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Research Article

PHARMACOGNOSTIC, PHYSICOCHEMICAL, AND PHYTOCHEMICAL STUDIES ON STEM BARK OF *ZANTHOXYLUM ARMATUM* DC.

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ABSTRACT

Objectives: To analyze the microscopic, macroscopic, physicochemical, and phytochemical standards of the *Zanthoxylum armatum* DC. (stem bark).

Methods: Pharamacognostic studies, namely macroscopic, microscopic, and powder microscopic analysis of stem bark were carried out. Physicochemical standards - ash content including total ash content, water soluble, and acid insoluble ash values, moisture content by loss on drying, solubility and extractive values of *Z. armatum* DC. were determined. Preliminary phytochemical screening, fluorescence analysis and quantification of alkaloids, flavonoids, phenol, tannins, saponins, and terpenoids were also studied.

Results: The macroscopic studies of stem bark showed the stem and its branches, armed with long, sharp prickles with variable size. The transverse section of bark showed the phellogen, phelloderm, cortex, phloem, and medullary rays that are characteristics to *Z. armatum* DC. The results of physicochemical standards give the identity and purity of the selected sample. Phytochemical studies revealed the presence of alkaloids, flavonoids, phenols, tannins, saponins, and terpenoids.

Conclusion: The present findings provide the pharmacognostic, physicochemical, and phytochemical information about the stem bark of *Z. armatum* and this might be useful by providing additional support with regard to its identification and standardization parameters.

Keywords: *Zanthoxylum armatum* DC., Pharmacognostic, Physicochemical, Phytochemical, Stem bark.

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INTRODUCTION

Plants are used as medicine from the time immemorial. About 2000 drugs of natural origin listed in Indian Materia Medica and most of them are derived from different traditional system and folklore practices [1]. The major sources of raw drugs are wild plants from local communities and herbal industries. The raw materials that are available in the markets are adulterated [2]. For safety and efficacy of the herbal product, accurate knowledge of crude drugs and its standardization studies becomes indispensable [3]. The importance of pharmacognosy has been widely felt in recent times. Standardization parameters, namely organoleptic, macroscopic, microscopic, physicochemical, phytochemical, and fluorescence studies helps to provide a unique identification of the plant even if the plant is in dry powder form and also helps to detect adulterations. Once the plant drug converted into dry powder, it loses its morphological identity and easily prone to adulteration. These kinds of studies will help in authentication of the plants and ensures reproducible quality of herbal products which will lead to safety and efficacy of plant drugs [4].

Zanthoxylum armatum (DC.) syn. *Z. alatum* a very important medicinal plant of Southeast Asia, belongs to the family Rutaceae commonly known as Timur (Nepal), Tejovathi (Sanskrit and Tamil), Indian prickly ash, Nepal pepper, or Tooth ache tree. It is an evergreen or sub deciduous shrub or occasionally very small tree with stem and branches, armed with long, sharp prickles found in Kashmir to Bhutan and also occurs throughout Northeast India. It is also found throughout China, Japan, Pakistan, Nepal, and Malaysia [5]. The *Z. armatum* (DC.) bark and fruit powder are used for the treatment of toothache [6]; its seeds and bark are used for the treatment of various diseases such as fever, cholera, heartburn, or indigestion [7]. It is reported for its antifertility [8], antiseptic, disinfectant, deodorant [9], antipyretic, and anti-diarrheal activities. It improves speech in children and increases saliva secretion [10]. Fruits and seeds of this plant are used in fever, dyspepsia, and skin diseases [11]. Leaves and bark were reported for hepatoprotective activity [12,13], anticancer [14,15], and antidiabetic activity [16,17]. However, no much scientific validation has been made for this plant; hence, the present study was aimed in the determination of pharmacognostic, physicochemical and phytochemical standards for the stem bark of *Z. armatum* DC.

MATERIALS AND METHODS

Collection of plant material

Plant source selected for the present study *Z. armatum* DC. (stem bark) and the authenticated bark material was bought from the Institute of Himalayan Bioresource Technology (Council of Scientific and Industrial Research), Himachal Pradesh, India. The bark was cleaned, shade dried and used for the present study.

