

**DIURETIC ACTIVITY OF QUERCETIN ISOLATED FROM
CANSJERA RHEEDII J.GMELIN (OPILIACEAE)**

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ABSTRACT

Quercetin (3, 5, 7, 3', 4'-Pentahydroxy flavone) isolated from the aerial parts of Cansjera rheedii J.Gmelin (Opiliaceae) has been tested for diuretic activity in rats. The parameters observed for each individual rat included body weight before and after test period, total urine volume (corrected for water intake during the test period), urine concentration of Na⁺, K⁺ and Cl⁻. The values of urine volume and cation excretion were increased with reference to saline control by quercetin (100mg per kilogram of body weight). Furesemide was used as a reference diuretic.

Keywords: *Cansjera rheedii, Diuretic activity, Flavonoids, Caffeic acid, Ferulic acid, Quercetin, Quercetin-3-O-β-D-glucoside, Rutin, Furesemide.*

INTRODUCTION

Cansjera rheedii J Gmelin (Opiliaceae) is a climbing shrub, sometimes armed, generally found in India through Malaya to Hong Kong and North Australia^[1-2]. The tribes of Nilgiris in Tamil Nadu, India using the plant extract for the treatment of post-natal pain^[3], intermittent fever^[4] and poisonous bites and skin diseases^[5]. In our earlier studies, the ethanol extract of aerial parts of *C.rheedii* has been reported to have hepatoprotective^[6], cytotoxic^[7], anthelmintic^[8], anti-inflammatory and membrane stabilizing property^[9], antipyretic^[10], anti-nociceptive^[11] and diuretic^[12] activities. The safety of this plant has also been proved by studying acute and sub-acute toxicity studies^[13]. The compounds such as 3, 4-dihydroxy cinnamic acid (Caffeic acid) (I), 4-hydroxy 3-methoxy cinnamic acid (Ferulic acid) (II), 3, 5, 7, 3', 4'-pentahydroxy flavone (Quercetin) (III), 5, 7, 3', 4'-tetrahydroxy -3-O-β-D-glucopyranosyl flavones (Quercetin-3-O-β-glucoside) (IV) and 5, 7, 3', 4'-tetrahydroxy-3-O-(6-O-α-L-rhamnopyranosyl)-β-D-glucopyranosyl flavone (Quercetin-3-O-β-rutinoside (or) Rutin). Structures of all these compounds were established by spectral and chemical methods^[14]. This was the first report of the above 5 compounds from the plant. The present study is focused on evaluation of diuretic activity of quercetin(III) isolated from aerial parts of *Cansjera rheedii* J.Gmelin (Opiliaceae).

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EXPERIMENTAL

Extraction and isolation

The air dried and coarsely powdered aerial parts (1.0Kg) were extracted with boiling 95% ethanol (3 X 5l) and the extract was concentrated to about 250 ml. The insoluble green residue was removed by filtration and the soluble in the filtrate (150 ml) were fractionated into C₆H₆, Et₂O, EtOAc and EtCOMe¹⁵. The C₆H₆ fraction after concentration yielded a pale yellow needle, recrystallized from MeOH and designated as compound **I** (910mg). The Et₂O concentrate was column chromatographed over sephadex LH-20 using MeOH. 35 fractions of 50ml each were collected. Fraction 4-29 gave colourless needles, recrystallized from MeOH and designated as compound **II** (800mg). The EtOAc concentrate was column chromatographed over Sephadex LH-20 using MeOH. 44 fractions of 50ml each were collected, fractions 7-32 gave yellow needles, recrystallized with MeOH and designated as Compound-**III** (1.1g). The EtCOMe concentrate was chromatographed on a column of Sephadex LH-20 using MeOH as eluent. 107 fractions of 50ml each were collected, fractions 6-35 deposited a homogenous yellow solid recrystallized from MeOH and designated as compound-**IV** (89mg). Fractions 36-98 gave a pale yellow homogenous solid, recrystallized from MeOH and were designated as compound-**V** (530mg). Quercetin isolated from aerial parts of *Cansjera rheedii* (Opiliaceae) was dissolved in 25ml/Kg of normal saline for the present study. Male albino rats with body weight between 140-170g supplied by the King Institute, Guindy, Chennai, were used for the study.

Characterization of Compound-III (3, 5, 7, 3', 4'-pentahydroxy flavone : quercetin):-

