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# HPTLC FINGERPRINTING AND PHYTOCHEMICAL ANALYSIS OF *TEJOVATI ZANTHOXYLUM ARMATUM* DC.

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## ABSTRACT

**Introduction:** Oral health is of prime importance to all individuals and oral hygiene habits are instilled in childhood itself irrespective of the nationality or geographic location of an individual. Oral diseases are perhaps, the most widespread of all diseases prevalent in the world. No population is free from caries and periodontal diseases – the most common of all such diseases and affecting almost 80% of the population. Poor oral hygiene or tooth disorders are very common and severely agonizing ailment leads to teeth loss or damaging of teeth, so it is the need of the hour to search for a potent drug which can fight this condition. *Tejovati* is first described in ayurvedic text *Charak Samhita as in Shirovirechan dravyas.* Later in some medicinal books *Tejovati* is mentioned as potent mediciene for *Dantvikar, agnimandaya, udarshoola etc. Zanthoxylum armatum* DC. of Rutaceae family is an alpine medicinal plant that is found in temperate Himalaya between 2000-3000 m The fruit of the plant is of good medicinal value and has been used as folk remedy for treating ailments like diabetes mellitus, gastric problems, peptic ulcers, diarrhoea, cancer, toothache, pyorrhoea, cough, cold and also has anthelmintic property. **Methods:** An attempt has been made to study the macroscopic and microscopic character, physicochemical analysis, phtochemical analysis, and chromatographic studies of its root following standard procedures. Two different extracts of *Tejovati* fruit were used to access the physicochemical and HPTLC analysis. Research work was carried out as per standard operating procedures and specified protocols. **Result:** Results showed that in physicochemical evaluation aqueous extract 2.9%, hydro-ethanol extract 3.6%, total ash 8.5%, water soluble ash 3.5%, acid and insoluble ash 2.0%. HPTLC results

for the quantification of linalool in EPF was analyzed for the first time by scanning at wavelength (λmax 366 nm) and the quantity of linalool present in *Tejovati* was estimated to be 1.48% w/w. In phytochemical analysis presence of carbohydrate, protein, glycosides, amino acid, protein in extract of *Tejovati*. **Conclusion:** *Tejovati* is a highly potent medicine for oral diseases due to presence of monoterpenes like linalool and limonene.

**KEYWORDS:** *Tejovati*, *Dantvikar*, pharmacognostical, TLC, HPTLC.

## INTRODUCTION

Oral health is of prime importance to all individuals and oral hygiene habits are instilled in childhood itself irrespective of the nationality or geographic location of an individual. Oral diseases are perhaps, the most widespread of all diseases prevalent in the world. No population is free from caries and periodontal diseases – the most common of all such diseases and affecting almost 80% of the population. Poor oral hygiene or tooth disorders are very common and severely agonizing ailment leads to teeth loss or damaging of teeth, so it is the need of the hour to search for a potent drug which can fight this condition. Oral diseases are one of the most important problems in public health and burden in many developing countries around the globe. Among 10 people out of them 9 may experience pain and problems with eating, chewing, smiling, disolouration, damaged teeth on their daily basis. According to worldwide data prevalence of dental caries is about to be 99% and among them prevalence of periodontal disease reported more than 90% of worldwide population. Most of oral issues are caused by microbes mainly bacteria, fungi etc. and medicinal plants such as *Tejovati,* posses good anti-microbial properties due to the presence of potential bioactive compound ,which help to reduce microbial load and prevent formation of plaque, dental caries, gingivitis etc. *Tejovati* (*Zanthoxylum armatum* DC.), known as Toothache tree, as the name justify it help to minimize tooth pain and according to *Acharya Bhavprakash*[1] drug is used in various disorders of *Netra*, *Karna*, *Oushtha*, *Shira*, and posses *Krimighan* property.

*Tejovati* is an armed shrub or a small tree, 3-8 m high. Prickles on trunk flattened, +2cm long and often with a corky base. Bark pale brown furrowed. Leaves 3-7, foliolate, unequally pinnate; leaflets 2-10 cm long, lanceolate or elliptic-lanceolate, glabrous beneath, petioles and rachis usually winged, petioles with 2 stipular prickles at the base. Flowers small, yellow in lax, panicles. Fruit dehiscent carpels, 4-5 mm across, sub-globose, reniform or ovoid, palered, tubercled. Seed solitary, black, globose, shining.

