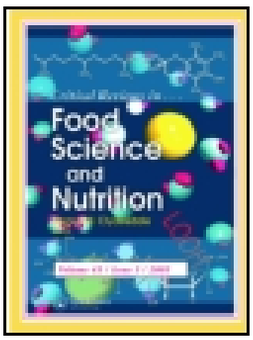

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Protective roles of flavonoids and flavonoid-rich plant extracts against urolithiasis: a review

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Abstract

In the urinary system, urolithiasis is the third prevalent disorder which causes severe pain in individuals. Urinary stones are composed of calcium oxalate (CaOx) and calcium phosphate in approximately 80% of patients. Although various drugs and surgery operations are used to treat the disease, side effects of drugs and the high recurrence after therapy in patients cannot be ignored. Flavonoids are a large group of plant polyphenols with presumed beneficial effects on several common diseases. Whereas, a very few have reached clinical use. The results of recent studies have shown that the plant flavonoids could effectively inhibit the formation of CaOx stones in vitro and in vivo, correlating with their diuretic, antioxidant,

anti-inflammatory, antibacterial properties and other protective effects. Thus, the flavonoids or flavonoid-rich plant extracts endowed with anti-urolithiasis activities and probable mechanisms of actions were reviewed. In addition, we also put forward some issues needed to be concerned in future investigations as well as offered prospects and challenges for developing the plant flavonoids into drugs for stone prevention.

Key words

Flavonoids, flavonoid-rich plant extracts, urolithiasis, antilithiatic effects, probable mechanisms, stone prevention

Introduction

Urolithiasis is a worldwide health problem among populations. It has been reported that it occurs in 12% of world population while the prevalence of the disease in female is only 25% that in male (Poonguzhali and Chegu. 1994; Tiselius 2003). Moreover, urinary calculi have a relapse rate of 50% in 5-10 years, which can lead to serious consequences throughout a patient's lifetime (Moe 2006; Dai et al. 2005). Depending on stone locations in the urinary system, urinary calculi are medically classified into renal stone, ureteral calculi, bladder stone and urethral calculus. Renal stone and ureteral calculi comprise the upper urinary calculi, while the calculus of lower urinary tract is made up of bladder stone and urethral calculus. Six types of urinary stones were found, including CaOx stone, calcium phosphate stone, magnesium ammonium phosphate stone, uric acid stone, cystine stone and hydroxyapatite stone; In approximately 80% of patients, the stones are composed of CaOx and calcium phosphate (Coe et al. 2005; Menon and Resnick. 2002). The mechanisms involved in the formation of urolithiasis have not been understood, however, it is believed that the risk of urinary calculi may be closely related with multiple factors, such as genetics, gender, age, occupation, metabolic disorder, drug, diet, urinary tract obstruction and infection (Fig. 1) (Gambaro 2004). In terms of metabolic disorder, the formation of stones may be induced by changes of urinary volume or pH, decreased level of constituents, such as citric acid or magnesium, and increased excretion of chemical components, such as calcium, oxalate, uric acid or cystine, in urine (Xiang et al. 2015). According to previous epidemiological data regarding diet and the risk of urinary stone disease, high consumption of

carbohydrate, fat, proteins, purine, dairy products, oxalate and sodium chloride increases the incidence of the disease (Arasaratnam et al. 2010).

Hematuria can be found in patients who have the upper urinary calculi with acute pain in body. By contrast, the cardinal symptoms of the calculus of lower urinary tract are interrupted micturition and significant dysuria. Imaging technologies have been utilized in the diagnosis of urinary stone disease for decades, consisting of B-ultrasonography, X-ray inspection, and computed tomography (CT) (Moe 2006). Drug therapy and operation are two common treatments for urolithiasis. In drug therapy, analgesia, infection control and urinary pH-adjustment of patients are considerably crucial. Whereas, certain drugs are not consistently effective and may have side effects due to long-term use, such as citrate and thiazide diuretic preparations (Liang et al. 2016). Regarding various surgical technologies, extracorporeal shock wave lithotripsy (ESWL) and endoscopic stone removal are widely applied to remove the calculi in patients (Ahmadi et al. 2012), yet the high recurrence rate remains an important unresolved problem in addition to the traumatic effects of shock waves, persistent residual stone fragments and the possibility of infection (Kishimoto et al. 1986; Begun et al. 1991). To be specific, in the majority of patients after ESWL and surgical intervention, the 1-year recurrence rate is approximately 10% (Brikowski et al. 2008). Thus, it is still urgent to develop of new agents against the urinary stone disease.

CaOx stone is hitherto the most common urolith, it has been demonstrated that generation of oxidative stress induced by CaOx may be the initial trigger of a vicious cycle of urolithiasis formation (Schepers et al. 2005; Khan 2006). On the one hand,

renal epithelial injury and apoptosis can be caused by reactive oxygen species (ROS) to provide sites for crystal attachment and eventual retention within the kidneys (Khan 2011a; Khan 2013). On the other hand, numerous membrane vesicles, which are effective crystal nucleators, may generate on account of cell degradation following renal epithelial injury (Fasano and Khan. 2001). Naturally, increasingly considerable attention has been given in recent years to develop safe antioxidants into drugs to inhibit the generation of ROS in patients, such as vitamin A, E, C, B_6, carotenoids and some antioxidant trace elements (Naghii et al. 2014).

