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# DIURETIC ACTIVITY OF RUTIN ISOLATED FROM CANSJERA RHEEDII J.GMELIN (OPILIACEAE)

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## ABSTRACT

Rutin 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 rutin (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 present study is focused on evaluation of diuretic activity of rutin isolated from aerial parts of *Cansjera rheedii* J.Gmelin (Opiliaceae).[14]

## 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 fractioned into $C_6H_6$, $Et_2O$, EtOAc and EtCOMe.[15] Fractions 36-98 gave a pale yellow homogenous solid, recrystallized from MeOH and were designated as compound (530mg). Rutin isolated from aerial parts of *Cansjera rheedii* (Opiliaceae) was dissolved in 25ml/Kg of normal saline and male albino rats with body weight between 140-170g supplied by the King Institute, Guindy, Chennai, were used for the study.

### Characterization of Compound Quercetin-3-O-β-D-rutinoside (Rutin)

Rutin ($C_{27}H_{30}O_{16}$) had UV $\lambda_{max}$ (MeOH) 257, 300sh, 359 nm and $R_f$ typical of flavonol glycoside. The compound on acid hydrolysis[16] with 2N HCl yielded an aglycone and two different monosaccharides in equimolecular ratio. The aglycone was found to be identical with compound Quercetin and sugars as D-glucose and L-rhamnose by $R_f$ values and co-PC with authentic samples. Glycosylation of 3-OH was inferred by the different UV fluorescence of the glycoside (Purple) from the aglycone (Yellow). The presence of free 7-OH was indicated by the bathochromic shift of 11nm in band II of $CH_3COONa$ spectrum compared to MeOH spectrum. A bathochromic shift of 70 nm in band I of $AlCl_3$ spectrum relative to the MeOH spectrum indicated the presence of free 5-OH. A bathochromic shift of 41 nm in band I of $CH_3COONa$ /H $_3BO_4$ revealed the presence of ortho dihydroxyl groups in ring B. Further evidence of free 3' and 4'-OH was obtained from a hypsochromic shift of 28nm in band I of $AlCl_3$/HCl relative to $AlCl_3$ spectrum. On $H_2O_2$ oxidation of compound[17] yielded a disaccharide, identified by Rf. It was not affected by enzyme β-glucosidase indicating that glucose was not the terminal sugar. Mass spectrum (positive and negative) showed peaks at 634 ($MH + N_a^+$, 30), 633 ($M + N_a^+$, 100), 611 ($MH^+$, 45), 465 ($MH^+$ -rhamnose, 10), 303 ($MH^+$-rutinose, 25) corresponding to a molecular formula $C_{27}H_{30}O_{16}$ and other fragment ions characteristic

of the attachment of rhamnosyl-glucose residue to the aglycone quercetin. The $^{1}H$ NMR spectrum exhibited signals corresponding to aromatic protons at 6, 8, 2', 5' and 6' and OH groups at 5, 7, 3' and 4'. The characteristic signals in the aliphatic region were assigned to the anomeric proton and other sugar protons showing the compound as a diglycoside of 3,5,7,3', 4'-penta oxygenated flavone. The position of inter linkage can be made by $^{1}H$ and mainly[13] C NMR have been employed to characterize the sugars. Thus the $^{1}H$ NMR spectrum exhibited a doublet at δ 5.35 (H-1'', J=3.0 Hz) and a broad multiplicate at δ 0.95 (H-6''', J=6.1Hz) clearly indicated the presence of rhamnose and its attachment to C-6 of glucose. The site at which a second sugar attached to the sugar of a flavonoid mono-O-glucoside is readily determined by[13] C NMR spectroscopy and this is perhaps the most significant information contained in the spectrum, as it is difficult to obtain it by other methods. A sizable downfield shift of δ 5.9 ppm in the resonance of C-6 of glucose carbon and up field shift of δ 0.5 ppm in the resonance of C-5 of glucose carbon and without affecting the rest of the spectrum compared to quercetin-3-O-β-D-glucoside clearly indicated that terminal sugar rhamnose is attached to C-6 of glucose. This fact was further proved beyond doubt on the basis of HMBC & HSQC data- Connecting rhamnose H-1 (δ 4.35) to C-6 of glucose (67.52 ppm). Similarly, connectivity between H-1 of glucose (δ 5.35 with C-3 of quercetin (133.81 ppm) was also established. Thus the structure of compound was established as 5,7,3',4'-tetrahydroxy 3-O-(6-O-α-L-rhamnopyranosyl) –β –D- glucopyranosyl flavone (or) Quercetin-3-O-β-D-rutinoside (Rutin) (Figure 1). The identity was further confirmed by co-PC, Mass, NMR and IR Spectrum data obtained from literature.[18-21]

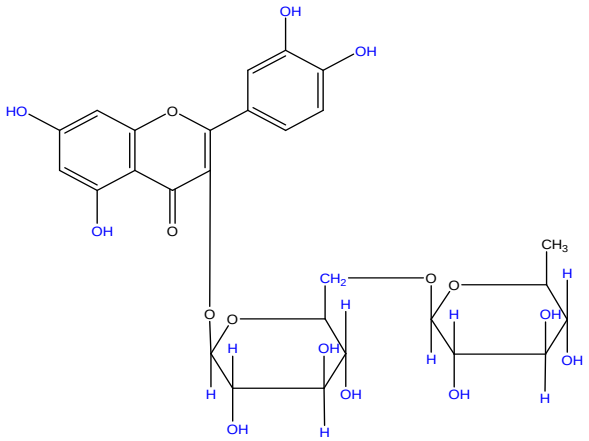


**Figure-1: 5, 7, 3', 4'-tetrahydroxy 3-O-(6-O-α-L-rhamnopyranosyl)–β –D-glucopyranosyl flavones (or) Quercetin-3-O-β-D-rutinoside (or) Rutin.**

**Diuretic activity**

The method of Lipschitz et al,[22] 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 rutin were dissolved. Control animals received saline only. Immediately after the dosing (100mg/kg), the rats (three in each cage) were placed in metabolic cages,[23] 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 rutin on excretory parameters are mentioned under the table-1.

**Table 1: Effect of rutin on excretory parameters.**

| Measured excretory Parameters (Electrolytes) | No. of animals | Saline control (25ml/kg) | Furosemide control (100mg/kg) | Rutin (100mg/kg) |
|---|---|---|---|---|
| Total volume of Urine(ml) | 6 | 1.88±0.14 | 4.82±0.22 | 5.48±0.94 |
| Total Sodium (Mcg moles/kg) | 6 | 2018±48 | 3240±68 | 3462±70 |
| Total Potassium (Mcg moles/kg) | 6 | 842±44 | 2078±408 | 1972±612 |
| Total Chloride (Mcg moles/kg) | 6 | 718±38 | 2462±110 | 2218±110 |

**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 indicator.[24]

**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 rutin 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 result reveals that rutin isolated from *Cansjera rheedii* J.Gmelin (Opiliaceae) has the diuretic activity on par with the furosemide control values.

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