

Morpho-Anatomical and Phyto-Pharmacognostical Characterization Coupled with HPTLC Fingerprinting of *Zanthoxylum armatum* DC. Bark: Novel Insights into Quality Evaluation of a Traditional Medicinal Plant

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ABSTRACT

The work provides a detailed Phyto-Pharmacognostical and analytical standardization profile on the bark of *Zanthoxylum armatum* DC. (Rutaceae), a key Ayurvedic medicinal plant for its multifold therapeutic efficacy. Phytochemical and morpho-anatomical studies were conducted by advanced microtomy and histochemical techniques, respectively. In contrast, physicochemical parameters such as ash values, extractive values, moisture content, and fluorescence analysis of these plants were done according to procedures provided by the WHO. Phytochemical screening indicated the presence of flavonoids, tannins, sterols, and carbohydrates in both types of extracts and quantitative estimation showed that flower alcoholic and aqueous extract contained higher amounts of total phenolic (216 ± 5.1 mg/g) and flavonoid contents (35 ± 2.8 mg/g) than stem alcoholic/water extract (210 ± 2.2 mg/g; 59 ± 3.0 mg/g). Microscopical elements such as sclereids, pericyclic fibers, oil cells, starch grains, and radially extended medullary rays could be observed for their diagnosis. High-performance thin-layer chromatographic (HPTLC) fingerprinting showed well-defined peaks of quercetin (Rf 0.47–0.52), which was developed as a standard chemical marker for identification purposes. The combined botanical, physicochemical, and chromatographic details offer reproducible diagnostic parameters for standardization and quality control of *Z. armatum* bark. These results support its Ayurvedic uses and will be helpful in the standardization of pharmacopoeial monographs of the plant, translating into purity, quality, and efficacy of herbal drugs used to prepare different dosage forms.

Keywords: *Zanthoxylum armatum* DC, Phyto-Pharmacognosy, HPTLC fingerprinting, Quality standardization, Ayurvedic medicine, Quercetin marker.

INTRODUCTION

Herbal drugs play a crucial role in the healthcare sector in most developing countries. The use of medicinal and aromatic plants has a long history throughout the world, including herbal extracts, which can be found in the pharmacopeias of numerous countries.⁽¹⁾ Thus, it led to an increase in resources and awareness for maintaining the quality and purity of the raw materials. Hence, a search for a valid

alternative for an authentic drug is going on to avoid over-exploitation of herbal resources from their natural habitat, which may increase the chances of adulteration and substitution in herbal material.⁽²⁾ The precise botanical identification can be achieved by step wise Phyto-Pharmacognostical studies which include morpho-anatomical, Physicochemical, preliminary phytochemical and Thin layer chromatography profiling of chemical constituents of respective raw material and hence characterization by

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qualitatively and quantitatively for medicinal plants that do not appear in official codes, is one of the important paradigm to ensure the authenticity, quality, safety and efficacy of herbal drug to be use as medicine.⁽³⁾

Zanthoxylum armatum DC, commonly known as Tumbru or Toothache tree, belongs to the family Rutaceae is an armed shrub or a small tree, up to 6 m in height, with dense glabrous foliage and straight prickles on stems, blaze yellowish brown rapidly darkening on exposure, which widely distributed in the Himalayas up to 2100 m and in Andhra Pradesh up to 1350 m. ⁽⁴⁾ The ethyl acetate fraction of shown significant anti-inflammatory activity in mice. ⁽⁵⁾ The anti-inflammatory activity exhibited may be due to the presence of some lignan-like compounds in the plant material. ⁽⁶⁾ The DPPH activity exhibited by the essential oil of the fruit of the plant is remarkable in scavenging the free radicals due to the presence of various forms of triterpenoids such as Cineole, linalool, and cubene. ⁽⁷⁾ The bark and fruits are acrid, bitter, aromatic, antiseptic, and anthelmintic. They are useful in vitiated conditions of *Kapha* and *Vata*, tumors, hepatopathy, cardiac debility, and for the treatment of various skin diseases. ⁽⁴⁾ Despite numerous medicinal properties attributed to this plant, detailed phyto-pharmacognostical reports are not yet documented. Therefore, the present study was undertaken to evaluate and establish quality control paradigms as per WHO guidelines for the correct authentication and identification of the plant material. WHO accepts HPTLC fingerprinting as a quality evaluation technique. Thus, HPTLC fingerprint has also been developed for *Zanthoxylum armatum* DC bark.