It is well-known that flavonoids are a large group of plant polyphenols with presumed beneficial effects on several common diseases, including flavonols, flavanones, isoflavones and anthocyanins (Cermark 2008; Samuel et al. 2017). Specific flavonoids and a series of plant extracts containing flavonoids have been administrated to cell or animal models of urinary stone disease and urinary stone patients in various investigations. The results showed that plant flavonoids may have preventive effects on urinary calculi in vitro or vivo and provided a potential therapeutic treatment for the disease. This paper summarized the flavonoids or flavonoid-rich plant extracts endowed with beneficial effects on urolithiasis, and briefly introduced the probable mechanisms of actions to provide certain references for further research.

Protective effects of flavonoids against urolithiasis

As the specific flavonoids, inhibitory effect of rutin, catechin, epicatechin and diosmin on the experimentally-induced urinary stone formation has been reported in rats (Fig. 2). Rutin exhibits remarkable anti-inflammatory and antioxidant effects,

which has been used for centuries in indigenous medicine for the treatment of many diseases (Janbaz et al. 2002; Maheshwari et al. 2006). Ghodasara et al. (2011) studied the effect of rutin [20 mg/kg body weight (BW), 28 days by oral route] on calcium and oxalate levels in urine, kidney tissues homogenate as well as the pathological structure of kidneys of rats exposed to ethylene glycol (EG) (0.75% v/v, 28 days) and ammonium chloride (NH_4Cl) (1% w/v, the first 3 days) in drinking water. They reported that calcium and oxalate levels in urine and kidney tissue homogenate of rats both supplied with rutin and drinking water containing EG and NH_4Cl were significantly lower than those rats only treated with EG and NH_4Cl. This is probably a consequence of the effects of rutin on inhibiting the synthesis of oxalate and increasing the bioavailability of nitric oxide to sequester calcium through the cGMP (3', 5' cyclic guanosine monophosphate) pathway in calculi-induced rats (Divakar et al. 2010). Additionally, it was revealed in histopathological examination that minimum tissue damage and less number of CaOx deposits in kidneys of animals both treated with rutin and drinking water containing EG and NH_4Cl as compared to those only supplied with EG and NH_4Cl. The researchers speculated that as an anti-inflammatory and antioxidant compound, rutin may interfere with the process of epithelial cell damage induced by CaOx crystals and exert inhibitory effect on the inflammation (Thamiselvan et al. 2003).

Green tea is widely consumed in China, Japan, Korea and Morocco (Cabrera et al. 2006). Due to the rich antioxidants in green tea, including flavonoids, phenolic acids and tannins, green tea exerts various effects on health, such as antimutation, antiatherosclerotic effect and antitumor activity (Maeda et al. 2003; Dos et al 2005;

Miao et al. 2008). It was well to remind that the antiurolithiasis activity of green tea on urinary stone was also demonstrated in a previous investigation (Itoh et al. 2005). In the study, green tea treatment remarkably decreased CaOx stone formation, osteopontin (OPN, an important soluable protein component of CaOx stone) expression and renal tubular cell apotosis, while increased superoxide dismutase (SOD) activity in rat kidney tissues compared with stone group (Kohri et al. 1993). The team tentatively put forward that the blockade of NF-κB activation (a pro-inflammatory cytokine which could manage the expression of other inflammatory cytokines as well as induce human aroticendothelial cell death and apoptosis) by antioxidants in green tea might account for its anti-apoptotic activity in a urolithiasis rat model (Wu and Lozano. 1994; Tardif et al. 1997). Basing on their findings, Zhai and his colleagues (2013) examined the effects of catechin, one of main components in green tea, on renal calcium crystallization both in vitro and in vivo. In vitro experiment, administration of catechin (0.4 μL/mL) in NRK-52 cells (renal proximal tubular cell line) exposed to CaOx monohydrate (COM) crystals (80 $\mu g/cm^2$) prevented the changes in mitochondrial membrane potential and expression of SOD, 4-hydroxynonenal (4-HNE, lipid peroxidation products), cytochrome c and cleaved caspase 3. It has been demonstrated that the levels of cytochrome c and cleaved caspase 3 in cells were closely related with apoptosis. In the vivo study, the mitochondrial collapse and increase of OPN, malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG, the general marker for monitoring DNA damage) expression induced by EG in kidneys of rats were diminished by low-dose (2.5 mg/kg BW per day, 14 days) and high-dose (10 mg/kg BW per day, 14 days) of catechin (Niimi et al. 2012). Thereby, the data of the

research implied that renal calcium crystallization in NRK-52 cells and rats could be prevented by catechin through decreasing the degree of COM-induced mitochondrial injury (Kawai et al. 2006).

