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# PHARMACOGNOSTICAL AND QUALITY CONTROL PARAMETERS OF KYLLINGA TRICEPS ROTTB

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## ABSTRACT

The present era witnesses herbal medication as providing better and safer mode of therapy for various diseases without any adverse effects. Kyllinga triceps is an herb belonging to family Cyperaceae. The present study endosses an effort to establish the pharmacognostical and quality control parameters for its standardization. The leaves of kyllinga triceps are as long as the stem, 1/8–1/6 in. broad. There are short, and sheath is thin membranous, pale to purplish in colour. The flower contains 3-4 dark brown leafy bracts at top.

#### INTRODUCTION

Kyllinga triceps is an herb belonging to the family Cyperaceae. It is also known as Nirbishi, Nirvishaa and Mustaa. The root is used as diuretic, refrigerant, demulcent and antipyretic. Traditionally it is used for the treatment of measles, diarrhea and other intestinal infections. Traces of hydrocyanic acid are reported to be present in the root, stems, and nutlets. Rhizome creeping; culms erect, 20-30 cm hight, leafy at the base only; leaves narrow linear, flat, scabrid towards the points; involucres 3-4 leaved, spreading.

Heads solitary, globose, pale; spikelets compressed, 1-flowered; two lower glumes minute, two upper nearly equal, ovate-lanceolate, sharply keeled, mucronate, about7-nerved; stamens 2; nut broadly ovate, finely punctuate, much shorter than the glumes.<sup>5</sup>

The present investigation was undertaken to establish pharmacognostical and phytochemical standards. Pharmacognostical study included Collection, identification & authentication of plant material, Drying & size reduction and

Microscopic evaluation (Transverse section of leaf, stem & root).

# MATERIAL AND METHODS Collection and authentication of Plant

#### Material

The plant (Kyllinga triceps) was collected from Venkateswara University, Tirupati, Andhra Pradesh, India, in the month of April, 2011. The plant was authenticated and identified by Dr. K. Madhava Chetty, Asst. Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. A voucher specimen has been deposited at the College of Pharmacy, TMU, Moradabad (TMU/Consult/2011-12/206) for future reference.

#### SUCCESSIVE SOXHLET EXTRACTION

About 1.0 kg of coarsely powdered drug was subjected to successive extraction in a soxhlet apparatus with solvents of increasing polarity viz petroleum ether (60°-80°C), chloroform, ethyl acetate, methanol and water. The individual extract was filtered, concentrated to a small volume in rotary vacuum evaporator followed by drying in china dishes on hot water bath. The extracts were then weighed individually. The respective percentage yield was calculated as shown in Table-1 and the obtained extracts were further used for phytochemical tests and pharmacological studies.<sup>6</sup>

#### MICROSCOPIC EVALUATION

For Microscopical studies, a portion plant material was stored in a solution containing 5ml formaldehyde, 5ml acetic acid and 90 ml 70% alcohol and the remainder was further size reduced and stored, for further use, in an air tight container.

# MICROSCOPY OF LEAF, STEM & ROOT

Free hand sections were taken for microscopical evaluation and studies were conducted as per standard. Lactophenol was used as clearing agent. Iodine, Potassium iodide (I<sub>2</sub> & KI) & Aniline Blue in lactophenol was used for differential staining along with Phloroglucinol & HCI. Concentrated nitric acid (50%) with a pinch of potassium chlorate was used as the macerating fluid.

#### PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening was performed for alkaloids, glycosides, flavonoids, tannins, etc with different extracts of the plant obtained through successive solvent extraction i.e. petroleum ether, chloroform, ethyl acetate, methanol and aqueous. 10,11 The results of various chemical tests performed therewith were recorded.

# CHEMICAL TESTS FOR DETECTION OF INORGANIC CONSTITUENTS

Ash of drug material was prepared. 50% v/v HCl or 50% v/v HNO<sub>3</sub> was added to ash, kept for 1hr and filtered. The filtrate was subjected to various tests to identify the presence of inorganic constituents. The obtained results were recorded.

#### **RESULTS**

# Phytochemical investigations of *Kyllinga* triceps

## Extractive values using successive extraction

The obtained percentage extractive values were found to be 4.6, 2.08, 1.96, 9.8 and 14.2 % for petroleum ether, chloroform, ethyl acetate, methanol and water respectively as shown in the **Table 1**.