## 2. MATERIAL AND METHODS

### 2.1. Plant collection and Authenticatiom

The plant fresh fruit was collected during the month of September from Aarokot village, Tehri district of Uttarakhand state. The botanical authentication of the specimen was done by by Dr. Gajendra Singh, Scientist/Engineer, Forestry and Climate Change Division, Uttarakhand Space Application Centre, Nalapani, Dehradun.

### 2.2. Pharmacognostical studies

#### 2.2.1. Morphological, histological evaluation

All the studies on morphological, and histological were performed based on the standard method. For morphological study, the fresh roots were evaluated for the texture, size, shape, colour, odour and taste. The sections were dehydrated with varying strength of absolute alcohol and then stained with safranin and fast green solution. Finally, the stained sections were mounted with glycerine for histological observation.

#### 2.2.2. Physicochemical evaluation[2]

The determination of various physicochemical constants such as foreign matter, total ash values, water soluble ash value, acid soluble ash value, different extractive values, was done as per the standard methods.

### 2.3. Phytochemical evaluation

#### 2.3.1. Preliminary phytochemical screening[3]

The preliminary phytochemical analysis of methanolic extract of *Tejovati* fruit extract was evaluated to detect the presence of various classes of phytochemicals such as alkaloids, glycosides, flavonoids, steroids, tannins, saponins, protein, amino acids and carbohydrates.

#### 2.3.2. Thin layer chromatography (TLC) and High-performance thin layer chromatography (HPTLC)

Preparation of extracts, thin layer chromatography and development of chromatogram was done as per the standard methods. Normal TLC silica gel G was used as the stationary phase and mobile phase toluene and ethyl acetate[4] for TLC and solvent system for developing of chromatogram were composed of solvent mixtures of varying chemical polarity. Spraying reagent used for detecting the phytochemicals are Fast blue salt reagent followed by 10% KOH (for polyphenolics classes), Liebermann Burchard reagent (saponins and steroidal components), and ninhydrin (for amino acids). The plates were also visualized under Ultra

violet light (366 nm) for the detection of different classes of UV active components containing active chromophore groups.

The quantification of Catechin in coarse powder of *Tejovati* sample was carried out by using HPTLC. Mobile phases of Toluene: ethyl acetate: acetone: formic acid (6: 6: 6: 1 v/v/v/v) were used for the identification of linalool. Preparation of standard solution, 10 mg of linalool was dissolved in 10 ml methanol, sonicated for 5 minutes and diluted from 0.1 ml to 1 ml. For identification 20µl and for quantification different amounts (1.2, 1.4, 1.8, 2, and 3µl) were applied on TLC plate. The 500 mg of sample was dissolved in 10 ml methanol, sonicated for 20 minutes and centrifuged to obtain clear solution. For identification 20µl and for quantification 10µl was applied on TLC plate. Quantification was done by application of the spots on the HPTLC plate which were coated with silica gel (plate size of 6.0 x 10.0cm). The plates were developed at 60F 254nm and 366nm under UV visible wavelength. The details of the instrument used in studies are CAMAG LINOMAT 5 with 5 application parameters, inert gas (nitrogen) as spray gas and methanol as sample solvent. The syringe size was 25µl with 3 tracks. The calibration parameter used was calibration mode multilevel, with CV statistics mode and the evaluation mode was based on peak areas.

The study was carried out at Drug Discovery and Drug Development, Patanjali Research Institute, Haridwar. Chemicals used; Standard biomarkers, Linalool is used from Sigma Aldrich/ WXBC6250V/ 98.8, Private Limited, Mumbai and all the chemicals used throughout the research were procured from institute.

## 3. RESULTS

### 3.1. Pharmacognostical studies

**Table no. 1: Organoleptic[5] characters of *Tejovati* fruit.**

| S. No. | Character | *Tejovati fruit* |
|---|---|---|
| 1. | Shape | Sub-globose to ovoid |
| 2. | Size | 4 to 5 mm in diameter |
| 3. | Odour | Aromatic |
| 4. | Colour | Black |
| 5. | Taste | Pungent |
| 6. | Surface | Rough |

### 3.1.1. Morphological, microscopical drug evaluation

**Macroscopic characters of different parts of *Tejovati (Zanthoxylum armatum* DC.)**

a) Leaf: imparipinnate or trifoliolate, 5-23 cm. long, often with flattened prickles: leaflets upto 5 pairs, opposite, ovate to lanceolate, entire to glandular-crenate, acute to obtusely acuminate.

b) Bark: Bark corky, channelled and single quilled with large marks of tubercular prickles;0.1-0.2 cm thick, external surface pale brown, rough with numerous scattered patches of lenticels, rather deeply furrowed; internal surface smooth, light yellow to pale brown; fracture, short; odour, aromatic; taste, aromatic pungent.