On account of the structure similarity, epicatechin can inhibit renal calculi in a manner similar to catechin as well. Precisely, Grases et al. (2009) found that epicatechin treatment (100 mg/L of epicatechin in drinking water, 24 days) effectively prevented the development of papillary and intratubular calcification in the kidneys of rats supplemented with 0.8% v/v EG plus 1% w/v NH_4Cl in the last 8 days. The results might be attributed to the effects of epicatechin on avoiding hyperoxaluria-induced peroxidatove damage to renal tubular membrane surface (lipid peroxidation) and the papillary tip epithelium, which could further suppress the formation of kidney stones (Sumathi et al. 1993).

Diosmin, which can be found in *Teucrium gnaphalodes* L'Her (Lamiaceae), is a naturally occurring flavonoid glycoside and belongs to the citrus flavonoid family (Barberan et al. 1985). In neuronal cells, anti-inflammatory and anti-apoptotic activites of diosmin have been already reported. In a recent animal experiment, kidney weight, urinary pH, total urinary protein, urinary calcium, phosphorus, serum potassium, sodium, magnesium, creatinine, uric acid and blood urea nitrogen levels (risk factors of stone formation) significantly decreased, while urinary volume, urinary magnesium, potassium, sodium, creatinine, uric acid and serum calcium levels (inhibitors of stone formation) remarkably increased in two diosmin groups (0.75% v/v EG + 2% w/v NH_4Cl + diosmin 10 or 20 mg/kg BW, 15 days) as compared to the group only treated with EG and NH_4Cl (Prabhu et al. 2016). It seemed that the

anti-urolithiatic activity of diosmin was similar to the standard drug cystone, and the team interpreted this to its antioxidant, anti-inflammatory effects as well as protective effects of microcirculation.

Protective effects of the flavonoid-rich leaf extracts against urolithiasis

Different organs or tissues of various plants, including the leaves, flowers, fruits, seeds and roots have been reported to be rich in flavonoids. In several latest studies, the anti-urolithiatic activities of extracts from leaves of *Copaifera langsdorffii*, *Hypericum perforatum*, *Desmodium styracifolium*, *Urtica dioica*, *Hibiscus sabdariffa*, *Phyllanthus amaru* and *Bryophyllum pinnatum* were demonstrated. Brancalion and his colleagues (2012) dired, grounded and macerated the leaves of *Copaifera langsdorffii* in a hydroalcoholic solution 7:3 to produce a 16.8% crude extract after solvent elimination, identifying the quercitrin (5.4%) and afzelin (7.4%) as the major flavonoids in extract through the HPLC analysis. After gavaging the rats (which had been introduced of a CaOx pellet into bladders for 30 days to induce urinary stone) with the extract (20 mg/kg BW, 18 days), they found that sodium, potassium and creatinine concentrations in urine and plasma of extract-treated animals were in the normal range, while both the mean number and mass of calculi remarkably reduced compared with animals only treated with CaOx pellet. Additionally, stones taken from extract treated group were more brittle and fragile than those from untreated group, which required merely half the amount of pressure as stones from untreated group (3.00 ± 1.51 vs. 6.90 ±3.45 kgf/mm^2) to be broken. Thereby, the team concluded that the extract rich in flavonoids from leaves of *Copaifera langsdorffii* might prevent rats from urolithiasis by dispersing the particles of CaOx in urine to

facilitate elimination, diminishing the number of calculi formed and reducing the pressure required to break the calculi (Laroubi et al. 2007). Similarly, the hydroalcoholic solution was also used to extract the leaves of *Hypericum perforatum* through the most common St. John's Wort preparations in research of khalili et al. (2012) and the leaf extract was proved to be consisted of glycoside flavonoids, such as quercetin and quercetin-3 (Ganzera et al. 2002; Khan et al. 2011b). The results showed that the low dose (300 mg/kg BW, 28 days) and high dose (500 mg/kg BW, 28 days) reduced the number of CaOx calculi by 43.91% and 44.07% in kidneys of groups exposed to EG (1%) plus NH_4Cl (0.5%) in drinking water, respectively. In the meantime, the size of CaOx deposits in the extract treated animals was much smaller than those only treated with EG and NH_4Cl. Apart from the anti-inflammatory, antioxidant mechanisms, and interference effects of flavonoids in the extracts from leaves of *Hypericum perforatum* with the process of epithelial cell damage induced by crystals (Yuen et al. 2010), the authors believed that the diuretic and antibacterial effects of the extracts should not be ignored because the role of bacterial origin, such as nanobacteria, was equally important in the formation of CaOx crystals (Saddiqe and Naeem. 2010; Izzo and Emst. 2009).

