Pharmacopoeia: [1]  

Official drug: [4]  
Nut grass Galingale Rhizome is the dried rhizome of *Cyperus rotundus* L. (Fam. Cyperaceae).

The drug is collected in autumn, burnt off the fibrous roots, boiled briefly or steamed thoroughly and dried in the sun, or dried in the sun directly after burning off the fibrous roots.

Origin: [2, 3, 19]  
Chinese Provinces Guangdong, Sichuan, Henan, Zhejiang, Anhui, Shandong and Hunan.

Descriptions of the drug: [1]  
Frequently fusiform, some slightly curved, 2–3.5 cm long, 0.5–1 cm in diameter. Externally dark brown or blackish-brown, longitudinally wrinkled and with 6–10 slightly prominent annular nodes with brown fibrous roots and broken roots; or slightly smooth and exhibiting indistinct annular nodes on the ones of fibrous roots completely removed. Texture hard, fracture of steamed rhizomes appearing yellowish-brown or reddish-brown, horny: fracture of the unsteamed ones white and starchy, an endodermis ring obvious, stele darkened in colour, with scattered dotted vascular bundles. Odour, aromatic; taste, slightly bitter.

Pretreatment of the raw drug: [1]  
Remove fibrous roots and foreign matter, pound to pieces or cut into thin slices.

Processing: [1]  
*Cyperi Rhizoma (processed with vinegar)*

Stir-bake the pieces or slices of *Cyperi Rhizoma* as described under the method for stir-baking with vinegar (Appendix II D) to dryness.

Medicinal use: [2]  
For the treatment of digestive disorders, vomitus, menstrual disorders, internal bleeding, acute hearing loss, otitis media, migraine, and depression.
Rhizoma Cyperi – Xiangfu

### Effects and indications of Rhizoma Cyperi according to Traditional Chinese Medicine\(^1,4,5\)

<table>
<thead>
<tr>
<th>Taste:</th>
<th>Acrid, sweet, bitter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature:</td>
<td>Neutral, with tendency to cold</td>
</tr>
<tr>
<td>Channels entered:</td>
<td>Orbis hepaticus, orbis lienalis, orbis tricolorii</td>
</tr>
<tr>
<td>Effects (functions):</td>
<td>To remove stagnation of qi, regulate menstruation and relieve pain (2005). To soothe the liver to resolve depression, regulate qi and soothe the middle, regulate menstruation and relieve pain (2010).</td>
</tr>
<tr>
<td>Symptoms and indications:</td>
<td>Stagnation of the liver-qi characterized by distending pain in the chest, hypochondria and epigastrum, indigestion, feeling of stuffiness in the chest and epigastrum, abdominal colic, distending pain in the breast, menstrual disorders, amenorrhea or dysmenorrhoea (2005). Liver depression and qi stagnation, distending pain in the chest and the hypochondrium, pain caused by genital disease, distending pain in the breasts. Spleen-stomach qi stagnation, stuffiness and oppression in the epigastrium and abdomen, pain, distention and fullness, menstrual irregularities, amenorrhea and dysmenorrhoea (2010).</td>
</tr>
</tbody>
</table>

### Main constituents:

- **Sesquiterpenoids**\(^6,7,10,12,17,20\)
  - Epi-guaidiol A, sugebiol, guaidiol A, sugetriol triacetate, cyperenoic acid, cyperotundone, rotundines A-C
- **Norsesquiterpenes**\(^7\)
  - norcyperone
- **Essential oil**\(^9,10-13,17,20\)
  - α-cyperone, β-cyperone, cyperol, isocyperol, cyperene, cyprotene, cyperotundone, cypera 2,4-diene, caryophyllene, rotundine, α-copaene, α-selinene, epi-α-selinene, β-selinene, rotundene, valercene, ylanga-2,4-diene, γ-gurjune, trans calamenene, δ-cadinene, γ-calacorene, α-murolene, γ murolene, cadalene, nootkatene, mustakone, α-copaene, isolongifolen-5-one + γ-gurjuneneoxide, (E)-pinocarveol, myrtenal, dihydrocarvone, verbenone, (E)-carveol, valencene
- **Flavonoids**\(^8,12-14,17\)
  - Vitexin, isovitexin, orientin, eporientin
- **Cardiac glycosides**\(^12,13,17\)
- **Alkaloids**\(^15\)
- **Saponins**\(^15\)
Fig. 1: Formulae of the main compounds of Rhizoma Cyperi [10]

