Research Article

Pharmacognostic, Phytochemical and Antidiabetic Activity Studies on Zanthoxylum Armatum Leaves

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Abstract
The present research work focused on pharmacognostic and phytochemical analysis of Zanthoxylum Armatum leaves. Zanthoxylum armatum commonly known as kondakasmi belong the family of Rutaceae. In pharmacognostic study to identify the calcium oxalate crystals in Zantoxylum armatum leaves. The phytochemical analysis revealed the presence of Phytosterols, alkaloids, glycosides, tannins, carbohydrates and triterpenes. For the observation of chemical constituents to performed to the TLC. The spots obtained on the TLC plate were observed in the U.V chamber initially and then by using the spraying reagent the spots were observed and reported. Zanthoxylum armatum leaves are extracted with methanol, hexane and ethyl acetate. Each individual extract it produce the different biological activity. These leaves are possess the several biological activities such as antioxidant, antimicrobial and anti diabetic activities. The present work describes that anti diabetic activity of zanthoxylum armatum leaves. Ethyl acetate extract of Zanthoxylum armatum showed good anti-diabetic activity against metformin.

Keywords: Zanthoxylum armatum, phytochemical, pharmacognostic, anti diabetic activity, calcium oxalate, ethyl acetate.

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1. Introduction
Zanthoxylum armatum DC (Rutaceae) is commonly known as Kondakasimi in telugu name. it is widely distributed in india. From Kashmir to Bhutan at altitudes up to 2,5000 m, also occurs through north east india. It is a small tree. Or large spiny shrub. The bark, fruits and seeds are extensively used in indigenous system of medicine as a carminative,
stomachic and anthelmintic. The stem has exhibited hypoglycemic activity in the preliminary trials. The fruits and seeds are employed as an aromatic tonic in fever and dyspepsia. A number of alkaloids have been reported from its stem-bark, wood and roots viz. berberine, dictamine, magnoflorine, xanthoplanine, sikimmianine, dictamine and γ fagarine.

2. Materials and Methods

Collection of plant material:
The plant material was collected in February 2013 from Sunkarametta Village, Arakuvalley, Andhra Pradesh, and authenticated by Prof. M. Venkaiah, department of botany, Andhra University, Visakhapatnam, Andhra Pradesh. The voucher specimen no. 22065, were deposited in the botany department herbarium, Andhra University.

Methods

Pharmacognostic study:
In pharmacognostic study microtome sections were taken, stained with safranin and mounted with glycerin and parts identification with the help of compound microscope 45x as usual and the cell content and cell wall structure were studied according to the method described by Sass (1940) Johnson (1940) and O'Brian et al (1964).

Microscopical Studies

Midrib:
In transactional view, the leaf appears dorsiventral with thick, broad elliptical midrib and thus winglike lamina. The midrib is biconvex in sectional view with more or less slightly curved adaxial and abaxial sides, the later showing more convexity than the former. It is 1.2 mm thick along median vertical plane, about 2.7 mm in horizontal plane and comprises of heterogeneous ground tissue within which lie two rows of vascular bundles three on the adaxial side and five on the abaxial side. The epidermis is thin and consists of small thick walled cells covered externally with a thin cuticle. Along the adaxial side, beneath the epidermis there exists a broad wide discontinuous band of chlorenchyma about 50 µm comprising of 5-7 layers of cells, which appears to be a lateral extension of the mesophyll tissue of the lamina. Along the abaxial side, above the epidermis is a continuous band of 4 or 5 layers of collenchyma cells, about 70 µm wide. The remaining ground tissue consists of fairly large, thin walled, compact angular or circular parenchymatous cells.

Lamina:
The lamina shows a dorsiventral arrangement of the mesophyll. The upper epidermis is composed of cubical to tangentially elongated type of cells covered externally with a thick cuticle. The cells of the lower epidermis are comparatively smaller but tangentially elongated. In the surface, view the cells of the upper and lower epidermis shows wavy and sinuous walls.

Phytochemical analysis:

Extraction process with methanol:
The freshly collected leaves of the plant were shade dried and powdered. The powdered materials were then subjected to Soxhlet extraction process. The dried powdered materials of leaves of the plant were extracted successively three times with methanol. The extracts thus obtained were concentrated under vacuum at temperature of 43°C by using rotary evaporator, dried completely, weighed and stored in a dessicator.