1.1 An account of *Zanthoxylum armatum* DC. Plant:



Figure 1.1: *Zanthoxylum armatum* plant

1.1.1 Botanical evaluation

a) Taxonomical Classification:

- Kingdom: Plantae
- Phylum: Tracheophyta
- Class- Magnoliopsida
- Order: Rutales
- Family: Rutaceae
- Genus: *Zanthoxylum*
- Species: *armatum*

b) Synonym:

- Sanskrit: Tumburuh
- Eng: Toothache tree
- Kannada: Tumburudu, Dhiva
- Tamil: Tumpunalu

c) Biological Source: It consists of the bark of *Zanthoxylum armatum* DC. Belonging to family: Rutaceae.

d) Geographical distribution: In the Himalayas up to 2100 m and in Andhra Pradesh up to 1350 m.

e) Botanical Description: It consists of lanceolate leaves, imparipinnate with leaflets. The outer surface of the bark is light brown in colour. Whereas the inner portion of the bark is light in colour. The taste of bark is bitter and astringent in taste.

f) Plant Parts used: fruits, leaves, and bark

g) Properties: It is used as an antipyretic, hepatoprotective, and tonic in the traditional system of medicine. It is also known to treat hepatopathy, leucoderma, cardiac debility, and general debility.⁽⁸⁾

h) Care and Maintenance:

- **Climate:** Prefers subtropical to temperate conditions; grows well at 800–2,100 m altitude.

- **Soil:** Thrives in well-drained sandy or clay loam soil with a pH 6.0–7.5 and good organic matter.
- **Light and Water:** Requires moderate sunlight and regular watering during early stages; mature plants tolerate mild drought.
- **Propagation:** Commonly propagated through seeds, stem cuttings, or root suckers; seed germination improves after pre-soaking in warm water.
- **Nutrition:** Application of compost or farmyard manure (5–10 kg/plant annually) enhances growth.
- **Pruning:** Annual pruning maintains plant shape and removes diseased or dead branches.
- **Pest and Disease Management:** Generally, pest-resistant; occasional aphid or fungal attacks can be controlled using neem-based formulations.
- **Harvesting:** Bark and fruits are harvested from 4–6-year-old plants; partial bark collection prevents girdling and allows regeneration.
- **Post-Harvest Care:** Shade-dry collected material and store in airtight containers away from moisture and sunlight.
- **Sustainability:** Encourage cultivation over wild collection to ensure conservation and long-term availability.⁽⁹⁾

2. MATERIAL & METHODS:

2.1. Plant material and extraction

The fresh and healthy plant material was collected in the months of August to September from the vicinity of Dhule district, Maharashtra, and authenticated by Sr. taxonomist from the Botanical Survey of India, Pune. The voucher specimen number is BSI/WC/Tech/2025/354-RRWZA-1. The herbarium was submitted to the Department of Pharmacognosy, SVKM NMIMS Global University, School of Pharmacy and Technology Management, Dhule. After authentication, the plant material was studied for its botanical characters such as vernacular

names, taxonomical characters, distribution, occurrence, properties, and parts used as per Ayurvedic literature. Thereafter, the bark of *Zanthoxylum armatum* was dried under the shade until they were free from moisture and subjected to morpho-anatomical and physicochemical studies.

Subsequently, *Zanthoxylum armatum* bark powder was subjected to successive hot extraction with organic solvents of increasing polarity, such as Petroleum ether, Chloroform, and Alcohol by using a Soxhlet apparatus. Each time before extraction with the next solvents, the powder material was dried in a hot air oven below 50 °C. Chloroform water IP 1996 was used for the preparation of the aqueous extract. The extracts were concentrated under reduced pressure (17 mm Hg, 45° °C, 10 -20 min) depending on the solvents. The extracts were stored in a cool place in the dark until use.

