

## **MACRO- MICROSCOPIC EVALUATION, PHYSICOCHEMICAL ANALYSIS, STANDARDIZATION AND HPTCL FINGUREPRINTING OF ARIL PART OF *KNEEMA ATTENUATA*(WALL).**

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### **ABSTARCT:**

*Kneema attenuata* (wall) family- Myrstickaceae is an evergreen tall aromatic tree found in the evergreen forests of western ghats from Konkan southwards to Travancore. Fruits ovoid, 1-1 ½ inch long with short point or beak, densely furaceously rusty tomentose; the fruit portion and the aril portion have been significantly used in the treatment of various ailments including male infertility according to the folklore healers of Udupi district.

**Key Words:** *Kneema attenuate*, folklore, male infertility

### **INTRODUCTION:**

India is a country with varied number of traditions, religion, customs & laanguages. All these have it's own scientific significance in maintaining the health of individual. Jatiphala (*Myristica fragrens Hoult*) is one such important herbal drug which mainly possess Shukra Stambhaka property<sup>1</sup>. But as per the habitat of western ghat is concerned, there are some wild varieties of Nutmeg by the source plant *Myristica malabarica* commonly called as Ramapatre gida and *Kneema attenuata* also called by the name Raktamara<sup>2</sup>. According to the folkelore healers of Udupi district,

*Kneema attenuata* also possess spermatogenetic activity. With this background, detailed quality control studies of *Kneema attenuata* (wall) were undertaken with the aim of developing standards of authenticity.

### **MATERIALS AND METHODS:**

**Collection and Identification:** Dried aril part of *Kneema attenuata* (wall) were collected from the Agumbe forest region. The plant material was authenticated at pharmacognosy deparment of S.D.M centre for research in Ayurveda and allied sciences Udupi, kuthpady.karnataka.

## **Part B: Methodology**

### **Macroscopy**

The external features of the test samples were documented using Canon IXUS digital camera. The macroscopic features were compared to local flora for authentication.

### **Microscopy**

Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with saffranine. The slides were also stained with iodine in potassium iodide for detection of starch. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.

### **Powder microscopy**

A pinch of powder was warmed with drops of chloral hydrate on a microscopic slide and mounted in glycerine. Slides observed under microscope and diagnostic characters were observed and photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures are indicated by the scale-bars

### **Loss on drying at 105°C**

10 g of sample was placed in tared evaporating dish. It was dried at 105°C for 5 hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample.

### **Total Ash**

2 g of sample was incinerated in a tared platinum crucible at temperature not exceeding 450°C until carbon free ash is obtained. Percentage of ash was calculated with reference to weight of the sample.

### **Acid insoluble Ash**

To the crucible containing total ash, add 25ml of dilute HCl and boil. Collect the insoluble matter on ashless filter paper (Whatmann 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccator for 30 mins and weigh without delay. Calculate the content of acid insoluble ash with reference to the air dried drug.

### **Water soluble ash**

Boil the ash for 5 min with 25 ml of water; collect insoluble matter on an ashless filter paper, wash with hot water, and ignite for 15 min at a temperature not exceeding 450°C. Subtract the weight of the insoluble matter from the weight of the ash; the

difference in weight represents the water soluble ash with reference to the air-dried sample.

#### Alcohol soluble extractive

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled Alcohol (approximately 95%). Shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours, cool in desiccator for 30 minutes and weigh. Calculate the percentage of Alcohol extractable matter of the sample. Repeat the experiment twice, and take the average value.

#### Water soluble extractive:

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled water, shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours. Cool in a desiccator and weigh. Repeat the experiment twice. Take the average value.

#### Volatile oil estimation

Volatile oil in the drug is estimated by distilling the drug with a mixture of water and glycerin in a Clavenger's apparatus.

The distillate was collected in a graduated tube in which the aqueous portion of the distillate is automatically separated and returned to the distilling flask. Volume of the oil collected on the surface of water in the graduated tube was measured. The content of the volatile oil is expressed as a percentage v/w.