Fractionation process with ethyl acetate and hexane:
100 gms of the methanolic extract was dissolved in a 500 ml of distilled water. This solution kept in a freezer condition for 6 to 7 days. After solution was filtered. Above solution was fractionated with successively with ethyl acetate and hexane. The extracts were obtained. dried completely, weighed and stored in a dessicator. After fractionation process three different extract (hexane extract, methanol extract, ethyl acetate extract) were obtained. Initially TLC (thin layer chromatography) was performed for these extracts with 96% hexane and 4% ethyl acetate and so on by increasing 2% each time up to 100% methanol. The spots obtained on the TLC plate were observed in the U.V chamber initially and then by using the spraying reagent the spots were observed and reported.

The extracts prepared was tested for the type of chemical constituent present by known qualitative tests.
The following tests were carried out on the extracts to detect various phyto constituent present in them.

Tests for Alkaloids

About 50 mg of solvent – free extract was stirred with little quantity of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows.

Mayer’s Test:
To a few ml of filtrate, two drops of Mayer’s reagent was added along with the sides of the test tube. If the test is positive, it gives white or creamy precipitate.

Wagner’s Test:
To a few ml of the filtrate, few drops of Wagner’s reagent were added along with the sides of the test tube. Formation of reddish brown precipitate confirms the test as positive.

Hager’s Test:
To a few ml of filtrate 1 or 2 ml of Hager’s reagent was added. A prominent yellow precipitate indicates positive test.
Dragendorff’s Test: To a few ml of filtrate, 1 or 2 ml of Dragendorff’s reagent was added. A prominent reddish brown precipitate indicates positive test.

Tests for Carbohydrates
About 100mg of the extract was dissolved in 5 ml of distilled water and filtered. The filtrate was subjected to the following tests.

Molisch’s Test: To 2 ml of filtrate, two drops of alcoholic solution of α – naphthol was added. The mixture was shaken well and 1 ml of concentrated sulphuric acid was added slowly along the sides of the test tube, the test tube was cooled in ice water and allowed to stand. A violet ring at the junction of two liquids indicates the presence of carbohydrates.

Fehling’s Test: 1 ml of filtrate was boiled on a water bath with 1 ml each of Fehling’s solution A and B. Formation of red precipitate indicates the presence of sugar.

Barfoed’s Test: To 1 ml of the filtrate, 1 ml of Barfoed’s reagent was added and heated on a boiling water bath for 2 minutes. Red precipitate indicates the presence of sugar.

Benedict’s test: To 0.5 ml of filtrate 0.5 ml of Benedict’s reagent was added. The mixture was heated on a boiling water bath for 2 minutes. A characteristic brick red precipitate indicates the presence of sugar.

Tests for Glycosides
For detection of glycosides, about 50 mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hrs on a water bath, filtered and the filtrate was subjected to the following tests.

Borntrager’s Test: To 2 ml of filtrate hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonium solution was added to it. Formation of pink color indicates the presence of anthraquinone glycosides.

Legal’s Test: About 50 mg of the extract was dissolved in pyridine. Sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide solution. Presence of glycoside is indicated by a characteristic pink color.

Tests for Saponins
Foam or Froth Test: A small quantity of the extract was diluted with distilled water to 20 ml. The suspension was shaken in a graduated cylinder for 15 minutes. A two centimeter layer of foam or froth which is stable for 10 minutes indicates the presence of saponins.

Tests for Phytosterols and Triterpenoids
Liebermann – Burchard’s test: The extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the side of the test tube. Red, pink or violet color at the junction of the liquids indicates the presence of steroids / triterpenoids and their glycosides.

Salkowski test: Few drops of concentrated sulphuric acid was added to the chloroform extract, shaken on standing, red colour in the lower layer indicates the presence of steroids and golden yellow colour indicates the presence of triterpenoids.

Tests for Phenolic Compounds and Tannins
Ferric chloride test: About 50 mg of extract was dissolved in distilled water and to this few drops of neutral 5% ferric hydrochloric acid solution was added. Formation of blue, green and violet color indicates the presence of phenolic compounds.

Gelatin test: A little quantity of extract was dissolved in distilled water and 2 ml of 1% solution of gelatin containing 10% sodium chloride was added to it. Development of white precipitate indicates the presence of phenolic compounds.

Tests for flavonoids
Alkaline reagent test: An aqueous solution of extract was treated with 10% ammonium hydroxide solution – yellow fluorescence indicates the presence of flavonoids.