Compound **III**, C₁₅H₁₀O₇, Yellow needles, mp.305.83°C, gave yellow colour with NH₃, Na₂CO₃ and NaOH, pink with Mg-HCl and olive green with Fe³⁺ [16]. It was yellow under UV and UV/NH₃. R_f characteristic of flavonoid aglycone and had λ_{max} (MeOH) 256, 271sh, 305sh, and 373 nm. A Characteristic bathochromic shift in band II of NaOAc spectrum with decomposition of band I suggested free 3', 7 and 4' -OH groups. A bathochromic shift in band II of NaOAc spectrum (10nm) suggested free -OH at C-7. A bathochromic shift in NaOH spectrum with fast decomposition showed the presence of 3', 4'-OH. A bathochromic shift of 53nm in band I of AlCl₃ / HCl spectrum was indicative of the presence of 3 and / or 5-OH groups. A hypsochromic shift of 21nm in band I of AlCl₃/HCl spectrum compared to AlCl₃ spectrum indicated orthodihydroxy in ring B, which was further supported by 53 nm bathochromic shift of band I in CH₃COONa/ H₃BO₄ spectrum. Further the ¹H NMR spectrum showed signal for five (5, 7, 4', 3 and 3') OH protons at δ 12.45, δ 10.75, δ 9.57, δ 9.37 and δ 9.29 ppm besides giving the characteristic -chemical shift and splitting pattern expected for the five (2', 6', 5', 8 and 6) aromatic protons at δ 7.65, δ 7.50, δ 6.87, δ 6.38 and δ 6.16ppm. Further the ¹³C NMR spectrum showed signals for five (C-7, C-5, C-4', C-3'and C-3) carbons with OH at δ 164.39, δ 161.24, δ 147.30, δ 145.57 & δ 136.26 ppm and C=O carbon at δ 176.35 ppm in addition to the other characteristic chemical shift for carbon at δ 156.60 (C-9), δ 121.92 (C-6'), δ 121.60 (C-1'), δ 116.48 (C- 5'), δ 115.71 (C-2'), δ 103.9 (C-10), δ 94.10 (C-6) and δ 93.40 (C-8). The compound had characteristic IR absorption frequencies at 3387 (Phenolic-OH), 1662 (C=O), 1608 (C=C). The compound yielded a penta acetyl derivative with mp.194.27°C and penta methyl ether mp.151.27 °C agreeing with the values reported. These properties led to the identification of the compound (**III**) as 3, 5, 7, 3', 4'- pentahydroxy flavone (quercetin) (Figure 1). The identity was further confirmed by co-PC, Mass, NMR and IR Spectrum data obtained from literature [17-21].

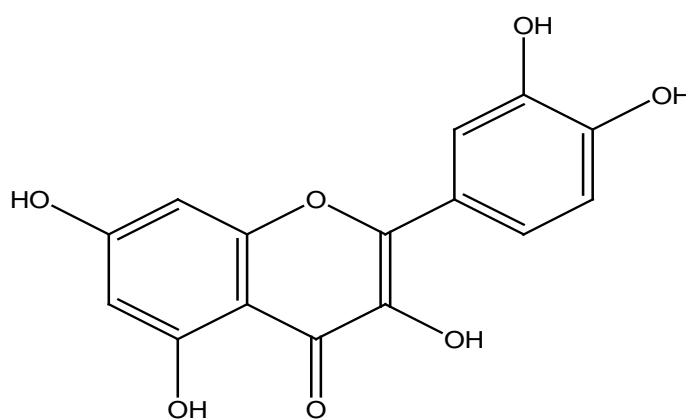


Figure-1: Structure of Quercetin (3, 5, 7, 3', 4'- pentahydroxy flavones)

Diuretic activity

The method of Lipschitz et al²² was employed for the assessment of diuretic activity. Groups of 6 male albino rats, each weighing 140-170g were kept fasted and deprived of water for 18 hours prior to the experiment. On the day of experiment, animals were given normal saline orally 25ml/kg of body weight in which the Furosemide and quercetin were dissolved. Control animals received saline only. Immediately after the dosing (100mg/kg), the rats (three in each cage) were placed in metabolic cages²³ specially designed to separate urine and faeces and kept at room temperature of 25±0.5°C. The urine was collected in measuring cylinders upto 5 hrs, after dosing. During this period, no food or water

was made available to animals. The total volume of urine collected was measured for both control and treated groups. The parameters observed for each individual rat were, body weight (before and after test period), total urine volume (corrected for water intake during the test period), urine concentration of Na⁺, K⁺, and Cl⁻. Where applicable, values were measured before and after the actual experiment. Overall effects of Quercetin on excretory parameters are mentioned under the table-1.

Table-1: Effect of Quercetin on excretory parameters

Measured excretory Parameters (Electrolytes)	No. of animals	Saline control (25ml/kg)	Furosemide control (100mg/kg)	Quercetin (100mg/kg)
Total volume of Urine(ml)	6	1.92±0.12	4.47±0.24	5.29±0.98
Total Sodium (Mcg moles/kg)	6	2026±48	3220±68	3458±78
Total Potassium (Mcg moles/kg)	6	846±42	2088±408	1956±610
Total Chloride (Mcg moles/kg)	6	714±32	2458±112	2112±108

Analytical Procedure

Na⁺ and K⁺ concentrations were measured by flame photometry and Cl⁻ concentration is estimated as sodium chloride by titration with silver nitrate solution (2.906g/l) using one drop of 5% potassium chromate solution as indicator²⁴.

Reference Diuretic

Furosemide-sodium salt was administered by stomach tube. Optimal dose-activity relation was found to be 100 mg Furosemide per kg body weight in a series of supportive experiments.

RESULTS

The quercetin isolated from *Cansjera rheedii* J.Gmelin (Opiliaceae) is active as diuretic in rodents. The data given in the table supports the conclusion that the extracts act as aquaretic. The values of urine volumes are elevated. The cation excretion is increased. The significant alteration of cation excretion is observed in quercetin treated animals, which is nearly equivalent to Furosemide control. A very high increase for the Cl⁻ excretion was also observed in the same range as with Furosemide.

DISCUSSION

The results clearly show that quercetin isolated from *Cansjera rheedii* J.Gmelin (Opiliaceae) enhances considerably the urine excretion equivalent to Furosemide control values. These findings may provide a lead for further investigation of the overall pharmacological actions of *Cansjera rheedii* J.Gmelin (Opiliaceae) in more appropriate models.

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