#### HPTLC

1g of *Knema attenuata* powder was extracted with 20 ml of alcohol, kept overnight and filtered. 3, 6 and 9 µl of the above extract was applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate: Formic acid (7.0: 3.0: 0.3). The developed plates were visualized in short UV, long UV, and then derivatised with anisaldehyde sulphuric acid reagent and scanned under UV 254nm, 366nm and 620nm. R<sub>f</sub>, colour of the spots and densitometric scan were recorded.

#### Part C: Results

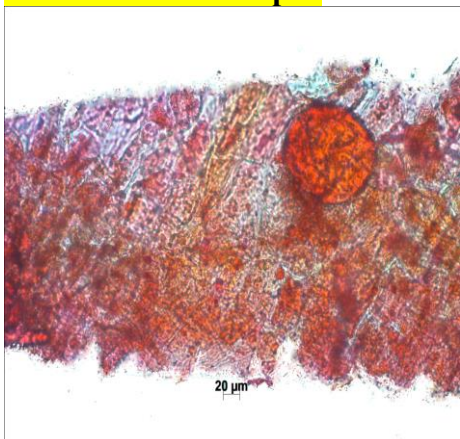
**Figure 1: Macroscopy of *Knema attenuata***





**Figure 2: Microscopy of *Knema attenuata***

**Pa→ SG→ OC→ Ep→**



**Fig 2a: T.S of aril**

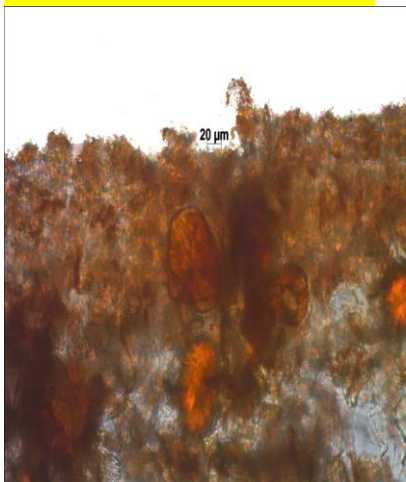
**Pa→ OC→**



**Fig 2b: T.S of aril without staining**

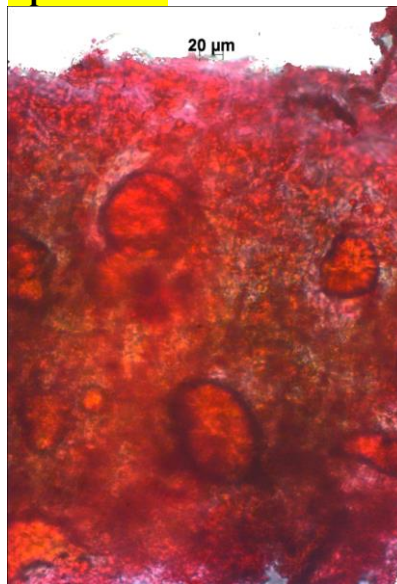
**Ep – epidermis; OC – oil cell; Pa – parenchyma; SG – starch grains;**