Shinoda test or Magnesium – Hydrochloric acid reduction: A little quantity of extract was dissolved in alcohol and few fragments of magnesium turnings and conc. hydrochloric acid (drop wise) were added. If any pink or crimson – red colour develops, presence of flavonol glycoside is inferred.

Zinc-hydrochloric acid reduction Test: The alcoholic solution is treated with pinch of zinc dust and few drops of concentrated hydrochloric acid - magenta colour is produced after few minutes.

Ant diabetic activity

Chemicals:
Alloxan monohydrate was purchased from sigma-Aldrich, (St.Louis, USA), metformin and tween 20 were purchased from SD fine chemicals (India), blood glucose kit was purchased from coral company, (India) and all other chemicals used in this experiment were of analytical grade.

Selection of animals:
Wistar albino rats of only male weighing between 200-250g were employed for the present study. All animals were procured from M/s mahavir enterprises, Hyderabad, and Andhra Pradesh (India). All rats were maintained under standard laboratory conditions like temperature (25±2°C) relative humidity (50±15%) and normal photoperiod (12 h dark/12 h light).they were fed with standard dry pellet diet supplied by ratti brothers, Hyderabad (India) and water were provide ad libitum animal ethical committee (IAEC) and by the regulatory body of the government (Regd no.1048/a/07/CPCSEA) (Ajitkar, B.K.Choudhary And N.G. Bandyopadhyay 2003).

Induction of diabetes:
Animals were allowed to fast for 18h and were injected with alloxan monohydrate dissolved in sterile normal saline at a dose of 150mg/kg b.w intraperitoneally. After stabilization of diabetes, the rats with blood glucose levels between 250-350mg/dl were used for the experiment. (Ragavan, B. and Krishnakumari, S. 2006).

Experimental design
In the experiment a total number of 40 surviving rats were used. The rats were divided into 8 groups; each group consists of five rats. Group I normal rats were treated with vehicle (2% tween 20) and served as normal control, group II were treated with metformin (mg/kg b.w) in normal and diabetic rats, group III& IV groups were treated with...
hexane extract of *Z. armatum* leaves at a doses of 150&300 mg/kg b. w respectively, group V & VI normal rats were treated with ethyl acetate extract of *Z. armatum* leaves at a doses of 150& 300 mg/kg b. w respectively. Group VII & VIII normal rats were treated with methanol extract of *Z. armatum* leaves at doses of 150& 300 mg/kg b. w respectively. All the doses were administered orally. (Chattopadhyay, S., 1997).

**Collection of blood samples and estimation of blood glucose:** The animal was restrained (unanaesthetised) in such a way that loose skin of the neck was tightened while handling the head with the left hand. With the help of index finger the eye was pressed just behind the angle of the jaw resulting in the engorgement of the retro orbital plexus. Then tip of the capillary was inserted at the medial canthus into the retro orbital plexus with gentle rotation by the other hand. As the vessels are ruptured, blood wells up in the retro orbital space. The tip of the capillary was then slightly withdrawn, so that the blood flows into the capillary, which was collected in micro centrifuge tube containing small quantity of potassium oxalate and sodium fluoride as anticoagulant. Blood samples were collected from retro orbital plexus at 0(before treatment) 2, 4, 6, 8 and 12h (after treatment). The plasma blood glucose levels were estimated by GOD-POD method. (T. Tomita, P.Lac, F.M. Matschinsky And M.L. Medaniel1974).

**3. Results and Discussion**

<table>
<thead>
<tr>
<th>Name of the test</th>
<th><em>Zanthoxylum armatum</em> leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hexane extract</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
</tbody>
</table>

*+- Present  - Absent*

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<td>+</td>
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<td>Carbohydrates</td>
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</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
</tbody>
</table>

![Table 1: Phytochemical analysis](image)

**Table 2: Effect of methanol extract of *Zanthoxylum armatum* leaves on fasting blood glucose in treated rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/k.g b.w</th>
<th>Blood glucose levels (mg/dl) at different time intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
<td>2h</td>
</tr>
<tr>
<td>Control (drug vehicle)</td>
<td>Tween 20</td>
<td>105.13</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Alloxan</td>
<td>299</td>
</tr>
<tr>
<td>Z. armatum methanol</td>
<td>150</td>
<td>290</td>
</tr>
<tr>
<td>Z. armatum methanol</td>
<td>300</td>
<td>293.62</td>
</tr>
<tr>
<td>metformin</td>
<td>3mg/kg</td>
<td>296.5</td>
</tr>
</tbody>
</table>