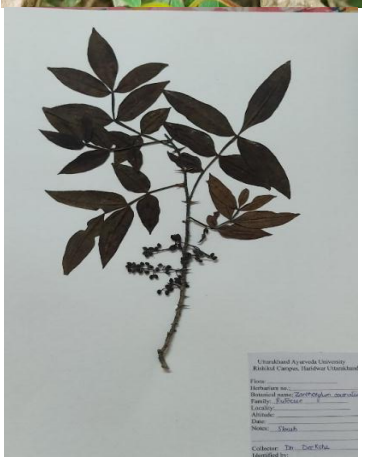
c) Flower: green or yellow, in dense terminal, and occasionally axillary sparse panicles

d) Fruit - Sub-globose to ovoid, trilocular, valvate capsule, 4 to 5 mm in diameter, frequently dehiscing half-way into two carpels, each with 2 loculi, exposing a solitary seed; surface rough, covered with compactly packed prominent spherical oily tubercles, apex is pointed, base rounded attached with short 1 to 2 mm long pedicel, outer surface dark brown, inner pale brown, exhibiting papery.

e) Seed: seeds solitary in a fruit, globose, shining black





## MICROSCOPIC STUDY

### 3.1.1 Microscopic characters of *Tejovati* fruit in Transverse section

- TS of fruit is circular in outline with undulated margin and centrally located wide seed, encircled by sclerenchymatous endocarp and wider mesocarp embedded with a row of oil glands.
- Pericarp shows a layer of epicarp with squarish to radially elongated thick-walled cells embedded with stomata, covered with cuticle 1 to 2 rows of thick-walled yellowish brown pigment cells of hypodermis
- Endocarp is sclerenchymatous, many layered, consisting of an outermost continuously running compactly arranged radially elongated thick-walled narrow lumened palisade like cells, followed by discontinuous rows of similar cells penetrated at places with groups of underlined tangentially running wide lumened sclereids, at places they being embedded with parenchymatous cells containing prismatic crystals of calcium oxalate, followed by a narrow band of hyaline layer.
- Seed - TS of seed is circular to oval in outline shows outermost sclerenchymatous testa, encircling the white endosperm embedded with two narrow horizontally placed cotyledons. LS of the seed is bean shaped, shows centrally located thin papery heart shaped cotyledon of the embryo and the radicle embedded in endosperm which is encircled by blackish testa band. The Seed coat is highly gritty, brittle and breaks into minute pieces and hence its cellular structure was hot possible to observe in TS.

### 3.2 Physicochemical and phytochemical study

Physicochemical parameters such as water-soluble extractives, alcohol-soluble extractive, n-hexane soluble extractive, chloroform-soluble extractive, ether soluble extractive, total ash value and moisture content of the samples were calculated and depicted (Table 2).

| S. No. | Test | Tejovati fruit |
|---|---|---|
| 1. | Foreign Matter | 0.1 |
| 2 | Aqueous soluble extract (%) | 2.9 |
| 3. | Hydro-ethanol soluble extract (%) | 3.6 |
| 4. | Total ash (%) | 8.50% |
| 5. | Water soluble ash (%) | 3.50% |
| 6. | Acid insoluble ash (%) | 2.00% |

### 3.3 Phytochemical study

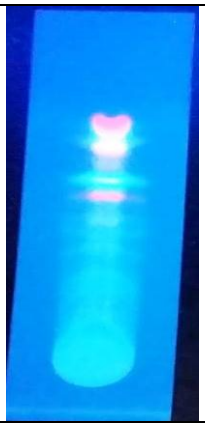
Preliminary phytochemical analysis of alcoholic extract of *Tejovati* was done. Phytochemical parameters such as test for carbohydrates, tanins, flavonoids, alkaloids, carbohydrate etc was calculated and depicted (Table 3).

| S.NO | PHYTOCHEMICAL TESTING | NAME OF TEST | ATV | HATV |
|---|---|---|---|---|
| 1. | Test for Carbohydrate | Molisch's test | - | + |
| 2. | Test for Alkaloids | Mayer's reagent test | + | - |
| 3. | Test for Amino acids | Ninhydrin test | + | + |
| 4. | Test for proteins | Millon's test | + | + |
| 5. | Test for Saponin | Foam test | - | - |
| 6. | Test for Glycosides | Borntragor's Test | - | + |
| 7. | Test for Tannin | Ferric chloride solution | + | + |

