Research Article

Pharmacognostic and Phytochemical study of Whole plant of *Quisqualis indica* Linn.

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**ABSTRACT**

Whole plant of *Quisqualis indica* Linn. (Family Combretaceae) is commonly known as Rangoon creeper (combretum indicum) or madhumalati, traditionally used as anthelmintic. The fresh plant was studied for pharmacognostic evaluations, including examination of morphological and microscopic characters, determination of plant constants, ash values and extractive values. The morphological studies revealed that the leaf is in dark green color with characteristic odour and slight bitter taste and the shape of *Quisqualis indica* leaves is as elliptical acuminate with entire margin, cordate base, and length varying from 7-12 cm. Dorsal side is glabrous and ventral surface is hairy. Powder study revealed the presence of covering trichomes, annular xylem vessels, calcium oxalate crystals and anomocytic stomata. The stomatal index 18.75, vein islet number is 7-10, vein termination is 3-5, palisade ratio 6-7. The Moisture content, Total ash, acid insoluble ash, watersoluble ash values and sulfated ash were observed to be 8%, 9%, 12.5%, 6.55% and 5.45% w/w respectively. Water-soluble extractive values, Alcohol soluble extractive value and petroleum ether soluble extractive value of the leaves were observed to be 10%, 3% and 1% w/w respectively. The phytochemical test revealed the presence of alkaloids, slight amount of glycosides, tannins, flavonoids and protein in both extract. Its flowers are used against diarrhea and eaten as vegetable. The flower extract gave high total polyphenol contents and showed strong antioxidant activity. In the search for new acetyl cholinesterase inhibitors from plant origin, it was demonstrated that methanolic extract of Q. indica flower exhibited this activity. The extract inhibited electric eel acetyl cholinesterase in dose dependent manner with an IC50 value of 0.77 g/ml.

**Keywords:** Chromen, Neoplasia, Antineoplastic activity DU-145, MOLT-4, SRB assay, IC50

**ARTICLE INFO**

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1. Introduction

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularize in developing and developed countries owing to its natural origin and lesser side effects. In olden times, vaidyas used to treat patients on individual basis, and prepared drugs according to the requirement of the patients. But the scene has been changed now; herbal medicines are being manufactured on a large scale in mechanical units, where manufacturers are facing many problems such as availability of good quality raw material, authentication of raw material, availability of standards, proper standardization methodology of drugs and formulations, quality control parameters and etc. [1,2] *Quisqualis indica* Linn (Combretaceae) is a strong climber, ligneous vine that can reach from 2.5 meters to up to 8 meters (Fig 1). It is commonly known as Rangoon creeper. It is indigenous in Africa, Indo Malaysian region and cultivated all over India [3]. Flower numerous, pendent, 7.5cm long, 3.8cm wide. At first they are white in colour then they become deep red. In amboynas the leaves are given in a compound decoction for flatulent distension of the abdomen. In China the ripe seeds are roasted and given in diarrhoea and fever and popular anthelmenic among the inhabitants of North Annan [4]. There was no report on the extensive pharmacognostic studies of this plant species. Meanwhile, in this investigation the phytochemical studies of the leaves extract are also carried out. To the best of my knowledge, this is the first time the leaf was screened for pharmacognostic study.

2. Materials and Methods

Plant Material Authentication

The mature green leaves of *Quisqualis indica* Linn were collected in the morning locally from Jaipur District, Rajasthan, India, in the month of August 2009. The plant was identified and authenticated by the Botanist, from the Department of Botany, University of Rajasthan, Jaipur, India. A voucher specimen (RUBL20663) is deposited in the Department of Botany, University of Rajasthan.
Pharmacognostic Studies

Macroscopy
Morphological studies were done by using simple microscope. The shape, apex, base, margin, taste and odor of leaves were determined.

Microscopy
Microscopic studies were done by preparing a thin hand section of midrib and lamina region of *Quisqualis indica* leaf. The section was cleared with chloral hydrate solution, stained with phloroglucinol and hydrochloric acid, and mounted with glycerin. (Fig 2) A separate section was prepared and stained with iodine solution for the identification of starch grains. Powder of the dried leaves was used for the observation of powder microscopical characters. The powdered drug was separately treated with phloroglucinol and HCl solution, glycerin and iodine solution to determine the presence of lignified cells, calcium oxalate crystals, trichomes and starch grains [5] (Fig 3).
3. Results and Discussion