**Pa→ CC→SG→ OC→E→**



**Fig 2c: Outer portion enlarged**

**Ep→ OC→**



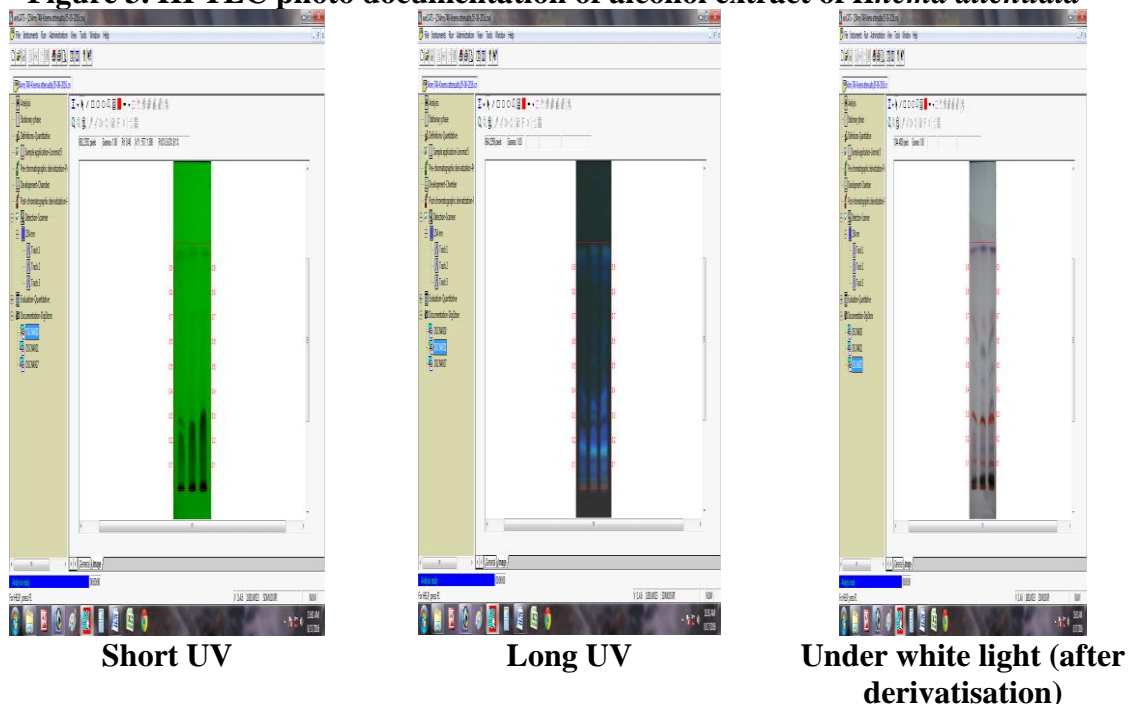
**Fig 2d: An outer Portion stained**

**CC – content cell; Ep – epidermis; OC – oil cell; Pa – parenchyma; SG – starch grains;**

**Table 1. Results of standardization parameters of *Knema attenuata***

Parameter	Results n = 3 %w/w
Loss on drying	22.31
Total Ash	4.05
Acid Insoluble Ash	0.59
Water soluble Ash	1.19
Alcohol soluble extractive value	47.95
Water soluble extractive value	49.50
Volatile oil percentage (%)	NIL

**Figure 3. HPTLC photo documentation of alcohol extract of *Knema attenuata***



Track 1- *Knema attenuata*– 3µl, Track 2–*Knema attenuata*– 6µl , Track 3- *Knema attenuata*– 9µl

**Solvent system- Toluene: Ethyl Acetate: Formic acid (7.0: 3.0: 0.3)**

**Table 2: R<sub>f</sub> values of *Knema attenuata***

At 254nm	At 366nm	At 620nm (after derivatisation)
-	0.05 (F. blue)	-
-	0.08 (F. blue)	-
-	0.11 (F. blue)	0.11 (Orange)
0.14 (D. green)	-	-
-	0.16 (F. blue)	0.16 (Blue)
0.18 (D. green)	0.18 (F. blue)	-
0.22 (D. green)	-	0.22 (Pink)
-	0.25 (F. blue)	-
0.27 (D. green)	-	-
-	0.28 (F. blue)	0.28 (Orange)
-	0.35 (F. blue)	0.35 (Purple)
-	0.39 (F. blue)	-
-	-	0.45 (Purple)
-	0.52 (F. green)	-
0.55 (L. green)	-	0.55 (Purple)
-	0.58 (F. blue)	-
0.66 (L. green)	-	-
-	-	0.68 (Pink)
-	0.88 (F. blue)	-
-	0.93 (F. blue)	-