By contrast, Zhang et al. (2014) used methanol (95%) to extract the leaves of *Urtica dioica*, which had a long history in the treatment of kidney, urinary tract, gastrointestinal tract and locomotor disorders. The flavonoids identified in extracts from the leaves of *Urtica dioica* were luteolin, gossypetin, rutin, kaempferol-3-O-rutinoside and kaempferol-3-O-glucoside. To evaluate the effects of the flavonoid-rich extracts on renal calculi formation, two doses of the leaf extracts

(700 and 1400 mg/kg BW, 15 days) were administrated by gastric intubation using a soft catheter to the rats which had already been treated with 0.55% EG and 1% NH_4Cl in drinking water for 10 days. Treatment with methanolic extracts from the leaves of *Urtica dioica* effectively reduced the renal deposition of calcium and oxalate in rats of two extract groups. It was assumed that the flavonoid-rich extracts might dissolve CaOx renal stones via disintegrating mucoproteins, which had a high affinity for CaOx surfaces to promote the growth and deposition of crystals (Leal and Finlayson. 1977). Ethnobotanical literature since ancient China revealed that *Desmodium styracifolium* was used for treatment of urolithiasis because of its heat-clearing and diuretic properties (Zhao et al. 2007). In study of Zhou et al. (2017), animal models of CaOx urolithiasis were established in rats by adding 5% w/w hydroxyl-L-proline in regular chow. The researchers extracted the powdered plant material twice with 50% ethanol for 1.5 h each at 80 ℃The total flavonoids content in the extracts from leaves of *Desmodium styracifolium* was 8.0 mg/mL, while vicenin 1, vicenin 2, vicenin 1, schaftoside, isoschaftoside and isovitexin were the major flavonoids in the extracts. After 4-week gavage, reduce of MDA content, increase of CAT and GSH-Px activities in renal homogenate as well as attenuation in the expression of MCP-1, OPN and TGF-β proteins of two extract groups (100 and 400 mg/kg BW) could be observed. It has been reported that the expression of MCP-1, OPN and TGF-β increased when renal epithelia cells exposed to high concentration of oxalate or CaOx crystals (Umekawa et al. 2006; Tsujihata et al. 2011). The results of the study suggested that the flavonoids in extracts from leaves of *Desmodium styracifolium* had the potential of inhibiting the urinary stone disease

by protecting the rats from oxidative stress changes and localized inflammation induced by hydroxyl-L-proline.

Interestingly, Woottisin et al. (2011) fed the rats with tablets containing the extracts from leaves of *Hibiscus sabdariffa* or *Phyllanthus amarus* instead of adding in the drinking water. Four weeks later, the serum oxalate and glycolate level in group received a 3% glycolate diet and tablets containing the extracts from leaves of *Hibiscus sabdariffa* (3.5 mg extract per tablet) significantly decreased, while urinary citrate and oxalate levels of the group were higher than the group only received a 3% glycoside diet. Alternatively, the extracts from leaves of both *Hibiscus sabdariffa* and *Phyllanthus amarus* decreased calcium crystal deposition in the kidneys of rats. This was probably a consequence of the effects of the extracts on promoting the creation of small CaOx dehydrate (COD) than COM particles. It has been demonstrated that COD binds less tightly than COM to epithelial cells (Wesson et al. 1998). Accordingly, CaOx crystal attachment to renal epithelia cells was effectively inhibited. *Bryophyllum pinnatum* (Lam.) Oken (Crasssulaceae) is a perennial herb widely distributed in eastern Asia, tropical America and Australia, which is also known as "dissciver of stones" (Kamboj and Saluja. 2009; Khare 2007). The pharmacological properties of leaves of *Bryophyllum pinnatum* were ascribed to the presence of flavonoids, glycoside and other active substances (Okwu and Nnamdi. 2011; Devbhuti et al. 2008). In-vitro inhibitory activity of extract from the leaves of *Bryophyllum pinnatum* on CaOx crystallization has been previously reported, therefore, its effects on experimentally induced in-vivo lithiatic model was investigated by Yadav et al. (2016). Renal calculi were induced in rats by

administration of 0.75% EG and co-treated with alcoholic extracts (total flavonoids: 4.19% rutin equivalent) or hydro-aclocholic extracts (total flavonoids: 3.67% rutin equivalent) in doses of 100, 200 and 400 mg/kg BW for 4 weeks. They found that treatment with two leaf extracts effectively reversed oxidative and histological damages in kidneys as well as attenuated the evaluation in urinary parameters and serum biochemical parameters of animals exposed to EG. It was supposed that the extracts from leaves of *Bryophyllum pinnatum* could protect the rats from kidney stone via increasing the stone inhibitory activity of endogenous stone inhibitor (glycosaminoglycans) (Fleisch 1978). Nevertheless, further studies were required to identify the composition of flavonoids in the extracts from leaves of *Hibiscus sabdariffa*, *Phyllanthus amarus* and *Bryophyllum pinnatum*.