Chemicals, reagents, and solvents

All chemicals, reagents, and solvents used for the study were analytical grade.

Pharmacognostic study

The stem bark of *Z. armatum* DC. was taken for macroscopic and microscopic analysis. The coarse powder was used for the physicochemical, fluorescence and phytochemical studies. The pharmacognostic studies such as macroscopic, microscopic, and powder microscopy were done by the procedure mentioned in standard literature [18-20].

Florescence analysis

Stem bark material was treated with various chemical reagents and exposed to visible, ultraviolet light to study their fluorescence behavior [21].

Physicochemical and phytochemical analysis

Physicochemical values such as the percentage of ash values and extractive values were determined according to the well-established procedure given in Indian Ayurvedic Pharmacopoeia [22]. Preliminary phytochemical screening was carried out to identify the presence of various secondary metabolites in stem bark of *Z. armatum* DC. [23] based on the change of color and/or precipitate formation after addition of specific reagents was observed. The quantitative analysis of major secondary metabolites such as alkaloids [24], flavonoids [25], phenols [26], terpenoids [27], tannins [28], and saponins [29] was determined according to the given standard procedures.

RESULTS

Macroscopic studies

Z. armatum DC. is an evergreen shrub or small tree (Fig. 1) The stem bark (Fig. 2) was taken for the macroscopic analysis. The color, appearance, taste, and odor of bark and powder were noted and given in Table 1. The macroscopic study of stem bark showed the stem and its branches, armed with long, and sharp prickles with variable size. The cork has large marks of tubercular prickles with 0.1–0.2 cm thickness. The external surface of stem bark looks pale brown in color and rough with numerous scattered patches of lenticels, slightly deeply furrowed. The internal surface was smooth, light yellow to pale brown with short fracture. The stem bark has aromatic odor with an aromatic pungent taste.

Microscopical studies of bark

The transverse section of stem bark was shown in Fig. 3a-c - The bark showed the presence of phellogen, phelloderm, cortex, phloem, and medullary rays. The phellogen was made up of 5–6 layered collapsed rectangular and thick walled dead cells and some of these cells contain yellow/golden yellow colored cell content. Phellogen was followed by phelloderm which was contains 2–3 cell layered rectangular cells; some of this phelloderm cells contain compound and simple globular and ovoid starch grains and prismatic calcium oxalate crystals. Cortexes are several cells layered which was made up of ovoid/globular/ polygonal shaped parenchyma cells.

Fig. 1: Zanthoxylum armatum DC.

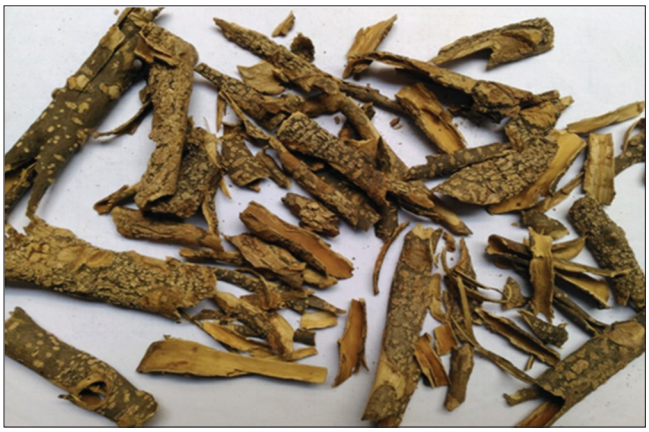

Fig. 2: Zanthoxylum armatum DC. Stem Bark

Powder microscopy

Powder microscopy studies of *Z. armatum* DC. (stem bark) indicated the presence of parenchymal cells containing yellowish brown content, prismatic calcium oxalate crystals, thick-walled fibers with narrow lumen and tapering ends, lignified parenchyma cells with thick lignified walls, macrosclereids with thick walls and pits, wide and branched central cavity, simple and compound, round, ovoid, polygonal, and irregular-shaped starch grains with centrally located hilum and the margins are striated, elongated sclereids, xylem vessels with spiral and pitted thickening and volatile oils are also seen in the study all these were shown in Fig. 4a-i.