Table 1: Percentage yield of whole plant of *Kyllinga triceps* after successive solvent extraction (Soxhlet Extraction)

| Extracts/Solvent | % Yield (w/w) | Colour      | Odour          | Consistency |
|------------------|---------------|-------------|----------------|-------------|
| Petroleum ether  | 4.6%W/W       | Green       | Characteristic | Waxy        |
| Chloroform       | 2.08 %W/W     | Light green | Characteristic | Waxy        |
| Ethyl acetate    | 1.96%W/W      | Pale yellow | Characteristic | Waxy        |
| Methanol         | 9.8 %W/W      | Dark green  | Characteristic | Sticky      |
| Water            | 14.2%         | Dark brown  | Characteristic | Powder      |

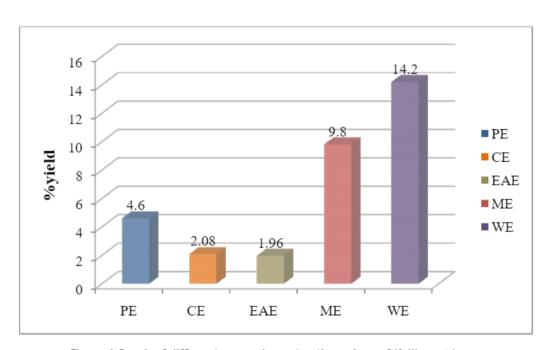


Figure 1 Graph of different successive extractive values of Kyllinga triceps

Where, PE = Petroleum Ether Extract, CE = Chloroform Extract, EAE = Ethyl Acetate Extract, ME = Methanolic

Extract, WE = Water (Aqueous) Extract

# **Preliminary Phytochemical Investigation**

The various extracts i.e. petroleum ether, chloroform, ethyl acetate, methanolic and

aqueous extracts were subjected to preliminary phytochemical screening through qualitative chemical tests.

Table 2: Qualitative chemical analysis of various extracts of Kyllinga triceps

| Test Name                        | Petroleum<br>Ether | Chloroform | Ethyl<br>acetate | Methanol | Aqueous |
|----------------------------------|--------------------|------------|------------------|----------|---------|
| Test for Carbohydrate            | -                  | -          | -                | -        | -       |
| Molish's test                    |                    |            |                  |          |         |
| Fehling's test                   | -                  | -          | -                | -        | -       |
| Benedict's test                  | -                  | -          | -                | -        | -       |
| Barfoed test                     | -                  | -          | -                | -        | -       |
| Selwinoff's test                 | -                  | -          | -                | -        | +       |
| Test for Protein                 |                    |            |                  |          |         |
| Biuret test                      | -                  | -          | -                | -        | -       |
| Millon's test                    | -                  | +          | -                | -        | +       |
| Xanthoprotein test for           | -                  | -          | -                | +        | -       |
| tyrosine and tryptophan          |                    |            |                  |          |         |
| Amino acid                       | -                  | +          | +                | +        | +       |
| Ninhydrine test                  |                    |            |                  |          |         |
| For tyrosine                     | -                  | -          | -                | +        | -       |
| For Cysteine                     | -                  | -          | -                | +        | -       |
| Test for Fats And Oils           | -                  | -          | +                | +        | +       |
| Filter paper test                |                    |            |                  |          |         |
| Test For Steroids                | -                  | +          | +                | +        | +       |
| Salkowski test                   |                    |            |                  |          |         |
| Liebermann's Burchard            | -                  | +          | +                | +        | +       |
| Liebermann's test                | +                  | -          | +                | +        | -       |
| Test For Glycosides              |                    |            |                  |          |         |
| Baljet's test                    | -                  | +          | +                | +        | +       |
| Legal's test                     | +                  | +          | +                | +        | +       |
| Keller's killani test            | -                  | -          | +                | +        | +       |
| Borntrager's test                | -                  | +          | +                | +        | -       |
| Test For Flavonoids              | +                  | +          | +                | +        | +       |
| Shinoda test                     |                    |            |                  |          |         |
| Lead acetate                     | +                  | +          | +                | +        | +       |
| NaoH Test                        | +                  | +          | +                | +        | +       |
| Alkaloids Test                   | -                  | +          | -                | +        | -       |
| Dragendorff's                    |                    |            |                  |          |         |
| Mayer's                          | -                  | +          | +                | +        | +       |
| Hager's                          | -                  | -          | +                | +        | +       |
| Wagner's                         | -                  | -          | -                | +        | +       |
| Murexide test for purine         | _                  | _          | -                | -        | _       |
| Tannins and phenolic             |                    |            |                  |          |         |
| compound (5% Fecl <sub>3</sub> ) | +                  | +          | +                | +        | +       |
| Lead acetate test                | -                  | _          | +                | +        | +       |
| Gelatin test                     | -                  | _          | -                | +        | _       |
| Acetic acid test                 | -                  | _          | -                | -        | _       |
| Potassium dichromate test        | -                  | -          | -                | -        | -       |
| Dil. lodine test                 | -                  | -          | _                | +        | -       |
| Dil. HNO <sub>3</sub> test       | +                  | -          | -                | -        | +       |
| Dil. NH4OH and potassium         |                    | _          | +                | _        | +       |
| ferricyanide test                | _                  | _          | '                | _        | ·       |
| Silver mirror test               | _                  | -          | -                | -        | _       |
| Dil. KMnO4 test                  | +                  | +          | +                | +        | +       |
| Posult: '+' = Present '-' = al   |                    | <u> </u>   |                  | '        |         |