2.2. Chemicals and instruments:

Staining reagents such as phloroglucinol, hydrochloric acid, 5 % Iodine, Sudan red, and all other chemicals used in the study were of analytical grade. Instruments: microscope (Make: Zeiss Company with Axis Vision Ac Rel 4.5 Software), Microtome (Make: Thermo Electron Corporation and Model Shandon Finesse), and CAMAG HPTLC system scanner 3, Reprostar 3, and WIN CATS-4 software.

2.3. Morpho-anatomical evaluation

The bark of *Zanthoxylum armatum* was subjected to morphological studies composed including organoleptic characteristics, viz, color, odor, taste, shape, and texture were examined as per standard WHO guidelines and evaluated by Khandelwal.^(10,11)

2.4. Microscopical evaluation

For histoarchitectural studies, the dried bark was removed from a healthy plant and washed with water. After proper washing, the killing and fixing of the specimen was carried out using a solution of (90 ml 70 % ethanol + 5 ml of glacial acetic acid + 5 ml of formaldehyde) for one week. Further, dehydration of the tissues was done with the help of different grades of tertiary butyl alcohol. Thereafter, the process of infiltration was followed by filling the cells with increasing order of paraffin. Furthermore, thin



transverse sections was taken using microtome (Make: Thermo Electron Corporation and Model Shandon Finesse) and histochemical tests were carried out using staining reagents such as phloroglucinol + hydrochloric acid (1:1) for (lignified cells), 5 % Iodine for (starch grains), Sudan red for (stone cells), Ruthenium red for (Oil cells) Photomicrographs of the microscopical sections were captured with the help of binocular microscope (Make- Zeiss Company with Axis Vision Ac Rel 4.5 Software).⁽¹²⁾

2.5. Physicochemical investigation

The shade-dried bark was subjected to size reduction to get fine powder (# 40 size mesh) and then evaluated for various qualitative and quantitative physicochemical paradigms such as total ash value, inorganic substance identification, extractive value, and moisture content as per the literature. For powder drug fluorescence analysis, the drug sample was treated with a specific reagent that exhibits specific fluorescence when observed under ultraviolet light.^(11,13)

2.6. Qualitative and Quantitative phytochemical investigation

The detection of various primary and secondary metabolites in the aqueous and alcoholic bark extracts subjected for preliminary phytochemical test using specific test reagent and chemicals such as: Alkaloids: Wagner, Mayer's, Hager's and Dragendorffs reagents, Flavonoids: Shinoda, and Lead acetate test, Tannins: Ferric chloride and Bromine water test, Carbohydrate: Molisch reagent, Barford test, Fehling test etc.⁽¹⁴⁾ On the other hand, the alcoholic and aqueous extracts were subjected to estimation of total phenolic and total flavonoid content using Folin-Ciocalteu and Aluminium chloride reagents, respectively.⁽¹⁵⁾

2.7. High Performance Thin Layer Chromatographic (HPTLC) fingerprinting

HPTLC study was carried out on aqueous and alcoholic extracts by the method of Harborne and Wagner et al.⁽¹⁶⁾

2.8. Development of solvent system

Several solvents were tried individually as well in combination for the separation and identification of quercetin from the respective extracts, but satisfactory resolution was obtained in the solvent system Ethyl acetate: Formic acid: glacial acetic acid: Water (99:12:11:27 v/v/v).⁽¹⁷⁾

2.9. Sample application and development of chromatogram

The *Zanthoxylum armatum* bark extracts were dissolved in respective HPTLC grade ethanol and water, which were used for sample application on precoated silica gel GF 254 aluminium sheets (Made-Merck). The samples (5 µL) were spotted in the form of bands of width 6 mm with a 100 µL sample using a Hamilton syringe on silica gel, which was precoated on aluminium plate GF-254 plates (20 cm X 10 cm) with the help of Lineman 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software. Prepared plates were developed in a previously saturated twin trough chamber (20 cm X 10 cm) in the linear ascending direction.