Protective effects of the flavonoid-rich flower extracts against urolithiasis

Lastly, several flower extracts were also demonstrated to have an inhibitory effect on urinary stone disease. *Flos carthami* is a traditional Chinese herbal plant containing carthamin, neocarthamine and kaempferol 3-rhamnoglucoside (Lu et al. 2008). Basing on its anti-coagulation, *Flos carthami* is initially used for improvement of blood circulation effects (Sun et al. 2009). Likewise, it has been shown in a previous research that the extracts from flowers of *Flos carthami* can effectively prevent stone disease (Lin et al. 2012). After 4-week experimental period, the crystal deposit score of the stone group (starch and 0.75% EG per day, intragastric administration) was 187.5% that of group gavaged with 0.75% EG and 1200 mg of flower extracts per day. However, the antilithic mechanism of the extracts remained

to be elucidated. Meanwhile, certain side effects such as a bleeding tendency also needed to be taken seriously. In Turkey, *Helichrysum* (Asteraceae) flowers were widely used in folk medicine to remove kidney stones due to their diuretic properties (Sezik et al. 2001; Sezik et al. 2005). Bayir's team (2011) prepared the extracts from dried flowers of *Helichrysum plicatum* DC. Subsp. *Plicatum* (HP) by using distilled water: absolute ethanol (1:1) (50-60 °C). To assess the effects of HP as a preventive agent in experimentally induced urolithiasis model in rats, different doses of HP extracts (125, 250 and 500 mg/kg BW) were orally administrated for 3 weeks in animals exposed to 1% EG and 1% NH_4Cl in drinking water, respectively. It was found that the BW gains of three HP groups were much higher than urolithiasis group. Alternatively, extensive intratubular crystal depositions and degenerative tubular structures were not revealed in HP groups. In another study, hot water was directly added on the dried capitulums of *Helichrysum graveolens* (M. Bieb) Sweet (HG) and *Helichrysum stoechas* ssp. *Barellier* (Ten) Nyman (HS) for preparation of the extracts (Onaran et al. 2016). Unlike the previous studies mentioned, the urinary calculi were induced by treating the rats with 70 mg/kg of sodium oxalate intraperitoneally for 5 days (Bouanani et al., 2010). After formation of stones, two different doses of extracts from the flowers of HG (62.5 and 125 mg/kg BW) and HS (78 and 156 mg/kg BW) were administrated to animals by mixing with drinking water for 10 days. Significant increase in the platelet count could be evaluated in serum of HG and HS groups (except HG 62.5 mg/kg dose group), while the level of alkaline phosphatase in serum of HG 125 mg/kg dose group was 77.7% that of sodium oxalate group. The results indicated that the degree of liver and red blood cell damage could be reduced by HG and HS extracts. Besides, a stone formation score (0-3) was used to determine

the stone formation rate of the kidney tissues of animals in different groups. The scores of two HS groups (1.00 and 0.33) were much lower than that of sodium oxalate group (2.33). Researchers of the two studies confirmed that the antioxidant activity of extracts from capitulums of *Helichrysum* species contributed to their preventive effect on the development of papillary calculi, as Asteraceae was a flavonoid-rich plant, for instance, helichrysins A and B, luteolin, kaempferol, quercetin, apigenin, naringenin, isoastragalin, and isosalopurposide, etc (Grases et al., 2009; Demir et al., 2009).

For the past few years, green nanotechnology has drawn world-wide attention for its simplicity, exclusion of harmful reagents and use of various prokaryotic and eukaryotic organisms such as plant extracts (Bhainsa and D'Souza. 2006). *Phlogacanthus thyrsiformis* Hardow (Mabb.) is a seasonal plant flowering in early spring, whose aqueous extracts are used for treating kidney and liver disorders in the ethnic system of medicine (Sharmistha and Jogen. 2012). Furthermore, flavonoids are the major phytoconstituents (72.53 mg/g extract) detected in the aqueous extract. In a recent investigation, Das et al. (2017) synthesized the biofabricated silver nanoparticles of aqueous extracts from flowers of *Phlogacanthus thyrsiformis* and envaluated its therapeutic activity against struvite urinary stones and CaOx kidney stones in rat models. Struvite stones were grown in tubes by gel diffusion technique while CaOx stones were induced by EG and NH_4Cl in rats for 2 weeks. It was demonstrated that treatment with the biofabricated silver nanoparticles (10 mg/kg BW, 14 days) remarkably reduced the size of struvite stones in vitro and eliminated CaOx stones in urolithiasis model rats in vivo. Anti-urolithiatic potency

could be attributed to the presence of flavonoids, while it was worth mentioning that the smaller size and increased surface area in case of the bio-fabricated silver nanoparticles resulted in enhanced drug distribution and greater efficacy.