### 3.4 Chromatography Study

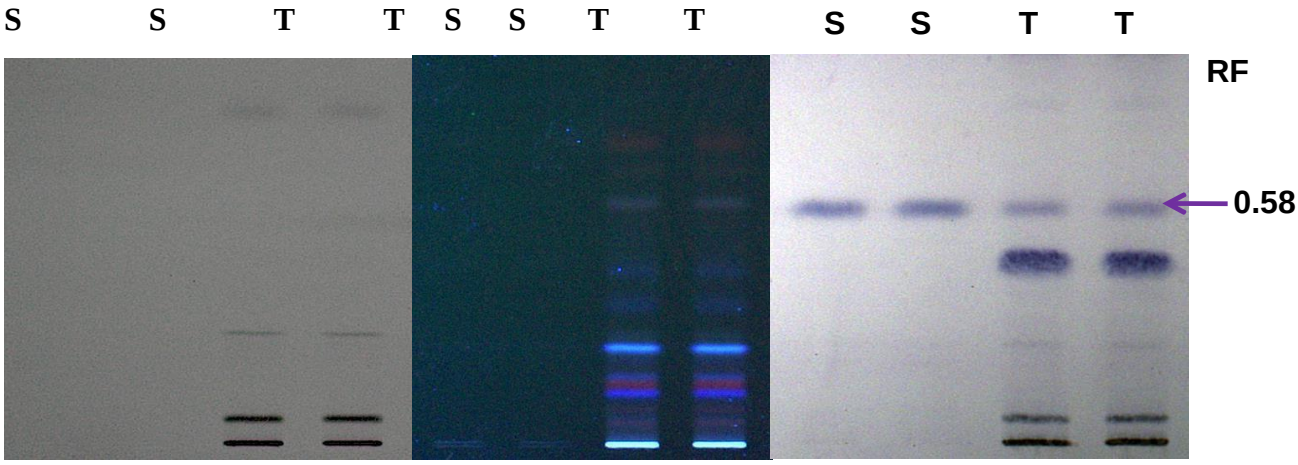
**Thin layer chromatography (TLC)**

The results from TLC analysis depicted in Table no.3

| | TEJOVATI SAMPLE |
|---|---|
| TLC plate |  |
| Rf value | 0.29, 0.34, 0.45, 0.55, 0.59, 0.62,0. 79, 0.84, 0.91 |
| No. of spots | 9 |

The ethanolic extracts of Tejovati and vajradanti was separated in mobile solution (Mobile phase: Toluene: ethyl acetate: acetone: formic acid (6: 6: 6: 1 v/v/v/v)) and visualized 254 rim, 366 rim and white light. which showed results for the quantification of linalool in EZA was analyzed for the first time by scanning at wavelength (λmax 366 nm) and the quantity of linalool present in Tejovati was estimated to be 1.48% w/w.

**HPTLC fingerprinting and Quantification Linalool in *Zanthoxylum armatum***




**254nm** **366 nm** **White light after derivatization**

## 5. DISCUSSION

*Tejovati* as it is very highly potent medicine for maintaining good oral health due to presence of phenols and flavonoids. *Zanthoxylum armatum* DC. contains monoterpenes like linalool and limonene and *Tejovati* in traditional ayurvedic medicine used for the treatment of toothache, stomach pain, tooth and digestive disorders. Various studies show that showed that linalool rich in antimicrobial and antioxidant properties, can prevent streptococcus mutants from adhering to tooth surfaces. The linalool with concentration of 100mg/L and their effects on bacterial adhesion to salivary coated were observed. The observed parameters of the root of *Potentilla fulgens*, like morphology physicochemical parameters and TLC, HPTLC profile always constant. It may be useful to establish certain botanical standards for standardization of *Potentilla fulgens* for the further studies. This paper explains the evidence-based information regarding the pharmacological activity of this plant.

## 6. CONCLUSION

Herbal medicinal plant contributed highly in the allophathic and various traditional system of medicine. *Zanthoxylum armatum* DC is a small tree almost entirely glabrous with a strong pungent and aromatic smell. Its local name is Timbar or timar (in Hindko), Tejmal, Nepali dhania (in Urdu). It is found in hot valleys of subtropical Himalaya. The seeds and bark are used as an aromatic tonic in fever, dyspepsia and in cholera. The fruit as well as branches and thorn are used as remedy for toothache, also as stomachic and carminative and employed as fish toxin.[6]

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