2.10. Detection of spots

The developed plates were dried by hot air to evaporate solvents from the plates. The developed plates were sprayed with 5 % Ferric chloride as spray reagent and dried at 100 °C in a hot air oven for 3 min. The plates were kept in a photo-documentation chamber (CAMAG REPROSTAR 3), and the images were taken under UV light at 254 nm. The R_f values and fingerprint data were recorded by the WIN CATS software.⁽¹⁸⁾

3. RESULTS

3.1 Morphological evaluation

The bark occurs in channelled pieces, which are about 0.5 mm thick. The external surface is gray due to the presence of cork. The inner surface is somewhat lighter and longitudinally striated. Fracture is short and splintery. The drug has a characteristic odour.



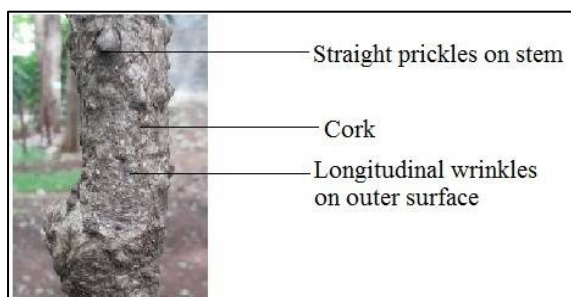


Figure 3.1: Morpho-Anatomy of *Zanthoxylum armatum* DC Bark

3.2. Microscopical and Histochemical evaluation

Transverse section of the bark shows many layers of cork, often lignified in nature, followed by a layer of pericycle, consisting of 3-4 layers of sclerenchymatous cells or sclereides with a small group of Pericyclic fibers occurring at intervals. Parenchymatous cells contain numerous starch grains. Oil cells are scattered in phloem parenchyma; cells are axially elongated and 2-3 times the diameter of phloem fibers. Medullary rays are composed of radially elongated cells, 7-14 cells high, uni-or bi-seriate near the cambium and widening slightly as they approach the pericycle.