Fluorescence analysis

The fluorescent analysis of stem bark of *Z. armatum* DC. on treatment with various chemical reagents showed different colors under day light and UV light. The results of fluorescent studies were given in Table 2. The green fluorescence indicates the presence of sterol, yellow fluorescence indicates the presence of flavonoids, and brown fluorescence indicates the presence of alkaloids.

Physicochemical standards

The physicochemical characteristics of stem bark powder, namely foreign matter, loss on drying, and ash values were given in Table 3. The result showed that the selected plant sample contained minimum amount of foreign matter (0.82%) and moderate amount of moisture content (6.134%). The total ash value was found to be 9.60% and water soluble ash content 3.12% was more than the acid insoluble ash 2.58%. The chloroform (9.67±0.58) and ethyl acetate (8.63±0.76)

Table 1: Morphological observation of *Zanthoxylum armatum* DC. (Stem bark)

Parameters	Observation
Stem bark	
Color	Brown
Shape	Presence of spines and its mark on the rough surface
Taste	Bitter and astringent
Odor	Bitter and aromatic odor
Stem bark powder	
Color	Yellow to brown
Taste	Bitter and astringent
Odor	Bitter and aromatic odor

Table 2: Fluorescence Analysis of powdered drug of *Zanthoxylum armatum* DC. (Bark)

S. No.	Treatment	Day light	UV light
1	Drug Powder	Green	Green
2	Drug powder+aq. 1N NaOH	Orange	Green
3	Drug powder+alc. 1N NaOH	Yellow	Light green
4	Drug powder+1N HCl	Light yellow	Light green
5	Drug powder+50% H2SO4	Dark brown	Dark green
6	Drug powder+Hexane	Colorless	Colorless
7	Drug powder+Benzene	Light yellow	Light yellow
8	Drug powder+Chloroform	Light yellow	Light yellow
9	Drug powder+Ethyl acetate	Light yellow	Light yellow
10	Drug powder+Alcohol	Light yellow	Light yellow
11	Drug powder+Acetone	Light yellow	Pink
12	Drug powder+Water	Yellow	Green

extractive values were higher when compared to hexane extractive value 4.2±0.26. This indicates the presence of high polar compounds. The water solubility was found to be higher when compared to alcohol solubility.

Qualitative and quantitative analysis of secondary metabolites

The preliminary phytochemical screening of dry powder and its various extracts of stem bark of *Z. armatum* DC. given in Table 4 and the results revealed the presence of variety of the phytochemicals in different extracts. Aqueous extracts revealed the presence of alkaloid, flavonoid, phenol, glycoside, lignin, coumarin, and tannin. Saponin, sterol, and terpenes were absent in aqueous extract. Ethanolic extract showed the presence of saponin, tannin, terpenes, flavonoid, alkaloid, coumarin, lignin, glycoside sterol, and phenol. Saponin, flavonoid, lignin, alkaloid, sugar, and phenol were present in chloroform extracts. Sterol, terpenes, flavonoid, coumarin, lignin, alkaloid, and phenol were present in ethyl acetate extract. In hexane extract, saponin, terpene, coumarin, and alkaloids were present. The drug powder showed the presence of alkaloid tannin, coumarin, glycoside, sugar, and phenol.