Protective effects of the flavonoid-rich fruit extracts against urolithiasis

Extracts from fruits of *Pinus eldarica, Bombax ceiba, Rosa canina* and pomegranate can protect animals or human beings from urinary calculi as well. P*inus eldarica* is a Tehran pine consisting of quercetin, pinene, myrcene and other active substances (Afsharypuor and San'aty. 2005). Hosseinzadeh and his colleagues (2010) investigated the effects of aqueous extracts from the fruits of *Pinus eldarica* on CaOx nephrolithiasis in rats. In the present study, the prohylatic and therapeutic groups received the extracts (500 and 1000 mg/kg BW) for 30 days and from the 14th day through the end of the experiment, respectively. After 1-month treatment, stone formation significantly decreased in the prohylatic group, whereas, there was no significant difference between the therapeutic group and urolithiasis group, which suggested a potent prohylatic effect of the *Pinus eldarica* fruit extracts on urinary stone disease. It is possible that the extracts prevented stone formation via antioxidant, anti-inflammatory and antibacterial mechanism (Busserolles et al. 2006; Brundig and Börner. 1987; Kajander et al. 2003). In 20th century, the fruit of *Bomax ceiba* was demonstrated to be rich in flavonoids, including mangiferin, kaemferol and quercetin (Seshadri et al. 1973). The dried fruits were given in calculus affections as well as chronic inflammation and ulceration of the bladder and kidneys (Kapoor 1990), however, the rationale behind its use was not scientifically established. To ascertain the scientific validity, the aqueous extract (400 mg/kg BW) and enthanol

extract (400 mg/kg BW) of *Bomax ceiba* fruit were orally administrated for 2 weeks in rats exposed to 0.75% EG in drinking water in a research of 2012 (Gadge and Jalalpure. 2012). The data indicated that the flavonoid-rich fruit extract was endowed with lithontriptic activity by increasing renal excretion of calcium and phosphate as well as reducing the elevated urinary oxalate in calculogenic rats, yet the mechanism of its anti-urolithiasis effects remained unknown.

Previously, the fruits of *Rosa canina* used to be applied in the therapy of gallstone and influenza (Orhan et al. 2007; Nojavan et al. 2008). Based on the study of Tayefi-Nasrabadi et al. (2012), the fruit could also be useful as a preventive agent against the formation of kidney stones. In the current investigation, the total flavonoids content of the hydromethanol extracts from the fruits of *Rosa canina* were 5.37±0.17 mg quercetin equivalents/g. The HPLC analysis exhibited the presence of hyperoside, isoquercitrin, quercitrin, *trans*- and *cis*-tiliroside as the major constituent components in the extracts. Supplementation of the hydromethanol extracts from the fruits of f *Rosa canina* (250 and 500 mg/kg BW, 30 days) effectively decreased the size and number of CaOx calculi in the kidneys as well as reduced the kidney and liver peroxides to optimum levels in rats treated with 1% EG. The excellent antioxidant activity and disruption effect of stone formation of the fruit extract might be responsible for its antilithiatic action (Grases et al. 2009). For a long time, the pomegranate has been known as a anthocyanin-rich plant (Tugcu et al. 2008). The preventive effects of pomegranate against the urinary stone disease through reducing the crystal deposition, inducible oxide synthase, p38-MAPK and p65-NF-κB activity and the level of oxidative stress markers in kidney tissues were

proved in considerable number of animal studies (Ilbey et al. 2009; Rathod et al. 2012). Accordingly, to examine the effects of the pomegranate extract on risk factors for nephrolithiasis in human beings, the team of Tracy (2014) collected the urine and blood samples from recurrent stone formers and non-stone formers receiving the extract (1 g/day) for 3 months. It was found that recurrent stone formers had significantly higher levels of oxidative stress at baseline as compared with non-stone formers, while the ability to prevent stone formation via supplement of the pomegranate extract could not be determined in the pilot study due to the failure of the team to demonstrate a positive or negative correlation between antioxidant supplementation with the extract and known risk factors development of nephrolithiasis. Whereas, there was 10% increase in paraoxonase1 arylesterase (an anti-atherosclerotic component associated with high-density lipoprotein) activity in recurrent stone formers after 3-month treatment with the extract, which correlated with a trend toward decreasing values of supersaturation of CaOx and were in line with finding of previous animal experiments (Getz and Reardon. 2004).

Protective effects of the flavonoid-rich seed extracts against urolithiasis

Oligomeric proanthocyanidins (OPCs, the grape seed extracts) were made up of dimmers or trimers of (+)-catechin and (-)-epicatechin (Silva et al. 1991). Various biological properties of OPCs have been reported, such as antioxidant, antibacterial, antiviral, anticarcinogenic and anti-inflammatory activity (Frankel et al. 1993; Bagchi et al. 1998). It has been shown in an in-vitro research that OPCs could protect against HK-2 cell injury induced by oxalate and COM via scavenging ROS and

increasing activity of SOD (Wang et al. 2016). Amounts of cells with vacuoles formed in cytoplasms, karyotheca dissolved and nucleolus disappeared in OPCs group (OPCs + oxalate + CaOx crystals) and vitamin E group (vitamin E + oxalate + CaOx crystals) were evidently less than those in the positive control group (oxalate + CaOx crystals), while the protection efficiency of OPCs was stronger than vitamin E. As a consequence, the results suggested a possibly antilithiatic effect of the OPCs, which needed to be demonstrated by further in-vivo experiments.