The morphological studies revealed that the leaf is in dark green color with characteristic odour and slight bitter taste. (Table 1). The shape of Quisqualis indica leaves is as elliptical-acuminate with entire margin, cordate base, and length varying from 7-12cm. Dorsal side is glabrous and ventral surface is hairy (Table 2). Microscopic studies showed the presence of covering trichomes and glandular trichome. Midrib is having hypodermis which is made up of collenchymas. Lamina showed the presence of chlorenchyma next to epidermis. Midrib region showed xylem towards upper epidermis. Protoxylem found to move towards upper epidermis and meta xylem towards lower epidermal cells, Phloem moves towards lower epidermis. (Table 3) Powder study revealed the presence of covering trichomes, annular xylem vessel, epidermal cell, and anomocytic stomata. (Table 4).

The fluorescence analysis of the powder drug was observed in day/visible light and UV light. (Table 5 and 6) The stomatal index 18.75-19.02, vein islet number is 7-10, vein termination is 3-5, palisade ratio 6-7. (Table 7) The Moisture content, Total ash, acid insoluble ash, water-soluble ash values and sulfated ash were observed to be 8%, 9%, 12.5%, 6.55% and 5.45% w/w respectively. Water-soluble extractive values, Alcohol soluble extractive value and petroleum ether soluble extractive value of the leaves were observed to be 10%, 3% and 1% w/w respectively (Table 8). The qualitative chemical test revealed the presence of alkaloids, slight amount of glycosides, tannins, flavonoids and protein in both extract. Gum and mucilage is present in PEE and absent in ME. Carbohydrate is present in ME and slight present in PEE. (Table 9)

### Table 1: Identification of morphological feature

<table>
<thead>
<tr>
<th>S.No</th>
<th>Features</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Colour (Upper surface)</td>
<td>Dark green color</td>
</tr>
<tr>
<td>02</td>
<td>Colour (Lower surface)</td>
<td>Light green color</td>
</tr>
<tr>
<td>03</td>
<td>Odour</td>
<td>Characteristic</td>
</tr>
<tr>
<td>04</td>
<td>Taste</td>
<td>Tasteless</td>
</tr>
<tr>
<td>05</td>
<td>Shape</td>
<td>Ellipticle</td>
</tr>
<tr>
<td>06</td>
<td>Size</td>
<td>7-12cm</td>
</tr>
<tr>
<td>07</td>
<td>Arrangement</td>
<td>Opposite</td>
</tr>
</tbody>
</table>

### Table 2: Botanical evaluation of Quisqualis indica linn. Leaf

<table>
<thead>
<tr>
<th>S.No</th>
<th>Features</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Trichomes</td>
<td>Present both glandular and covering</td>
</tr>
<tr>
<td>02</td>
<td>Upper epidermis</td>
<td>Present</td>
</tr>
<tr>
<td>03</td>
<td>Midrib</td>
<td>Hypodermis is made up of collenchymas</td>
</tr>
<tr>
<td>04</td>
<td>Lamina</td>
<td>After epidermis collenchyma is present</td>
</tr>
<tr>
<td>05</td>
<td>Midrib (vascular bundles)</td>
<td>Xylem towards upper epidermis. Proto xylem towards upper epidermis and meta xylem towards lower epidermal cells, Phloem towards lower epidermis.</td>
</tr>
<tr>
<td>06</td>
<td>Sclerenchyma</td>
<td>Present</td>
</tr>
</tbody>
</table>
Table 3: Transverse section of leaf

<table>
<thead>
<tr>
<th>S. No</th>
<th>Leaf Portion</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Apex</td>
<td>Acuminate</td>
</tr>
<tr>
<td>02</td>
<td>Margin</td>
<td>Entire</td>
</tr>
<tr>
<td>03</td>
<td>Shape</td>
<td>Ellipticle</td>
</tr>
<tr>
<td>04</td>
<td>Lamina</td>
<td>Pinnate</td>
</tr>
<tr>
<td>05</td>
<td>Venation</td>
<td>Reticulate</td>
</tr>
<tr>
<td>06</td>
<td>Midrib Continuous</td>
<td>from base to apex</td>
</tr>
<tr>
<td>07</td>
<td>Dorsal surface</td>
<td>Glabrous</td>
</tr>
<tr>
<td>08</td>
<td>Ventral surface</td>
<td>Hairy</td>
</tr>
<tr>
<td>09</td>
<td>9 Petiole size</td>
<td>1 cm</td>
</tr>
<tr>
<td>10</td>
<td>10 Petiole shape</td>
<td>Cylindrical</td>
</tr>
<tr>
<td>11</td>
<td>Colour</td>
<td>Green</td>
</tr>
<tr>
<td>12</td>
<td>Leaf base</td>
<td>Cordate</td>
</tr>
</tbody>
</table>