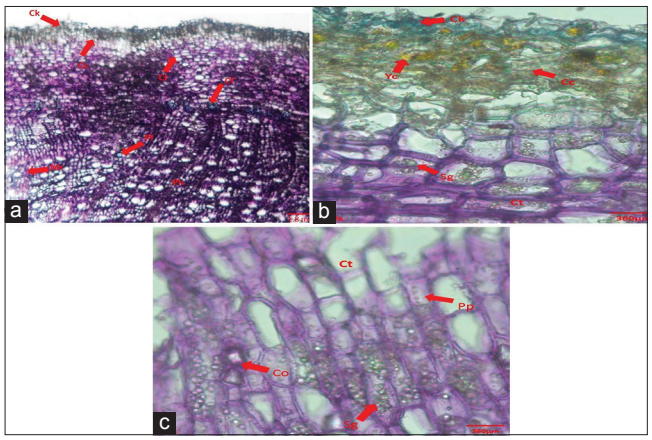

Fig. 3: (a) TS of *Zanthoxylum armatum* DC. (Bark). (b) TS of *Z. armatum* DC. (Bark). (c) TS of *Z. armatum* DC. (bark) Enlarged view. Cc - Cork cambium, Cf - Cortical fiber, Ck - Cork, Co – Calcium oxalate crystals (prismatic), Ct - cortex, Sc - Sclereids, Mr - medullary ray, Pf - Phloem fiber, Pp - Parenchyma with pits, Sg - Starch grains, Yc - Yellow cell content

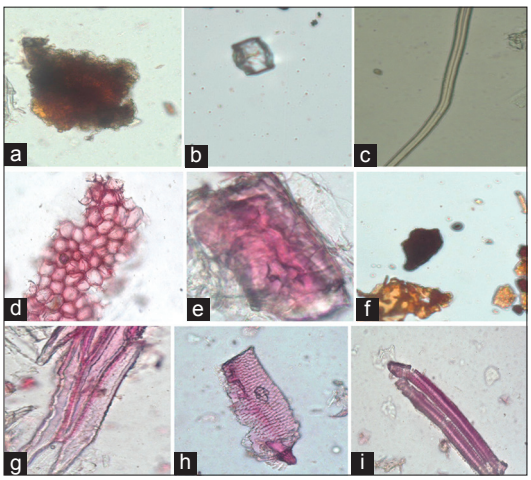

Fig. 4: Powder microscopy of *Zanthoxylum armatum* DC. Bark. (a) Brown content (×400). (b) Prismatic calcium oxalate crystal (×400). (c) Fiber (×400). (d) Lignified parenchyma (×400). (e) Sclereids (×400). (f) Starch grain (×400). (g) Elongated sclereids (×400). (h) Xylem vessels with pitted thickening (×400). (i) Xylem vessels with spiral thickening (×400)

The quantitative analysis of major secondary metabolites in the *Z. armatum* DC. shows in Table 5. Alkaloid content was found to be higher, followed by saponin, terpenoid, and flavonoid.

DISCUSSION

The standardization studies of a crude drug are an integral part for establishing its correct identity, purity, and quality of the raw drug material. Before including any crude drug an herbal pharmacopeia, pharmacognostic parameters such as anatomy, macroscopy, and microscopy including powder microscopic standards must be established. The microscopic method is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials [30-34]. In the present study, from the results obtained, the macroscopic and microscopic features of stem bark of *Z.armatum* DC. were identified and depicted (Fig. 3a-c).

The crude drugs from plant origin were the major source of drug used in the Indian medicinal system. To check the identity, purity and quality of the crude drug the pharmacognostic and physicochemical standards were followed before it comes to use or marketing. This will helps to assess the quality of the drugs. The presence of a decreased level of foreign matter indicates the purity of the collected plant materials of the selected plant [35]. According to the World Health Organization (WHO), the macroscopic and microscopic description of a medicinal plant is the first step toward establishing its identity and purity and should be carried out before any tests are undertaken [36]. In the present study, the macroscopic features of stem bark of *Z. armatum* were studied and it serves as diagnostic parameter. Microscopic study of stem bark including powder microscopy resulting the presence of cork interrupted by lenticels, stone cells, phloem arrangements, calcium oxalate crystals, oil globules, and starch grains indicates the characteristics of stem bark.

Physicochemical standards such as ash content, moisture content using the loss on drying, extractive values give the identity, purity, and strength of the selected drug (Table 3). Ash values are used to determine the quality and purity of crude drug. It indicates the presence of various impurities such as carbonate, oxalate, and silicate in the drug source decrease the quality and efficiency of selected drug. The water-soluble ash is used to estimate the amount of inorganic compound and the acid insoluble ash consists mainly of silica and indicate contamination with earthy material. Moisture content of drugs could be at minimal level to discourage the growth of bacteria, yeast, or fungi during storage and it is mentioned in Ayurvedic Pharmacopoeia of India for number of medicinal plants. In the present study, the ash values including water soluble and acid insoluble ash of stem bark of *Z. armatum* DC. were determined and do not exceeding the prescribed values given in Ayurvedic Pharmacopoeia. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent [37].