In Morocco, the seeds of *Trigonella foenum graecum* were widely used to treat lithiasis patients. Flavonoids in the extracts from the seeds of *Trigonella foenum graecum* have been observed to possess the activitiy of preventing lipid peroxidation (Kaviarasan et al. 2004). However, the anti-urolithiasis property of the extracts has rarely been demonstrated before. Hence, Laroubi et al. (2007) made full use of urinary calculi model in rats to investigate the effects of extracts from the seeds of *Trigonella foenum graecum* on stone formation. Animals exposed to 0.75% EG and 2% NH_4Cl were given 1 mL/day of 100 and 200 mg/kg BW of the aqueous extracts by gavage, respectively. Ultimately, the amount of calculi in the kidneys and the total calcium amount of the renal tissue in two extract groups were significantly lower than those of the group only treated with EG and NH_4Cl. The inhibitory activity of the extracts from the seeds of *Trigonella foenum graecum* on urinary stone disease were suspected to be closely related with its antioxidant activity, effects of increasing dieresis and lowering levels of stone formation constituents in urine (Thirunavukkarasu et al. 2003).

Protective effects of other flavonoid-rich plant extracts against urolithiasis

Due to the high content of citrate in lemon fruits, few previous studies focused on the potential effects of lemon peels on urinary stone disease (Agarwal et al. 2011). On the basis of a recent investigation, the extracts of lemon peels might be applied both in stone prevention and treatment (Sridharan et al. 2015). The aqueous methanol extracts of lemon peels were prepared according to methods described by Nogata et al. (2010), and hesperidin, eriocitrin, narigenin and rutin were the major bioflavonoids of the extracts. The preventive and treatment groups received the extracts of lemon peels (100 mg/kg BW) for 7 weeks and from the 4^{th} week to the 7^{th} week, respectively. Meanwhile, two extract groups were also administrated to the drinking water mixed with 0.75% EG throughout the whole experiment. Adverse impacts on urinary and serum parameters, tissue antioxidant enzymes as well as expressions of Tamm-Horsfall protein and NF-κB induced by EG were improved in both preventive and treatment goups. Tamm-Horsfall protein was reported to be a potent crystal inhibitor, which has a tendency to get expressed more in the diseased state in order to interfere with crystal aggregation process and prevent them from adhering to the tubules (Mo et al. 2006). Thus the extracts of lemon peels had the potential of curing the progression of the urolithiasis via reducing the oxidative stress and inflammation-mediated cellular damage, maintaining the membrane integrity while increasing the expression of the crystal inhibitors in kidneys of rats.

Radix Paeoniae Alba is the dried root of *Paeoniae lactiflora Pall* without bark and has been used as a medical herb in traditional Chinese medicine for centuries,

containing kaempferol-3-O-glucoside and kaempferol-3,7-di-O-glucoside (Kamiya et al. 1997). Yet there are few investigations regarding the effects of *Radix Paeoniae Alba* on urinary calculi. Li and his team (2017) carried out a study to estimate the possible effects of an aqueous extract of *Radix Paeoniae Alba* on the crystal formation in vitro and CaOx nephrolithiasis in vivo. In vitro, treatment with 64 mg/mL of the aqueous extracts remarkably dissolved formed crystals (8.99±1.43) and inhibited crystal formation (2.55±0.21) compared with the control (55.10±4.98; 54.57±5.84). To establish the rat models of CaOx nephrolithiasis, 1% (v/v) EG was added in distilled water throughout the whole study. The prophylactic and treatment groups were orally administrated of the extracts of *Radix Paeoniae Alba* (220 and 660 mg/kg BW) for 28 days and from the 15th day to the end of the experiment, respectively. Reduced urinary and renal oxalate levels, decreased OPN expression, renal crystallization and pathological changes as well as increased urinary citrate and calcium levels were merely observed in preventive group as compared to the group treated with EG. Interestingly, the team aspirated the urine from the bladder of rats to access microbiological variation. They found that the pyelonephritis rate of the high-dose prophylactic or treatment group was 20% or 90% that of the group exposed to EG. The results in the current study suggested that *Radix Paeoniae Alba* could be a potent agent for prevention of kidney stones. The probable mechanisms may be connected with the antioxidant, antimicrobial, anti-infective activity and inhibitory effects on the crystallization modulators of the extracts of *Radix Paeoniae Alba* (Clayman 2004; Wang et al. 2014; Tavichakorntrakool et al. 2012).

As a local plant in Tamil, *Aerva lanata* is well-known for its therapeutic effects in controlling kidney disorders. It has been shown in literatures that the plant is endowed with flavonoid components, such as kaemepferol-3-O-rhamnoside and kaempferol-3-O-rhamnogalactoside (Afaq and Afridi. 1991). In research of Soundararajan et al. (2006), the aqueous extracts from the aerial parts of *Aerva lanata* (2000 mg/kg BW, 4weeks) were orally given by using an intragastric tube to rats exposed to 0.75% (v/v) EG in drinking water. Extract-treated rats showed significant decrease in the markers of crystal deposition in the kidney as well as activities of oxalate synthesizing enzymes including glycolic acid oxidase and lactate dehydrogenase in liver and kidney comparing with animals only treated with EG. Therefore, the extracts from the aerial parts of *Aerva lanata* was likely to inhibit the CaOx urolithiasis in rats by maintaining the balance of oxalate metabolism, increasing the solubility of crystal deposits and restoring the normal renal architecture (Liao and Richardson. 1972).