Table 4: Powder microscopy

<table>
<thead>
<tr>
<th>S.no</th>
<th>Feature</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Nature</td>
<td>Coarse powder</td>
</tr>
<tr>
<td>02</td>
<td>Colour</td>
<td>Light green</td>
</tr>
<tr>
<td>03</td>
<td>Odour</td>
<td>Characteristic</td>
</tr>
<tr>
<td>04</td>
<td>Taste</td>
<td>Slight bitter</td>
</tr>
<tr>
<td>05</td>
<td>Covering trichome</td>
<td>Present</td>
</tr>
<tr>
<td>06</td>
<td>Xylem vessel</td>
<td>Present (Annular)</td>
</tr>
<tr>
<td>07</td>
<td>Epidermal cell</td>
<td>Present</td>
</tr>
<tr>
<td>08</td>
<td>Stomata</td>
<td>Present (Anomocytic)</td>
</tr>
<tr>
<td>09</td>
<td>Fibres</td>
<td>Present</td>
</tr>
<tr>
<td>10</td>
<td>Starch grain</td>
<td>Present</td>
</tr>
<tr>
<td>11</td>
<td>Calcium oxalate crystals</td>
<td>Present</td>
</tr>
</tbody>
</table>

Table 5: Analysis of powdered drug through naked eye

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Colour observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder as such</td>
<td>Fade green</td>
</tr>
<tr>
<td>Powder + conc.HCL</td>
<td>Green</td>
</tr>
<tr>
<td>Powder + Conc.HNO3</td>
<td>Brown</td>
</tr>
<tr>
<td>Powder + Conc.H2SO4</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Powder + Glacial acetic acid</td>
<td>Green</td>
</tr>
<tr>
<td>Powder + 5%NaOH</td>
<td>Brownish green</td>
</tr>
<tr>
<td>Powder + 5%KOH</td>
<td>Brownish green</td>
</tr>
<tr>
<td>Powder + 5%Ferric chloride</td>
<td>Dark green</td>
</tr>
<tr>
<td>Powder + Picric acid (saturated Aq. Solution)</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>Powder + Ammonia</td>
<td>Brownish green</td>
</tr>
</tbody>
</table>

Table 6: Fluorescence analysis of powder drug

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Fluorescence Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder as such</td>
<td>Green</td>
</tr>
<tr>
<td>Powder + 1N NaOH in methanol</td>
<td>No fluorescence</td>
</tr>
<tr>
<td>Powder + 1N NaOH in water</td>
<td>Green</td>
</tr>
<tr>
<td>Powder + 50%HCL</td>
<td>Brown</td>
</tr>
<tr>
<td>Powder + 50%HNO3</td>
<td>Brown</td>
</tr>
<tr>
<td>Powder + 50%H2SO4</td>
<td>Green</td>
</tr>
<tr>
<td>Powder + Petroleum ether</td>
<td>Green</td>
</tr>
<tr>
<td>Powder + chloroform</td>
<td>Black</td>
</tr>
<tr>
<td>Powder + picric acid</td>
<td>Brown</td>
</tr>
<tr>
<td>Powder + 5% Ferric chloride</td>
<td>Green</td>
</tr>
<tr>
<td>Powder + 5% Iodine solution</td>
<td>Green</td>
</tr>
<tr>
<td>Powder + Methanol</td>
<td>Green</td>
</tr>
<tr>
<td>Powder + HNO3 + NH3</td>
<td>Green</td>
</tr>
</tbody>
</table>

Table 7: Data representing values of microscpical study

<table>
<thead>
<tr>
<th>S. No</th>
<th>Microscopical Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phloem fibre</td>
<td>Length:8.52-82.36 Width:1.09-1.42</td>
</tr>
<tr>
<td>2</td>
<td>Calcium oxalate crystals</td>
<td>Length:1.6-3.2 Width:1.4-1.6</td>
</tr>
<tr>
<td>3</td>
<td>Starch grains</td>
<td>1.42-7.1</td>
</tr>
<tr>
<td>4</td>
<td>Trichomes</td>
<td>Length:15.62-5 Width:1.42-2.84</td>
</tr>
<tr>
<td>5</td>
<td>Stomatal no.</td>
<td>0.23-0.28</td>
</tr>
<tr>
<td>6</td>
<td>Stomatal index</td>
<td>18.75-19.02</td>
</tr>
<tr>
<td>7</td>
<td>Vein islet</td>
<td>7-10</td>
</tr>
<tr>
<td>8</td>
<td>Vein termination no.</td>
<td>3-5</td>
</tr>
<tr>
<td>9</td>
<td>Palisade ratio</td>
<td>6-7</td>
</tr>
</tbody>
</table>

