem Bark of *Ficus amplissima*.

parameters	
Condition	Dried
Colour	Outer bark is rough, light greenish to ash in colour, outer stem bark split into rectangle to irregular pieces, fissures deep, longitudinally as well as transverse. Inner surface of bark smooth, reddish in colour; longitudinally striated and fibrous; fracture difficult, fibrous, fracture irregular.
Odour	Odourless
Taste	astringent
Texture	Hard, outer is granular, inner is splintery.
Fracture	Fracture difficult, fibrous, fracture irregular.
Size	Length 40-62 cm Thickness 17-22 mm
Shape	Dried bark forms no quelling.

Anatomy of Bark: -

Cork consists of 6-7 layers square to rectangular in shape, 8-15×6-9µ. Below the cork cortex consists of 40-42 layers parenchyma cells are arranged vertical, barrel, elongated, square to rectangular in shape, 9-25×7-19µ. Outer cortex consists of 18-20 layers parenchyma cells are arrange vertical to cross line, square, rectangular, elongated to barrel in shape, 9-23×7-16µ. Inner cortex consists of 20-23 layers parenchyma cells are arranged vertical as well as horizontal, both are barrel, rectangular, square to elongate in shape, 7-18 ×6-12µ. Below the cortex medullary rays are arranged in several group oval to irregular in shape. Medullary rays are thick; double walled, hollow, tangle, rhombus to diamond in shape, 5-9×4-7µ. Medullary rays groups are arranged vertical as well as horizontal. Below each medullary rays group parenchyma cells are arranged horizontal, compactly arranged, elongated, barrel to rectangular in shape, 9-17×7-13µ.

Maceration of Bark:

Four types of fibres; one is very long, thick, single wall, pointed at both the ends, broad at middle irregular shape measuring from 440-470×6-12µ. Second is thin, single wall divided into several segments or cells which square to rectangular in shape measuring from 130-160×10-17µ. Third is long, thin, single wall, pointed at both the ends, broad at middle measuring from 120-150×7-13µ. Fourth is very long, thick, double wall, pointed at both the ends, broad at middle irregular in shape measuring from 350-380×5-15µ. Two types of parenchymatous cells; one is thick, single wall, square to rectangular in shape with having intercellular space measuring from 22-33×18-21µ. Second is thick, double wall, square to rectangular in shape with having intercellular space measuring from 27-45×18-23µ. One sclenchymatous cell is thick, single wall without any intercellular space, hexagonal in shape measuring from 25-30×8-23µ.

Table: 2 Reactions of stem bark powder of *Ficus amplissima* with different chemical reagents.

Sr. No.	**Chemical Reagents**	**Observation**
1	Conc. Sulphuric acid	Dark Black
2	Conc. Hydrochloric acid	Light Brown
3	Conc. Nitric acid	Reddish yellow
4	Picric acid	Chocolate color
5	Glacial Acetic acid	Light grey
6	Iodine solution	Light yellow
7	Sodium hydroxide solution (aq. 5%)	Cream color
8	Potassium hydroxide solution (aq. 5%)	Yellowish white
9	Ferric chloride solution (aq. 5%)	Dark brown
10	Powder as such	Light yellowish
11	Methanol	Light Brown
12	10% NaOH	Yellowish white

13	Chloroform	Light yellow
14	Petroleum ether	Dark brown
15	Distilled water	Light brown

Table: 3 Physico-Chemical Properties of *Ficus amplissima* stem bark.

Sr. No.	Quantitative Standards	%	Sr. No.	Quantitative Standards	%
1.	Dry matter	64.80	13.	Non-Reducing Sugar	0.89
2.	Bulk Density mg/cm3	264	14.	Total Sugar	5.74
3.	Ash	10.98	15	Crude Fibre	35.46
4.	Acid soluble ash	10.19	16.	Crude Fat	3.62
5.	Acid insoluble ash	0.79	17.	Cellulose	25.50
6.	Water soluble ash	8.75	18.	Hemicellulos	11.40
7.	Water insoluble ash	2.23	19.	Lignin	3.90
8.	Nitrogen	0.68	20.	Tannins	5.20
9.	Water Soluble Nitrogen	0.14	21.	Gross Energy K/cal	3.42
10.	Crude Protein	4.25	22.	Calcium	4.10
11.	Carbohydrates	45.50	23.	Phosphorus	0.268
12.	Reducing Sugar	4.85	24.	Potassium	0.245