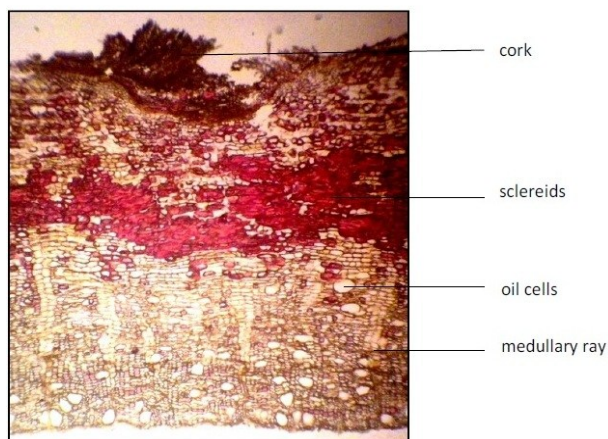


Figure 3.2a: Microscopy of *Zanthoxylum armatum* DC Bark

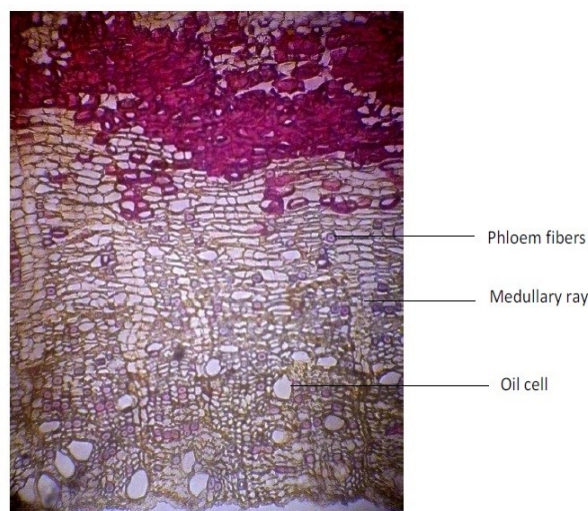


Figure 3.2b: Microscopy of *Zanthoxylum armatum* DC inner bark

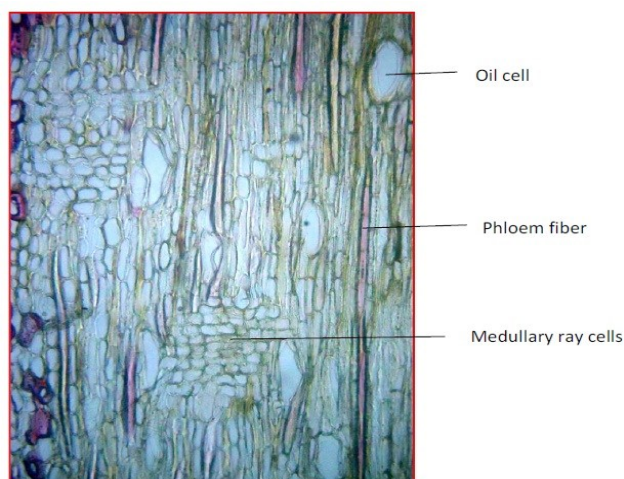


Figure 3.2c: Microscopy of longitudinal section of *Zanthoxylum armatum* DC bark

3.3. Powdered drug analysis

The powder was dark brown in colour, with a characteristic odour, slightly sweet and bitter, and astringent in taste. After shaking the powder with water in a test tube, no persistent foam was formed, indicating the absence of saponins. Powdered drug under ultraviolet and ordinary light, when treated with different reagents, emitted various colour radiations which help in identifying the drug in powder form. The behaviour of powder with different chemical reagents is summarized in Table 1. The microscopic powder analysis showed the presence of cork fibers, stone cells, and abundant sclereides, parenchymatous cells attached to medullary rays, and minute starch grains were scattered in the powder.

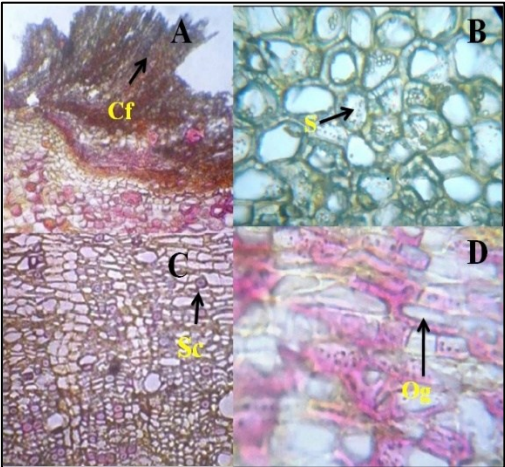


Figure 3.3: Powder Microscopic characters of *Zanthoxylum armatum* DC bark

Table 1: Fluorescence analysis of *Zanthoxylum armatum* bark powder

Sr.no	Treatment	Observation under		
		Ordinary light	Ultraviolet light	
			254 nm	366 nm
1	Powder as such	Light brown	Brown	Dark brown
2	Powder + 1 N NaOH in methanol	Dark brown	Light brown	Dark brown
3	Powder + 1 N NaOH in Methanol + nitrocellulose in amyl acetate	Brownish black	Black	Black
4	Powder+1N HCl Brown	Lightly dark brown	Black	Brown
5	Powder+1N HCl+ nitrocellulose in amyl acetate	Brown	Dark brown	Dark brown
6	Powder + 1 N NaOH in water	Brownish black	Dark brown black	Dark brown
7	Powder + HNO ₃ (1:1)	Brownish black	Dark brown black	Dark brown
8	Powder + H ₂ SO ₄ (1:1)	Brownish black	Dark brown black	Dark brown black
9	Powder + nitrocellulose	Light brown	Brown	Brown
10	Powder + Powder + 1 N NaOH in water, dried and mounted in amyl acetate	Brownish black	Dark brown	Brownish

3.4. Physicochemical evaluation

The ash content of the drug also showed the presence of calcium, magnesium, and sulphate, while the

absence of sodium, potassium, and phosphate types of inorganic compounds. The physicochemical parameters, such as ash value, extractive value, and

moisture content, were important to determine the purity of the drug, as shown in Table 2.

