PHARMACOGNOSTIC EVALUATION OF THE RHIZOME OF COPTIS TEETA WALL.

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ABSTRACT
Elaneer kuzhambu and Karpuradi kuzhambu are two ocular preparations referred in Sahasrayoga. According to tradition, Pitaka rohini is added in these preparations. Pitaka rohini is considered as stem bark of Gambhari, Gmelina arborea Roxb. according to Ayurvedic Formulary of India. However according to tradition, Pitaka rohini is considered as Coptis teeta Wall and its rhizomes are used in these preparations. Coptis teeta Wall is a perennial stemless herb belonging to the family Ranunculaceae. HPTLC evaluation of the above two preparations have shown the presence of the active ingredient Berberine. Berberine is present in Daruwaridra, Berberis aristata. HPTLC studies showed the presence of Berberine in the rhizomes of Pitaka rohini, Coptis teeta Wall. But Berberine was not detected in the stem bark of Gambhari, Gmelina arborea Roxb. The present study deals with Pharmacognostic evaluation (macroscopic, microscopic, physicochemical, powder microscopy) and HPTLC studies of the rhizomes of Coptis teeta Wall. This study will help in standardization of samples of whole, cut or powdered rhizome samples of Coptis teeta Wall and also help to identify the Berberine content in the rhizomes as well as in the formulations.

Keywords: Pitaka rohini, Coptis teeta Wall, Pharmacognostic evaluation, HPTLC.

INTRODUCTION
Herbal system of medicine has been in practice since historical times and traces its roots to ancient civilizations. Before the availability of synthetic drugs, plant - based remedies formed the basis of primary healthcare system. Herbal infusion, decoction and tinctures were house-hold remedies for common ailments. Elaneer kuzhambu and Karpuradi kuzhambu are preparations referred in Sahasrayoga for ocular disorders. According to tradition, Pitaka rohini is added in these preparations. Ayurvedic Formulary of India considers stem bark of Gambhari as Pitaka rohini. Gmelina arborea Roxb., is the accepted botanical source of Gambhari, belonging to the family Verbenaceae. This is an important ingredient of the group ‘Dasamoola’ which enters in to the composition of many of the Ayurvedic formulations. The plant is astringent, bitter, digestive, cardio tonic and laxative. It promotes digestive power, improves memory, overcome giddiness and it is useful in burning sensations. Pitaka rohini used by Ayurvedic Physicians in Elaneer kuzhambu and Karpuradi kuzhambu has been botanically identified as the rhizomes of Coptis teeta Wall instead of Gmelina arborea. WHO Monographs describe the use of dried rhizomes of Coptis chinensis Franch, Coptis deliodes, Coptis japonica Makino or other berberine containing species of the same genus. The variety indigenous to India is Coptis teeta Wall. It is a perennial stemless herb, belonging to the family of Rununculaceae. It is present more commonly in the hilly areas, especially that of the Himalayan region. It is also found in Bhutan, Nepal and China. In India it is found in Assam (Mishmi Mountains), Arunachal Pradesh, and Sikkim. The plant is 20-50 cm in height, rhizome is 5-6 cm long, brownish yellow, densely covered with numerous nodes and often with rootlets. It is used for applying in inflammatory eye diseases, decreased vision, cataract, skin-related problems, indigestion, constipation, jaundice and fever especially in malaria, gonorrhea, and urine disorders. The root is a pungent, very bitter and cooling. The root contains 8-8.5 % berberine, and is widely used as antibacterial, stomachic and anti-inflammatory. Berberine is one of the major active ingredients in the plant. It contains alkaloids like copine or coptina, fixed oil, albumin, coloring matter, lignin and sugar. Thus it is used widely in ocular ailments in the Ayurvedic system of medicine as a drug of choice and has proved beneficial and safe. The present study is undertaken with the objective to delineate the pharmacognostical profile of rhizomes of Coptis teeta Wall, as it may assist in standardization of samples of whole, cut or powdered plant material which could guarantee accurate means of identifying the crude drug and also fill the lacuna of our understanding about botanical pharmacognosy of Coptis teeta Wall.