![Data 1](image)

**Fig 3:** Effect of methanolic extract of *Zanthoxylum armatum* leaves on fasting blood glucose in treated rats
Table 3: Effect of ethyl acetate extract of *Zanthoxylum armatum* leaves on fasting blood glucose in normal rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/k.g.b.w</th>
<th>Blood glucose levels (mg/dl) at different time intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (drug vehicle)</td>
<td>Tween 20</td>
<td>104.16 101.16 98 96.66 94.16 90.5</td>
</tr>
<tr>
<td>Diabetic control (Alloxan)</td>
<td>299</td>
<td>311 310 301 295 286.66 284.16</td>
</tr>
<tr>
<td><em>Z. armatum</em> ethyl acetate</td>
<td>150</td>
<td>299.66 296.16 (3.51) 293 290 (9.69) 286.66 (13.04)</td>
</tr>
<tr>
<td><em>Z. armatum</em> ethyl acetate</td>
<td>300</td>
<td>297.5 292.6 (5.02) 286.8 282.66 (15.22) 281.16 (16.75)</td>
</tr>
<tr>
<td>Metformin</td>
<td>3mg/k.g</td>
<td>292.5 271.33 (22.99) 268.83 269.5 264.16 (21.4)</td>
</tr>
</tbody>
</table>

Fig 4: Effect of ethyl acetate extract of *Zanthoxylum armatum* leaves on fasting blood glucose in treated rats

Table 4: Effect of hexane extract of *Zanthoxylum armatum* leaves on fasting blood glucose in normal rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/k.g b.w</th>
<th>Blood glucose levels (mg/dl) at different time intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Drug Vehicle)</td>
<td>Tween 20</td>
<td>104.17 101.17 98 96.66 94.16 90.5</td>
</tr>
<tr>
<td>Diabetic control (Alloxan)</td>
<td>299</td>
<td>311 310 301 295 287.5 (16.57)</td>
</tr>
<tr>
<td><em>Z. armatum</em> Hexane</td>
<td>150</td>
<td>287.5 284.66 (3.23) 280.83 (7.61) 278.5 (10.28) 275.33 (13.9)</td>
</tr>
<tr>
<td><em>Z. armatum</em> Hexane</td>
<td>300</td>
<td>269.16 264.33 (6.98) 260.33 (12.77) 256.66 (18.07) 251.83 (25.06)</td>
</tr>
<tr>
<td>Metformin</td>
<td>3mg/kg</td>
<td>292.5 271.33 (22.99) 268.83 261.5 254.16 (21.4)</td>
</tr>
</tbody>
</table>

Fig 5: Effect of hexane extract of *Zanthoxylum armatum* leaves on fasting blood glucose in normal rats
Discussion:
The present study was conducted to evaluate the hypoglycemic and anti-hyperglycemic activity of Z. armatum leaves. In this study the methanolic, ethyl acetate, hexane extracts of Z. armatum leaves produced dose dependent blood glucose reduction in normal and diabetic group. In normal group treated with Z. armatum leaves produced percentage blood glucose reduction was observed up to 12 h and maximum percentage blood glucose reduction was observed at 6h where as in diabetic group the percentage blood glucose reduction with Z. armatum leaves was up to 12h and maximum at 8h the percentage blood glucose reduction produced by Z. armatum leaf extracts at 300mg/kg b.w in diabetic group were significant and were nearly equal with metformin (standard) treated group.

4. Conclusion
The present research work concluded that the forgoing observations it is seen that the leaf of Zanthoxylum armatum appears dorsiventral with thick, broad elliptical midrib and thus wing like lamina. There are eight prominent and collateral vascular bundles, three on the adaxial side and five on the abaxial side, juxtaposed and alternate with each other. The xylem elements are located in the centre and towards the outer part of which is phloem. The cells of the spongy mesophyll in general contain cluster of calcium oxalate crystals was observed and reported. The preliminary phytochemical tests indicated the presence of alkaloids, glycosides, tannins, and flavonoids in the different extracts. Several such compounds are known to possess potent antioxidant activity; some of these constituents have already been isolated from this plant. From the results in present study the author concludes that the Z. armatum leaves has hypoglycemic and antihyperglycemic activity and it is further needed to isolate bioactive molecule responsible for Anti-Diabetic activity and the traditional usage of the plant (Z. armatum) was scientifically justified now.

5. References

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