Table 2: Physicochemical study of *Zanthoxylum armatum* bark powder

Parameters		% w/w (Mean ^a ± SEM)
Ash Values	Total Ash value	5.52 ± 0.27
	Acid-insoluble ash	0.54 ± 0.02
	Water-soluble ash	1.02 ± 0.04
Extractive values	Water-soluble	19.33 ± 0.88
	Alcohol soluble	18.2 ± 0.44
Moisture content (Loss on drying)		10.2 ± 0.10
A mean value of three readings		

3.5. Qualitative and quantitative phytochemical investigation

Successive Soxhlet extractive values colour and consistency of bark extracts of *Zanthoxylum armatum* was found to be: Pet ether (1.9 w/w, light brown, semi solid sticky mass); Chloroform (3.6% w/w, light brown, semi solid sticky mass); alcohol (5.5% w/w, light brown, semi solid sticky mass) and aqueous extract (7.6% w/w, brownish-black, dry powder), respectively.

Preliminary phytochemical analysis of the alcoholic extract revealed the presence of alkaloids, steroids, tannins, and flavonoids, whereas aqueous extract showed the presence of polyphenolic compounds and glycosides. The quantitative estimation of the total phenolic and flavonoid content in alcoholic and aqueous bark extract was found by 216 ± 5.1 , 35 ± 2.8 , 210 ± 2.2 , and 59 ± 3.0 , respectively, mg/g of extract.

3.6. HPTLC analysis

The HPTLC chromatogram at 214 nm showed the presence of quercetin and exhibited a blackish

(visible) band in the R_f range of 0.47 to 0.52. According to the literature on flavonoids, the R_f range is found to be 0.4 to 0.6. Therefore, the Chromatogram fingerprint suggests the presence of quercetin in aqueous and alcoholic extracts of *Zanthoxylum armatum*, has been shown in figures 3.6a-3.6d.