Reported Pharmacological Activities

- Anti-inflamatory\textsuperscript{[6, 7, 12, 13, 15, 17, 20]}
- Anti-estrogenic activity\textsuperscript{[3, 7, 14]}
- Antimicrobial\textsuperscript{[14, 16]}
- Anthelmintic\textsuperscript{[7, 14]}
- Anti-histaminic\textsuperscript{[14]}
- Anti-emic\textsuperscript{[7, 14]}
- Antipyretic\textsuperscript{[7, 12–15, 17, 20]}
- Antidiabetic\textsuperscript{[6, 7, 14, 20]}
- Anti-diarrhoeal activity\textsuperscript{[3, 7, 20]}
- Antimalarial\textsuperscript{[7, 15, 16, 20]}
- Antispasmodic\textsuperscript{[17]}
- Hepatoprotective\textsuperscript{[7]}
- Acetylcholinesterase inhibitory activity\textsuperscript{[6]}
- Protein glycation inhibitory activity\textsuperscript{[6]}
- Antidepressant\textsuperscript{[20]}
- Inhibition of nitric oxide and superoxide production\textsuperscript{[6, 20]}
- Hypotensive\textsuperscript{[7, 12, 13, 17]}
- Aphrodisiac\textsuperscript{[7]}
- Diuretic\textsuperscript{[7]}
- Sedative\textsuperscript{[7, 17]}
- Carminative\textsuperscript{[7]}
- Anticolic\textsuperscript{[7]}
- Stomachic\textsuperscript{[7]}
- Removes renal calculi\textsuperscript{[7]}
- Emmenagogue activity\textsuperscript{[16]}
Rhizoma Cyperi – Xiangfu

TLC-Fingerprint Analysis

<table>
<thead>
<tr>
<th>Drug samples</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Rhizoma Cyperi/Cyperus rotundus</td>
<td>Sample of commercial drug obtained from HerbaSinica (origin: Zhejiang)</td>
</tr>
<tr>
<td>2 Rhizoma Cyperi/Cyperus rotundus</td>
<td>Sample of commercial drug obtained from Herbasin (origin: unknown)</td>
</tr>
<tr>
<td>3 Rhizoma Cyperi/Cyperus rotundus</td>
<td>Sample of commercial drug obtained from TCM-Clinic Bad Kötzting (origin: unknown)</td>
</tr>
<tr>
<td>4 Rhizoma Cyperi/Cyperus rotundus</td>
<td>Province Shandong (China)</td>
</tr>
<tr>
<td>5 Rhizoma Cyperi/Cyperus rotundus</td>
<td>Province Hebei (China)</td>
</tr>
<tr>
<td>6 Rhizoma Cyperi/Cyperus rotundus</td>
<td>Province Anhui (China)</td>
</tr>
</tbody>
</table>

Reference compound: Fig. 2a and 2b

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>α-Cyperone</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Reference compound: Fig. 2c and 2d

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>α-Cyperone</td>
<td>0.34</td>
</tr>
</tbody>
</table>

1. Extraction: 2 g powdered drug are extracted with 20 ml methanol for 1 h under reflux, filtered and evaporated to dryness. The residue is dissolved in 1 ml methanol.

2. Reference compound: 1 mg is dissolved in 1 ml ethyl acetate

3. Separation parameters:
   - Plate: HPTLC Silica gel 60 F254, Merck
   - Applied amounts: Rhizoma Cyperi extracts: each 10 μl
                     Reference compound: 10 μl
   - Solvent system: Toluene + ethyl acetate + glacial acetic acid (92+5+5)
Detection:

1. Without chemical treatment *(Fig. 2a)*

254 nm

2. Dinitrophenylhydrazine reagent *(Fig. 2b)*

1.5 g 2,4-dinitrophenylhydrazin are dissolved in 20 ml sulphuric acid (25%), filled up with water to 100 ml and filtered.

After spraying with 10 ml, the plate is evaluated after 10 min in VIS.

3. Anisaldehyde – Sulphuric acid reagent *(Fig. 2c and 2d)*

0.5 ml anisaldehyde is mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid, in that order.