Table- 4: Successive Extractive Values of the stem Bark of *Ficus amplissima.*

Sr. No.	Solvent	Weight of Drug	Average Extractive Value (%w/w)
1	Methanol	10gm	7.40
2	Alcohol	10gm	5.70
3	Benzene	10gm	2.89
4	Petroleum ether	10gm	2.71
5	Chloroform	10gm	0.65
6	Acetone	10gm	4.60
7	Water	10gm	8.30

Table- 5: Distribution of Phenolic acids and chemical compounds in bark of *Ficus amplissima.*

Sr. No.	chemical compounds	Results	Sr. No.	chemical compounds	Results
1.	Vanillic acid	+	12.	Saponins	+
2.	Syringic acid	+	13.	Iridoids	-
3.	Ferulic acid	+	14.	Quercetin	-
4.	Protocatechuic acid	+	15.	Kaempferol	-
5.	*p*-hydroxybenzoic acid	-	16.	Catechin	-
6.	*p*-coumaric acid	-	17.	Coumarin	-
7.	Phloretic acid	-	18.	6,7-Dimethoxy coumarin	-
8.	Melilotic acid	-	19.	5-Methoxy genistein	-
9.	Tannins	+	20.	Anthocyanin	-
10.	Phenols	+	21.	Proanthocyanin	-
11.	Alkaloids	+			

The **HRLCMS** analysis revealed a diverse range of bioactive compounds, categorized into various pharmacological groups:

1. **Carbohydrates & Glycosides** – Act as prebiotics, antioxidants, and antimicrobial agents.
2. **Alkaloids** – Show analgesic, neuroactive, antimicrobial, and anticancer properties.
3. **Aromatic & Phenolic Compounds** – Possess antioxidant, antimicrobial, and anti-inflammatory effects.
4. **Lactones & Furanones** – Exhibit antibacterial and antifungal activity.
5. **Triterpenoids & Diterpenes** – Known for immunomodulatory, anticancer, and anti-inflammatory benefits.
6. **Steroids & Brassinosteroids** – Involved in hormonal regulation, anti-inflammatory activity, and growth modulation.

7. **Fatty Acids & Lipids** – Play roles in skin protection, inflammation reduction, and lipid metabolism.
8. **Antibiotics & Antimicrobial Agents** – Include natural antibiotics with broad-spectrum antibacterial and antifungal properties.
9. **Pharmaceutical Drugs & Bioactive Agents** – Contain therapeutic compounds used in antihistamines, antiarrhythmics, hormone therapy, and parasitic treatments.
10. **Marine & Organometallic Compounds** – Feature cytotoxic and anticancer properties, with potential medicinal applications.

These findings highlight the **pharmacological diversity** of the studied bark drugs, supporting their potential use in drug discovery and traditional medicine.

Discussion

The Pharmacognostic and phytochemical evaluation of *Ficus amplissima* Sm. stem bark provides valuable insights into its medicinal potential. The macroscopic and microscopic characteristics revealed distinct anatomical features such as cork, cortex, and medullary rays, which can serve as diagnostic markers for identification and standardization. Physicochemical parameters, including ash values and extractive yields in various solvents, were within acceptable limits, indicating the purity and quality of the raw material.

The phytochemical screening confirmed the presence of bioactive compounds such as alkaloids, flavonoids, tannins, saponins, and phenolic acids, which are known for their therapeutic properties like antioxidant, anti-inflammatory, and antimicrobial activities. HR-LCMS analysis identified a wide range of secondary metabolites including carbohydrates, glycosides, triterpenoids, steroids, and aromatic compounds. These compounds possess diverse pharmacological activities, such as neuroprotective, immunomodulatory, and anticancer properties, supporting the ethnomedicinal use of *Ficus amplissima* in traditional systems of medicine.

The quantitative estimation of nutritional and mineral content such as crude protein, calcium, and phosphorus further highlights its potential as a nutraceutical source. The combined findings emphasize the pharmacological significance of the species and validate its traditional applications for treating ailments like diabetes, ulcers, and skin disorders.

Conclusion

This comprehensive study on *Ficus amplissima* stem bark highlights its rich phytochemical profile and Pharmacognostic parameters, establishing a scientific basis for its ethnomedicinal uses. The presence of multiple bioactive constituents underscores its potential for developing herbal formulations and novel drugs. Future research focusing on bioassay-guided isolation and characterization of individual compounds can pave the way for pharmaceutical applications. Thus, *Ficus amplissima* serves as a promising candidate in natural product research and modern herbal medicine.

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