**\*F – fluorescent; L –light; D – dark**

Figure 4. Densitometric scan of *Knema attenuata*

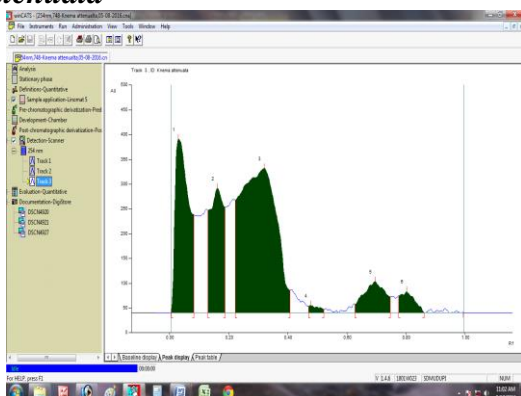


Fig 4a. At 254nm

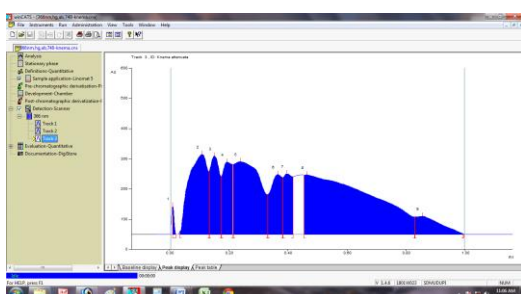


Fig 4b. At 366nm

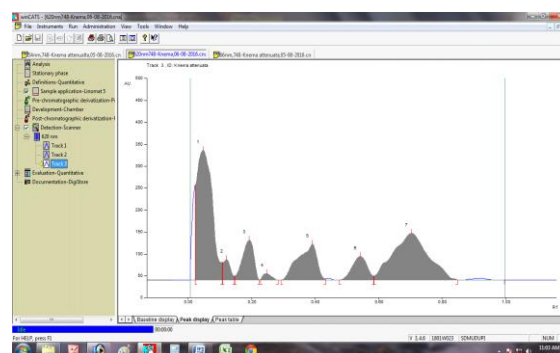


Fig 4c. At 620nm (After derivatisation)

#### Part D: Remarks

Test sample *Knema attenuata* has been standardized as per standard testing protocol. Results of macro-microscopy, standardization parameters and HPTLC (photo documentation, densitometric scan and  $R_f$  values) are presented in respective tables and figures.

#### Discussion:

The seed portion and aril portion of *Kneema attenuata* are well known shukra stambhaka dravyas in folklore system of medicine. Morphological and anatomical standardization of herbal drug needs the information from basic disciplines of plant sciences for identification of plant drug.



Simultaneously for identification of chemical nature of plant in term of physico-chemical analysis, qualitative and quantitative analysis for the detection of active constituents, expertise are required. According to Kunle et.al, Standardization of herbal drug is a series of Protocol which assure the quality, efficiency and safety of plant drug.<sup>3</sup> Macro-microscopic analysis helps in the identification of plant characters anatomically and helps in identification of botanical background. Standardization and authentication of plants was done by evaluating physico chemical testing.<sup>4</sup> The values obtained in the study will serve as constants for quality standard measures for standardization of drugs in the dried form.

High performance thin layer chromatography (HPTLC) serves as the quality assessment tool which helps in identification of variation in chemical composition of plants. TLC identity is a part of every herbal monograph of international standards.<sup>5</sup> HPTLC fingerprinting shows different R<sub>f</sub> values at different wavelengths and reported values can be used as a quality indicating fingerprint for *Kneema attenuate* in the dried form .Findings reported in the present investigations are in support of API and QSIMP2012.<sup>6</sup>

### Conclusion:

Pharmacognostical characterization of *Kneema attenuata* has been done as per pharmacopeial methodology. Present study explores the botanical (in terms of macro microscopic observations) physico-chemical observations (in terms of total ash, AIA, ASA, ASE, USE and loss on drying ) and HPTLC fingerprint profile can serve as excellent standard for the identification and authentication of drug in dried form.

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