MATERIALS AND METHODS
Plant material
Rhizomes of Coptis teeta Wall. were collected from the raw material store of Sreedhareeyam Ayurvedic Medicines (P) Ltd., Koothattukulam, Kerala, India. The authentic identification of the drug was made by CMPR, AVS, Kottakkal, India and the voucher herbarium specimen (SARDI/SP/92) was prepared and preserved in...
previously saturated with mobile phase acetate: Formic acid: Methanol (4:4:1:1) developed with Linomat equipped with Linomat as bands of 8 mm l of the rhizome of Coptis teeta Wall. were collected, shade dried and powdered. The powder was passed through Sieve No. 60 for obtaining fine powder and that was further subjected to powder microscopy. Photographs were taken with Trinocular digital microscope.

Physicochemical Studies
The shade dried rhizomes powder was used for the study. Physicochemical parameters like foreign matter, total ash, acid insoluble ash, water soluble extractives and alcohol soluble extractives were determined as per procedures described in the Ayurvedic Pharmacopoeia of India.

HPTLC Studies
Berberine Standard
The reference standard berberine was purchased from Sigma Aldrich India

Reference standard 1
Berberine standard was prepared by dissolving 0.1 mg of berberine in 1 ml methanol. Reference standard 2: Coptis teeta rhizome extract was prepared by refluxing 20 g powdered rhizome with 50 ml Chloroform for 30 minutes; filtered and evaporated completely by water bath and the residue was dissolved in 1 ml methanol. Reference standard 3: Gmelina arborea stem bark extract was prepared by refluxing 20 g powdered stem bark with 50 ml Chloroform for 30 minutes; filtered and evaporated completely by water bath and the residue was dissolved in 1 ml methanol. High performance Thin layer chromatography (HPTLC) was carried out on an Aluminum sheet pre coated with silica gel 60 F254 (E Merck). 5 µl of the samples of RS 1, 2 and 3 were applied as bands of 8 mm length using CAMAG HPTLC system equipped with Linomat - V sample applicator. Win cats software was used for interpreting data. The plates were developed to a distance of 80 mm with Toluene: Ethyl acetate: Formic acid: Methanol (4:4:1:1) as mobile phase in a CAMAG twin trough glass chamber (20 cm x 10 cm) previously saturated with mobile phase vapor.

Physicochemical Evaluation
The Physicochemical parameters obtained for rhizomes of Coptis teeta Wall. are given in Table 1.

HPTLC Studies
High Performance Thin Layer Chromatography analysis of the extract shows that the rhizome of Coptis teeta (T2) shows the Rf value 0.61, which is similar to that of berberine standard (T1). But the sample of Gmelina arborea (T3) shows no corresponding spots at region showing Rf values 0.61. Scanned results also show that the berberine standard peaks at an Rf range 0.55 to 0.65. The same peak is shown in rhizome sample Coptis teeta Wall. but there was no corresponding peaks in Gmelina arborea in the same Rf range.

Table 1: Physico chemical parameters of Coptis teeta Wall.

<table>
<thead>
<tr>
<th>Physico chemical parameters</th>
<th>Results</th>
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<tbody>
<tr>
<td>Foreign matter</td>
<td>Not more than 0.5 %</td>
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<tr>
<td>Total ash</td>
<td>Not more than 3.5 %</td>
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<tr>
<td>Acid insoluble ash</td>
<td>Not more than 0.9 %</td>
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<tr>
<td>Alcohol soluble extractives</td>
<td>Not less than 16.0 %</td>
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<tr>
<td>Water soluble extractives</td>
<td>Not less than 10.0 %</td>
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</tbody>
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Figure 1: Dried rhizome of *Coptis teeta* Wall.
Ct- Cortex, Stc- Stone cells, Sg- Starch grains, Cam- Cambium, Mr- Medullary rays, Pi- Pith

Figure 2: Powder microscopy of *Coptis teeta* Wall.
Powder microscopy: A- Fragments of cork cells, B- Fragments of parenchymatous cells in surface view, C- Stone cells, D- Xylem vessels

Figure 3: HPTLC Finger print of *Coptis teeta* and *Gmelina arborea* with Berberine standard
CONCLUSION
The present study has shown that the pharmacognostic characters which include morphology, anatomy, powder microscopy and HPTLC studies of the dried rhizome. Berberine is one of the active ingredients in Elaneer Kuzhambu for its medicinal properties. From this study it is clear that the presence of berberine in the rhizomes of Coptis teeta is more prominent than in the stem bark of Gmelina arborea. This study will help in laying down pharmacopoeial standards for Coptis teeta Wall. It is also helpful for the further isolation and identification of phyto-constituents of Coptis teeta Wall. This may be useful to the pharmaceutical industries for the authentication of the commercial samples.

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