Table 8: Data representing physiological parameter

<table>
<thead>
<tr>
<th>01</th>
<th>Parameter</th>
<th>Values (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on Drying</td>
<td>8% w/w</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>02</th>
<th>Ash values</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Ash</td>
<td>9% w/w</td>
<td></td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>12.5% w/w</td>
<td></td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>6.55 w/w</td>
<td></td>
</tr>
<tr>
<td>Sulphated ash</td>
<td>5.45% W/W</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>03</th>
<th>Extractive Values</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Water soluble extractive</td>
<td>10% w/w</td>
<td></td>
</tr>
<tr>
<td>Alcohol soluble extractive</td>
<td>3% w/w</td>
<td></td>
</tr>
<tr>
<td>Petroleum ether soluble Extractive</td>
<td>1% w/w</td>
<td></td>
</tr>
</tbody>
</table>
Table 9: Phytochemical analysis of the petroleum ether and methanol extract of leaves of Quisqualis indica linn.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Constituents/tests</th>
<th>Petroleum ether extract</th>
<th>Methanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dragendorff’s</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Mayers</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Wagners</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>Hagers</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>Tannic acid test</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>1</td>
<td>Legal test</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>Baljet test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Borntrager’s test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Keller killiani test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>Molish test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Fehlings test</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>Barfoeds test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Test for starch</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>Test with ruthenium red</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>1</td>
<td>Salkowski test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Libermanns burchard reaction</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>Shinoda test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Lead acetate test</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>Ferric chloride test</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>Reaction with alkali and acid</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>1</td>
<td>Lead acetate test</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>Ferric chloride test</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>Potassium dichromate test</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Assay of protein content of AChE

The protein content of AChE preparation was estimated by Bradford method using BSA (0-40 g/ml) as a standard (Bradford, 1976). All experiments were done in triplicate (n = 3). 22Silpakorn U Science & Tech J Vol.1(2), 2007 Kinetics of Acetylcholinesterase Inhibition.

In-vitro analysis of AChE activity

In order to select the proper concentration of enzyme, the AChE activity was measured in vitro by Eillman method (Eillman et al., 1961), and each assay was done in triplicate (n = 3). The assay contained 1 ml of mixture of 0.25 mM ASCh and 0.25 mM DTNB in 50 mM sodium phosphate buffer pH 8 and 200 L of AChE in different concentrations (0.01-0.243 g/ml). The final volume was adjusted to 3 ml with 50mM sodium phosphate buffer pH 8. The enzymatic reaction of AChE was the hydrolysis of acetyl group of ASCh and gave thiocholine (SCh) as the product. The SCh could react with DTNB to form 5-thionitrobenzoate, a colored anion, which absorbed UV at 412nm. The absorbances were measured at 0, 0.5 min and every 1 min interval starting from 0.5 min (0, 0.5, 1.5….. 20.5). The rate of product formation (A) was measured by the difference of absorbance (A) in every 1 min time intervals within 20.5 min. Then the product formation was calculated for each AChE concentration.

The effect of plant extract on AChE substrate Hydrolyzation. For studying the effect of plant extract on AChE activity, the enzyme was preincubated with each plant extract for 10 min before the addition of ASCh.

Estimation of the IC50 value

The concentration of the extract that inhibited 50% of AChE activity (IC50) was estimated by method described by Kamal et al., 2000 and Alhomida et al., 2000. The method was performed by plotting % activity and %inhibition of AChE versus extract (inhibitor) concentrations on the same graph. The concentration at this intersection of these two curves was the IC50 value. The assay contained 200 l of 0.0948 g/ml AChE, chosen from in vitro analysis of AChE activity, 1 ml of mixture of 0.25 mM DTNB and 0.25 mM ASCh in 50mM sodium phosphate buffer pH 8 and 200 l of plant extract in reaction concentration range of 0-2.22 g/ml (final
et al., Q. indica, 976, “Indian Pharmacopoeia” 310. showed low inhibition (Bisswanger, 2002). The 50 -
2005 -
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