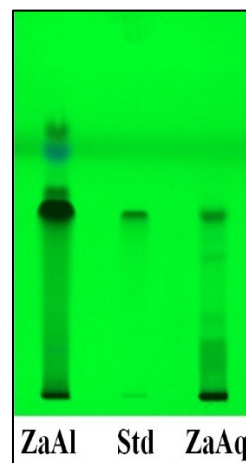


Figure 3.6a: HPTLC profile of *Zanthoxylum armatum* bark extracts

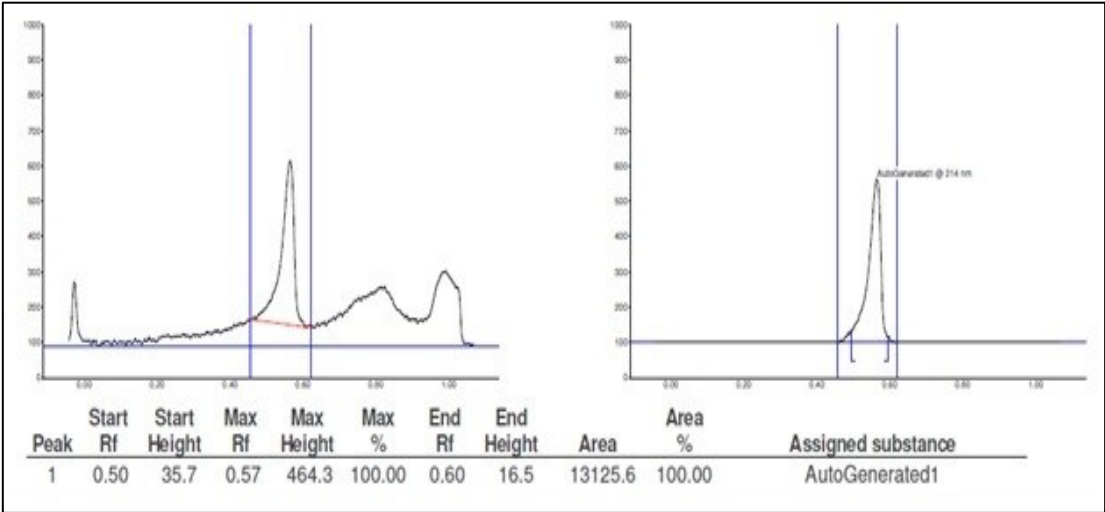


Figure 3.6b: HPTLC chromatogram of standard Quercetin

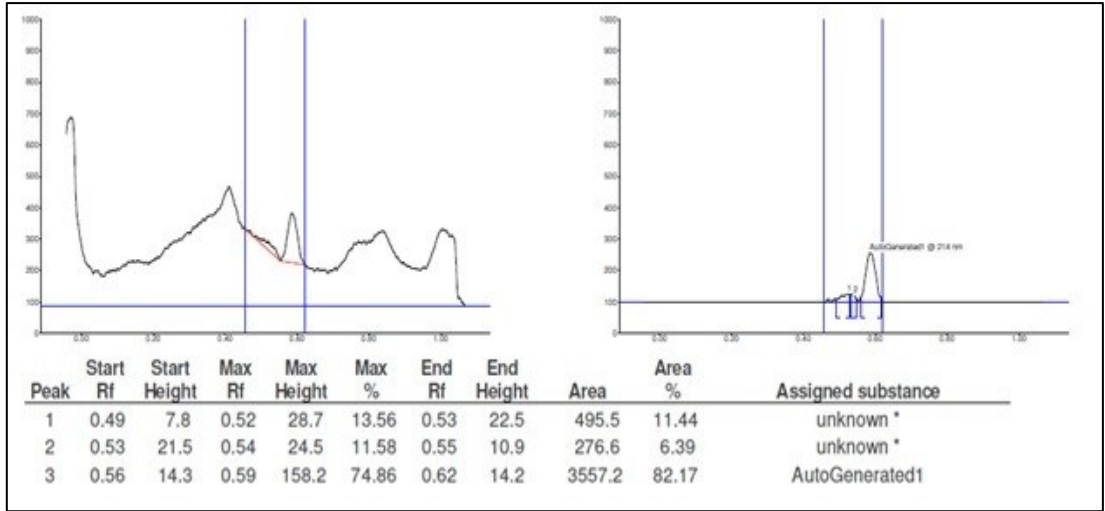


Figure 3.6c: HPTLC chromatogram of alcoholic extract of *Zanthoxylum armatum* bark showed the presence of Quercetin

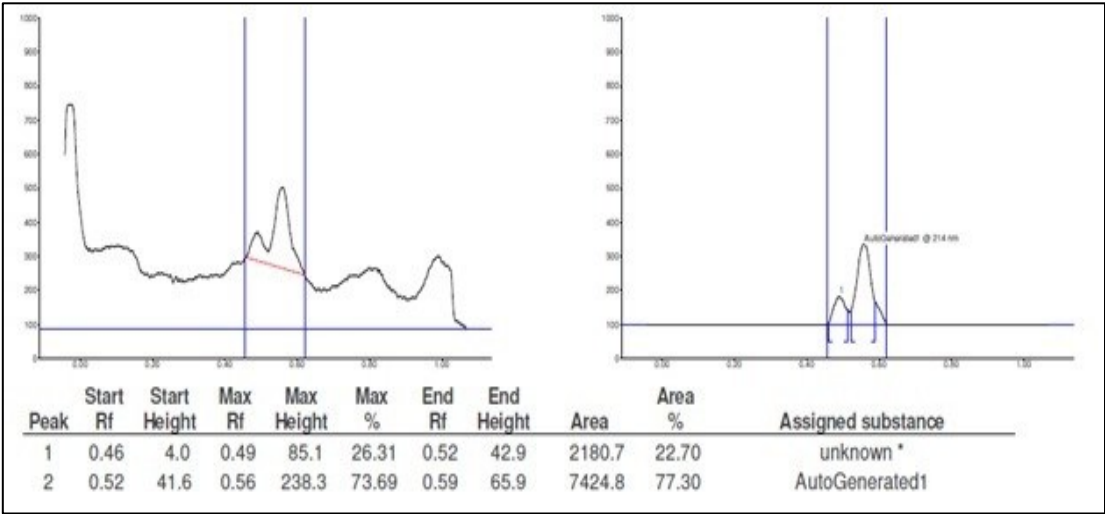


Figure 3.6d: HPTLC chromatogram of aqueous extract of *Zanthoxylum armatum* bark showed the presence of Quercetin

