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Plant flavonoids as inhibitors of calcium oxalate urolithiasis

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Abstract. Flavonoids, including flavones, flavonol and their glycosides, showed inhibitory activity on the growth of calcium oxalate crystals. Kaempferol and luteoline exhibited the highest inhibitory activity among the nine flavonoids studied. Results on the monitoring of the urine of patients suffering from kidney stones who were given meshed juice of *Raphanus sativus* are promising, and studies are on-going for a larger sample population.

Abstrak. Flavonoid-flavonoid, termasuk flavon, flavonol dan glikosidanya, menunjukkan aktiviti perencatan terhadap pertubuhan hablur kalsium oksalata. Kaemperol dan luteolina menunjukkan aktiviti perencatan yang tertinggi antara sembilan flavonoid yang dikaji. Hasil dari pengawasan air kencing pesakit batu buah pinggang yang diberi jus kisar *Raphanus sativus* adalah menggalakkan, dan kajian sedang dijalankan atas sampel populasi yang lebih besar.

Introduction

Metabolic or environmental disturbance that alters the biochemical-physicochemical balance is the most common characteristic of a urinary stone-forming environment. The absence or low concentration of inhibitory substances in the urinary system such as magnesium, pyrophosphate, citrates, phosphocitrates, diphosphonate, mucoproteins and various peptides is cited as the major causes of stone formation [1]. The prevention of a recurrence, the efficacy of a prophylactic program, the inhibition of spontaneous passage, the reduced need for stone removal and cost effectiveness justify the medicinal approach for treating the disearse, unlike the surgical approach, which involves the removal of stones.

Natural products are used to treat the urinary calculi of the renal system; studies of local plants used traditionally in for such a purpose confirm the presence of flavonoid. In the present study, flavonoids from a local plant, lobak putih, *Raphanus sativus*, were tested for the inhibition of calcium oxalate crystal formation.

Experimental

Urine from normal (N), stone former (SF) and recurrent stone former (RSF) patients were obtained from the Urology Department, Penang General Hospital. Precautions were taken to ensure minimum contamination or deterioration. Schneider's gel slide method [2] was modified by coating the slide with 1.5 mL of 1% purified agar; this was divided into three equal parts. In each division, 4 wells of 3 mm diameter were made by vacuum suction; 2 wells were made 1.25 cm apart along the horizontal axis of the slide and two others were made 1 cm opposite each other along the perpendicular axis.

A 5 μL quantity of of 0.2 M calcium chloride and an identical volume of 0.2 M amonium oxalate volume were pipetted into the opposite wells along the longer axis; 5 μL of undiluted urine and 5 μL of the inhibitor solution were then pipetted into the remaining wells. The

inhibitor solutions were 100 ppm solutions of various local plant flavonoids in dissolved in dimethyl sulphoxide (DMSO). A solution of 10 ppm methylene blue were used as a comparison solution. The size of the calcium oxalate crystals that formed in the gel slide was measured by using a Quantimet Image Analyser (Q520) at 2, 4, and 24 hours intervals. The clinical studies involved 4 selected recurrent stone former patients who were required to drink extracts of *Raphanus sativus* every morning for several days. Urine samples were collected after the drinks were consumed for at least 3 days. The urine was tested in the usual manner and the surface area of the individual crystals was measured after 24 hours. The efficacy of this treatment was observed by comparing the results before and after the intake of *R. sativus* extracts.

Results and discussion

The calcium oxalate inhibition index for the N, SF and RSF patients was negative relative to the control, which as distilled water in place of urine. The relative difference between the size of the crystals in N and RSF urine compared with their controls was insignificant ($P > 0.1$) whereas the SF urine showed a significant difference ($P > 0.001$). This finding is consistent with our earlier reported studies [1].

In the presence of flavonoids, kaempferol(86.8%) and luteolin (84.5%) exhibited the highest inhibitory activity followed by quercetin (82.1%). Morin (76.8%), quercetin-3-*O*- rhamnoside (73.0%), fisetin (66.4%) and rutin (64.2%) (Table 1). Calcium oxalate crystallisation inhibition is related to the ability of complex formation or crystal poisoning by chelators like methylene blue. This ability was exhibited by flavonoids present in a number of medicinal plants used for problems related to kidney stones [3].

Flavonoids with a free 3-OH group complexed sufficiently strongly with calcium ions to result in a bathochromic shift in the UV spectra; the effect of which is reversed in the presence of HCl. Compounds with alkylated 3-OH group or without a C-3 substituent do not show any change in the UV spectrum on addition of the calcium chloride solution. Compounds like such as luteolin, quercetin-3-*O*-rhamnosode, kaempferol-3-*O*-glycoside, chrysoeriol and rutin have a free 5-OH group and some have *ortho*-dihydroxy groups in the B-ring. These groups do not respond to calcium ions, which suggests that calcium chloride may be specific for detecting free 3-OH and as it probably induces a complex formation [4]. Even though flavonoid glyosides do not possess a free 3-OH, the results indicated that some degree of Ca^{2+} complexation does possibly occur in other free-OH positions like C-5 and *ortho*-dihydroxy groups, as shown for Al^{3+} complexation [5].

Selected patients with recurrent stone forming tendencies who were given extracts of *Raphanus sativus* showed inducements of diuretic effects and changes in urine colour and appearance. The size of the calcium oxalate crystals grown *in vitro* in urine was significantly reduced after the intake of the extracts. Quercetin present in the *Raphanus sativus* extracts [6] might be responsible for this activity.

Flavonoids isolated from local plants used traditionally have a good potential in inhibiting the growth of calcium oxalate grown under various conditions. Kaempferol and luteolin exhibited the highest inhibitory activity amongst the flavonoids studied. Extracts of *Raphanus sativus* taken by recurrent stone former patients exhibited good inhibition of calcium oxalate crystals growth; the extracts might serve as a useful prophylactic agent for calcium oxalate urolithiasis. Further studies are needed with a larger sample population to confirm this conclusion.

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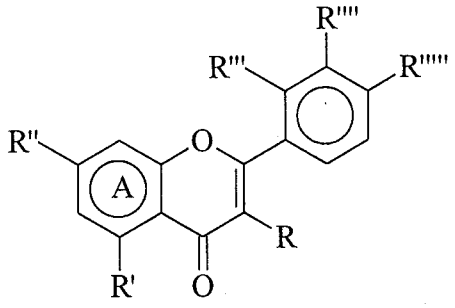
Table 1. Inhibition of calcium oxalate crystallisation flavonoids



Compound (100 ppm)		R	R' (C3)	R'' (C5)	R''' (C7)	R'''' (C2')	R''''' (C3')	(C4')	Mean Area ± S.D.
1	Quercetin		OH	OH	OH	H	OH	OH	9.13±1.5
2	Kaempferol		OH	OH	OH	H	H	OH	6.75±0.8
3	Fisetin	OH	H	OH	H	OH	OH		17.15±1.9
4	Morin	OH	OH	OH	OH	H	OH		11.85±1.8
5	Quercetin-3-*O*-rhamnoside		O-gly	OH	OH	H	OH	OH	12.23±1.8
6	Rutin	O-gly	OH	OH	H	OH	OH		18.28±1.3
7	Chrysoeriol		H	OH	OH	H	OMe	OH	13.75±1.5
8	Luteolin	H	OH	OH	H	OH	OH		7.88±1.4
9	Kaempferol-3-*O*-glycoside	O-gly	OH	OH	H	H	OH		12.25±1.6
10	Control (DMSO)								51.00 ±7.5
11	Methylene Blue (10 ppm)								5.93±0.8