Table 3: Physicochemical characteristics of *Zanthoxylum armatum* DC. (Bark)

Parameters	Value % W/W
Foreign matter	0.82
Loss on drying	6.134±0.15
Total ash content	9.60±0.35
Water soluble ash	3.12±0.26
Acid-insoluble ash	2.58±0.69
Extractive values (%)	
Hexane	4.2±0.26
Chloroform	9.67±0.58
Ethyl acetate	8.63±0.76
Solubility values	
Alcohol	11.21±0.40
Water	16.11±0.55

Values are expressed in Mean±SD where n=3

Table 4: Preliminary phytochemical screening of *Zanthoxylum armatum* DC. (Bark)

S. No	Test for	Drug powder	Hexane	Chloroform	Ethyl acetate	Ethanol	Water
1	Saponin	−	+	+	−	+	+
2	Tannin	+	−	−	−	+	+
3	Sterol	−	−	−	+	+	−
4	Terpene	−	+	−	+	+	−
5	Flavonoid	−	−	+	+	+	+
6	Coumarin	+	+	−	+	+	+
7	Lignin	+	−	+	+	+	+
8	Alkaloid	−	+	+	+	+	+
9	Glycoside	+	−	−	−	+	+
10	Sugar	+	−	+	−	−	+
11	Phenol	+	−	+	+	+	+

+: Positive indicates presence, −: Negative indicates absence

Table 5: Quantitative analysis of secondary metabolites

S. No	Content	Value (mg/g)
1	Total alkaloids	2.73±0.23
2	Total flavonoids	0.05±0.02 QE equivalent
3	Trpenoids	153±3.21
4	Saponin	0.13±0.01
5	Tannin	0.041±0.01
6	Phenol	25.92±1.36 GAE equivalent

Values are expressed in mean±SD where n=3. QE: Quercetin equivalent, GAE: Gallic acid equivalent

The extractive values of the selected drug are useful to evaluate the chemical constituents present in crude drug and also help in estimation of specific constituents soluble in particular solvents. The water solubility was found to be higher followed by alcohol extractive in the stem bark chosen for the current study. The fluorescent analysis under day light and UV light by treatment with different chemical reagents showed different colors (Table 2). It indicates the presence of active ingredients, and it is the preliminary test for the presence of various secondary metabolites. The preliminary phytochemical screening and quantitative analysis of *Z. armatum* DC., stem bark showed the presence of various secondary metabolites such as alkaloids, flavonoids, phenol, tannin, saponin, terpenoids, glycosides, and coumarin and some of the major secondary metabolites quantity were also determined. The phytochemical compounds are known to exhibit bioactive properties. Different alkaloids have been isolated from several medicinal plants and investigated for their possible antidiabetic activity in different animal models. The preliminary phytochemical screening and its estimation (Tables 4 and 5) may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.

Qualitative and quantitative analysis of secondary metabolites is very essential for identifying the presence of phytochemicals which is important for the contribution of medicinal as well as physiological properties to the plants. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, and steroids, etc. [38]. The present study revealed that the stem bark of *Z. armatum* DC. is a rich source for phytochemicals and can be used as the best source for the treatment of different ailments.

CONCLUSION

In the current study, the macroscopic, microscopic, physicochemical, and phytochemical studies of *Z. armatum* DC. (stem bark) were determined. The pharmacognostic and physicochemical and phytochemical parameters provide the authentication source, standardization and therapeutical information of the selected drug source. In conclusion, the above finding gives the pharmacopeial and pharmacological information for further studies.

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AUTHORS' CONTRIBUTIONS

Designed the experiments Jothi G. and Sridharan G, Performed and drafted by Keerthana K and Jothi G. All authors contributed for the final version.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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