The plate is sprayed with 10 ml, heated at 100 °C for 5 min, then evaluated in VIS and under 366 nm.

Note: The reagent has only limited stability and is no longer useable when the colour has turned to red-violet.

4. Description:

*[Fig. 2a]*: Thin layer chromatogram of the methanol extracts of Rhizoma Cyperi without chemical treatment (UV 254 nm)
Fig. 2b: Thin layer chromatogram of the methanol extracts of Rhizoma Cyperi sprayed with 2,4-dinitrophenylhydrazine (VIS)

Figure 2a shows the six samples of Rhizoma Cyperi under UV 254 nm without chemical treatment. In all samples several black zones are detectable in the Rf range from the start up to 0.5. The main zone at Rf = 0.41 (T) could be identified as α-cyperone. The second zone at Rf = 0.39 might be β-cyperone.

After spraying with 2,4-dinitrophenylhydrazin (Fig. 2b) the zones appeared in yellow/orange colours. In all samples the orange spot of α-cyperone at Rf = 0.41 is clearly detectable.

Fig. 2c: Thin layer chromatogram of the methanol extracts of Rhizoma Cyperi sprayed with Anisaldehyde – Sulphuric acid (VIS)
Fig. 2d: Thin layer chromatogram of the methanol extracts of Rhizoma Cyperi sprayed with Anisaldehyde – Sulphuric acid (UV 366 nm)

Fig. 2c and d: With the solvent system generally used for essential oils several pink and violet zones from the start up to R_f=0.85 are detectable. In VIS (Fig. 2c) α-cyperone is not exactly distinguishable, but under UV 366 nm (Fig. 2d) the compound can be detected by a light blue coloured spot at R_f=0.34.

HPLC-Fingerprint Analysis [18]

1. Sample preparation: 2 g powdered drug are extracted with 20 ml methanol for 1 h under reflux, filtered and evaporated to dryness. The residue is dissolved in 1 ml methanol and filtered over Millipore® filtration unit, Type 0.45 μm.

2. Injection volume: Rhizoma Cyperi extract: each 10.0 μl

3. HPLC parameter:
   - Apparatus: MERCK HITACHI D-6000 A Interface
     MERCK HITACHI L-4500 A Diode Array Detector
     MERCK HITACHI AS-2000 Autosampler
     MERCK HITACHI L-6200 A Intelligent Pump
   - Separation column: LiChroCART® 250-4 LiChrospher® 100 RP-18 (5 μm), Merck
   - Precolumn: LiChroCART® 4-4 LiChrospher® 100 RP-18, Merck
   - Solvent: A: water (Millipore Ultra Clear UV plus® filtered)
     B: methanol (VWR)
   - Gradient: 10–100 % B in 45 min, total runtime: 45 min
   - Flow: 1 ml/min
   - Detection: 254 nm
Rhizoma Cyperi – Xiangfu

Retention times of the main peaks recorded at 254 nm

<table>
<thead>
<tr>
<th>Peak</th>
<th>Rt (min)</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40.8</td>
<td>β-Cyperone ?</td>
</tr>
<tr>
<td>2</td>
<td>43.2</td>
<td>α-Cyperone</td>
</tr>
</tbody>
</table>

4. Description of the HPLC-Figures

In the Rt – range 27.0–39.0 there a several minor peaks in both samples. The two main peaks at Rt 40.8 and 43.2 can be assigned to β- and α-cyperone, respectively.

Fig. 3a: HPLC-fingerprint analysis of the methanol extract of Rhizoma Cyperi, sample 2

Fig. 3b: HPLC-fingerprint analysis of the methanol extract of Rhizoma Cyperi, sample 6
**Note:** Rhizoma Cyperi should contain not less than 1.0% of volatile oil, according to the Chinese Pharmacopoeia [1].

**Conclusion**

The identity of Rhizoma Cyperi can be easily determined by TLC- and HPLC-analysis using MeOH-extract or essential oil by means of the characteristic $\alpha$-$\beta$-cyperone dublett in HPLC.

**References**

Rhizoma Cyperi – Xiangfu


19. Zhao, Z.Z.: An illustrated Chinese materia medica in Hong Kong. School of Chinese Medicine, Hong Kong Baptist University, Hong Kong (2004)

Chromatographic Fingerprint Analysis of Herbal Medicines Volume III
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