Result: '+' = Present, '-' = absent.

The tests revealed the presence of tannins, carbohydrates, and phenolic compounds, alkaloids, glycosides, flavonoids, steroids, fixed oils and fats as reported in Table 2.

# **Detection of Inorganic constituents**

Various inorganic constituents i.e. iron, sulphate, phosphate & chloride were identified & the obtained results were as shown in table 3.

Table 3 Inorganic Elements of Kyllinga triceps

| Tests     | Treated<br>with HCl | Treated with HNO <sub>3</sub> |
|-----------|---------------------|-------------------------------|
| Calcium   | +                   | +                             |
| Magnesium | -                   | -                             |
| Potassium | +                   | +                             |
| Iron      | +                   | +                             |
| Sulphate  | -                   | -                             |
| Phosphate | +                   | -                             |
| Chloride  | -                   | -                             |

Result: '+' = Present, '-' = absent.

# Microscopic Evaluation of Kyllinga triceps Transverse Section of leaf

The leaf differentiated into the upper adaxial surface which was smooth than that of abaxial lower surface. The adaxial surface contained the large bulliform cells (fig-II)

evident as one of the characteristic features of the most of the grasses. Bulliform cells were somewhat rectangular to polygonal in shape and of varying size. Each side of lamina contained 18-19 vascular bundles dotted from midrib to the margin (fig-II). The abaxial surface contained polygonal epidermal cells of varying sizes. The mesohyll cells were thin walled irregular with no intercellular spaces. The vascular bundles were collateral with phloem towards the abaxial surface and xylem towards the adaxial surface. They were surrounded by bundle sheath (Fig-III). Xylem tissues, specially the vessels were quiet prominent where as the phloem was inconspicuous. The midrib contained bundle of sclerenchymatous cells on either side to provide mechanical strength to the midrib. Xlyem elements and sclerenchymatous cells were lignified and got stained red with phloroglucinol and HCl.

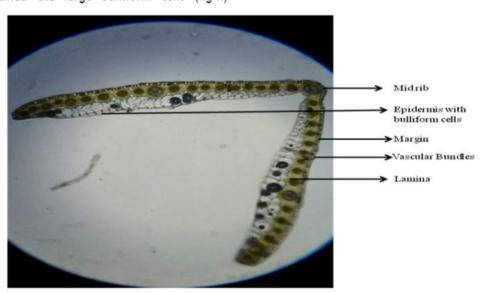


Fig-II: T.S of Entire leaf of Kyllinga triceps magnification at 10X

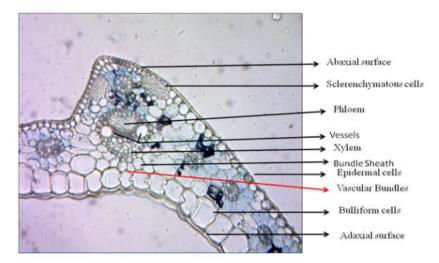


Fig -III: Magnifed T.S of Leaf of Kylling triceps-at 20X

## Transverse Section of stem

The epidermis of kyllinga tricpes was single layered with tangentially elongated cells with no inter cellular spaces. Hypodermis was not present. Scattered patches of sclerenchyma were present near the periphery to give strength to the stem. The ground tissue consisted thin system of parenchymatous cells having well defined intercellular spaces. The ground tissue was not clearly differentiated into cortex, endodermis, pericycle and pith. The vascular bundles were collateral, closed and scattered in the ground tissue. A large number of

vascular bundle lied towards periphery and a few were found scattered in the ground tissue (fig 4&5). Each bundle was covered by a sheath which was more defined towards upper and lower side of the bundle. The bundle consisted of two parts, i.e xylem and phloem. The xylem constituted bigger vessels of metaxylem and smaller vessels of protoxylem as well as tracheids. Phloem consisted of sieve tubes and companion cells. Phloem parenchyma was absent (fig-6). The central part of the stem consisted of parenchymatous cells with air space and lot of intercellular spaces. Starch grains were also seen (fig 4).