4. DISCUSSION

Due to the paucity of authentic data on genuine herbal drug identification, it becomes challenging for the herbal formulation industry to use the correct source of raw material for formulation. However, the modernization and sophistication of analytical techniques in biochemical analysis have greatly altered drug discovery and development in the field of botanical and phyto-pharmacognostical research.⁽¹⁹⁾ Hence, the present study was undertaken to establish systematic and scientific botanical morpho-anatomical, physicochemical, and phyto-pharmacognostical standards of *Zanthoxylum armatum* bark for its correct authentication and identification. Some of the important diagnostic features of bark are its special characters, such as oil cells, starch grains, sclereides, radially elongated medullary rays, and stone cells. Pharmacognostical parameters, including HPTLC, are helpful for the future identification and authentication of this plant in the herbal industry.⁽²⁰⁾ Physiological ash is composed of various inorganic and non-volatile components. Therefore, ash value estimation is the physicochemical marker for quality evaluation of drug material. On the other hand, the extraction of any crude drug with a specific solvent yields an extract containing different phytochemicals. Hence, determination of extractive values facilitates evaluation of crude drugs as drug constituents get soluble in a specific quantity in the prescribed quantity of solvents.⁽²¹⁾ Moreover, the estimation of the moisture content of the drug is an essential requirement in evaluation, as it helps in the identification of the quality of the drug and also signifies the storage conditions and deterioration with bacteria, fungi, or yeast growth.⁽²²⁾ Plants are considered to be a biochemical laboratory that biosynthesizes enormous primary and secondary metabolites of therapeutic importance via enzyme-mediated chemical reactions. As the richest source for the biosynthesis of major secondary metabolites, such as polyphenolic compounds that exhibit significant physiological effects.⁽²³⁾ The presence of important plant secondary metabolites such as tannin, phenolic substances, steroids, and glycosides in *Zanthoxylum armatum* could make the plant useful for treating different ailments of living organisms because the therapeutic efficiency of any plant is usually traced by

its chemical compounds. Thus, a preliminary screening test may be useful in the detection of bioactive principles. HPTLC results indicate the presence of constituents and further facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds.⁽²⁴⁾ The bark has shown the presence of a remarkable number of polyphenols in alcoholic and aqueous extracts. An important observation from a Phytochemistry point of view is the presence of quercetin in the bark, quantified by using HPTLC.

Herbs and herbal formulation drugs are widely used worldwide as over-the-counter drugs, home remedies, and in the form of raw materials for the phytopharmaceuticals industry and represent a significant proportion of the global drug market. Hence, it is necessary to ensure the reproducible quality of herbal products. Since the plant *Zanthoxylum armatum* is a popular drug used in traditional medicine for the treatment of various ailments, it is necessary to provide scientific information with respect to its quality, purity, and identity. Thus, the phyto-Pharmacognostical finding of this plant and the diagnostic microscopic features reported in this work could be useful for the assessment of its authenticity and also for the compilation of a suitable monograph and proper identification, as well as distinguishing between closely related species.

5. CONCLUSION:

The current research has successfully accomplished standardization of botany, physico-chemistry, and phyto-pharmacognosy for the bark of *Zanthoxylum armatum* DC, successfully. The identification of microscopic characters, quantitative phytochemical standards, and HPTLC fingerprint shows the dependable qualitative and quantitative standards for its quality control. The results confirm its folk use and justify the preparation of pharmacopoeial standards for the purity, safety, and therapeutic potential of the herbal drug derived from the plant.

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7. AUTHOR CONTRIBUTION

Mr. Kalpesh Yeole: Conducted pharmacognostic studies, physicochemical analyses, HPTLC profiling, and manuscript formatting.

Dr. Raju Wadekar: Supervised the study design and phytochemical analysis.

Dr. Kalpana Patil: Contributed to result interpretation and manuscript drafting.

8. CONFLICT OF INTEREST:

The authors declare no conflicts of interest.

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