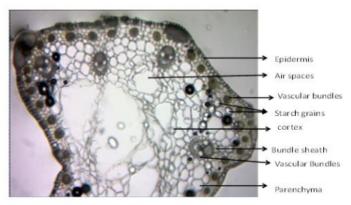


Fig 4. T. S of Stem of Kyllinga triceps

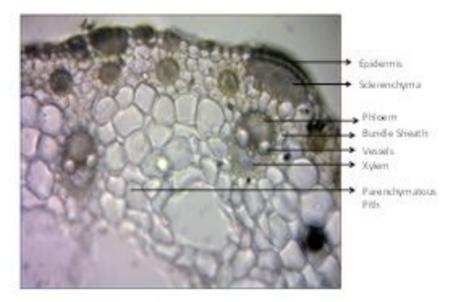


Fig. 5: Enlarged view of more of kyllings tricepi-

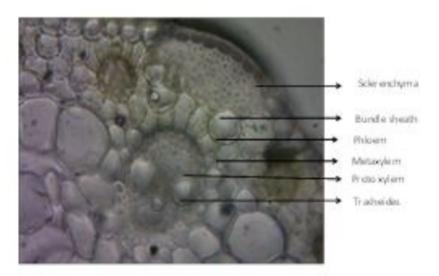


Fig.6 Enlarged view of Viscolar bundle of Kylinga triesps.

# Transverse Section of root

The epidermis was uniserate and composed of compact tubular cells having no intercellular spaces, The root hairs, though normally present, were not found in this section. Just below the epidermis lied a large cortex region consisting of thin walled polygonal parenchyma with very little intercellular spaces between them (Fig-7). Brown coloured tanniniferous cells were found scattered in the cortex region (fig-8). Pericycle was not

clearly seen and differentiated. The vascular tissue consisted of alternating strands of xylem and phloem. The xylem was exarc and the protoxylem lied towards the periphery while metaxylem lied towards the centre (Fig-9). The phloem strands consisted of sieve tubes, companion cells and phloem parenchyma . The parenchymatous and sclerenchymatou tissues were also seen between the xylem and phloem strands. The central part of stele was occupied by well developed pith.

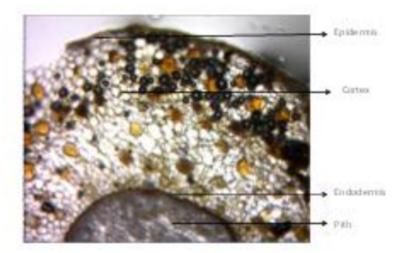


Fig.7: T5 of floot of root of Kytlings triags;

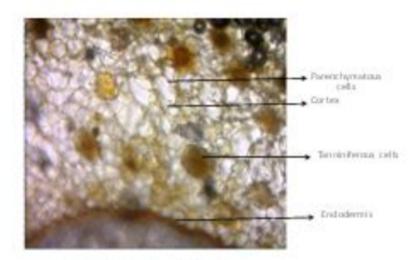


Fig.-8: Enlarged year of Tau ( Xyllings trice):

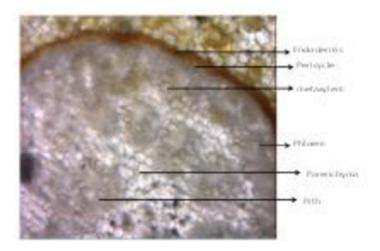


Fig. 8.13 of Boot of Fyllingstrueps showing the pith region.

## DISCUSSION

present study encompassed microscopical and phytochemical investigation of various extracts of Kyllinga triceps (whole plant) to ensure the quality, consistency and therapeutic efficacy, it was felt necessary to develop standardization procedures to provide ease for regulatory authorities to keep a check on the drug adultration and its formulations. It was found that the aqueous extract had the highest (14.2%) yield than the other types i.e. 4.6 % (Petroleum Ether), 2.08% (Chloroform), 1.96% (Ethyl acetate) and 9.8% (Methanolic). Microscopical findings, specially presence of parenchymatous pith. trachides sclerenchyma in stem, tanniniferous cells, metaxylem in the root and lamina, bulliform cells and vascularbundles in leaves.

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