Concluding remarks

Here, the flavonoids and flavonoid-rich plant extracts which might be applied in the prevention of the urinary calculi were summarized. In particular, plant flavonoids were appropriate for administrating to those patients under drug treatment due to the small size of stones. The anti-urolithiasis activity of plant flavonoids was supposed to be implicated in their diuretic, antioxidant, anti-inflammatory, antibacterial, anti-infection, anti-apoptotic effects, protective effects of microcirculation, effects on modulating the synthesis and expression of endogenous stone activators or inhibitors, maintaining the balance of oxalate metabolism or reducing the size and number of crystals (Fig. 3). Other possible mechanisms related to antilithiatic effect of flavonoids still remained to be elucidated. Additionally, several issues should be concerned and paid abundant attention in the following studies. First, the specific flavonoid constituents in a plenty of plant extracts have not been identified yet (Table 1). Meanwhile, those identified flavonoids of the extracts, such as hyperoside, isoquercitrin, quercitrin, afzelin, etc, needed to be purified and given to animal models of urinary stone disease, respectively, for further determination of the functional components (Tayefi-Nasrabadi et al. 2012; Brancalion et al. 2012). Second, emerging technology could be made full use of to develop new and highly efficient drugs for modern urinary stone therapy, such as green nanotechnology (Das et al. 2017). Third, the models utilized by present research are almost CaOx calculus models in animals, while calcium phosphate is also a major component of urinary stones. It is natural that the potential roles of plant flavonoids on the formation of calcium phosphate stone in vivo should be

studied in further investigations. Afterwards, some plant flavonoids endowed with anti-urolithiasis activity may cause certain side effects which must be taken seriously, for example, the extracts from flowers of *Flos carthami* can induce a bleeding tendency in animals (Lin et al. 2012). Thereby, the drugs containing those flavonoid components must be strictly recommended to specific patient groups after a series of clinical trials. Last but not the least, currently, more than 95% of studies regarding the antilithiatic effects of plant flavonoids were conducted in experimental animals. On account of the differences between experimental animals and human beings (for instance, the ability to prevent stone formation via supplement of the pomegranate extract could not be determined in the pilot study despite previously proved anti-urolithiasis activity of the extract in animal models), clinical trials are urgently needed to ascertain that which flavonoids can indeed play protective roles against the urinary calculi in individuals and have promise to be applied in stone precaution (Tracy et al. 2014). Whilst, the bioavailabilty of plant flavonoids needs to be concerned in humans. In future research, it is also advisable to evaluate the potential effects of plant flavonoids on the recurrence of urinary stone disease in patients after shock wave lithotripsy which is known to generate ROS and cause renal damage (Khan et al. 2005).

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References

Afaq, S. H., and Afridi, T. (1991). Bisehri booti (Aerva lanata) - some lesser known uses and pharmacognosy. *Der Hautarzt*, **53**(7):447-455.

Afsharypuor, S., and San'aty, F. (2005). Essential Oil Constituents of Leaves and Fruits of Medw. *J Essent Oil Res*, **17**(3):327-328.

Agarwal, M. M., Shwaran, K., Singh, S. K., et al. (2011). Preventive fluid and dietary therapy for urolithiasis: An appraisal of strength, controversies and lacunae of current literature. *Indian J Urology*, **27**(3):310-319.

Ahmadi, M., Rad, A. K., Rajaei, Z., et al. (2012). *Alcea rosea* root extract as a preventive and curative agent in ethylene glycol-induced urolithiasis in rats. *Indian J Pharmacol*, **44**(3):304.

Arasaratnam, V., Balakumar, S., Senthuran, A., et al. (2010). A study of *Tribulus terrestris* extract on risk factors for urinary stone in normal subjects and urolithic patients. *J Natl Sci Found Sri*, **38**(3):187-191.

Bagchi, D., Garg, A., Krohn, R., et al. (1998). Protective effects of grape seed proanthocyanidins and selected antioxidants against TPA-induced hepatic and brain lipid peroxidation and DNA fragmentation, and peritoneal macrophage activation in mice. *Gen Pharmacol*, **30**(5):771–776.

Barberán, F. A. T., Gil, M. I., Tomás, F., et al. (1985). Flavonoid Aglycones and Glycosides from Teucrium gnaphalodes. *J Nat Prod*, **48**(5):859-860.

Bayir, Y., Halici, Z., Keles, M, S., et al. (2011). *Helichrysum plicatum* DC. subsp. plicatum, extract as a preventive agent in experimentally induced urolithiasis model. *J Ethnopharmacol*, **138**(2):408-414.

Begun, F. P., Knoll, C. E., Gottlieb, M., et al. (1991). Chronic effects of focused electrohydraulic shock waves on renal function and hypertension. *J Urology*, **145**(3):635-639.

Bhainsa, K. C., and D'Souza, S. F. (2006). Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*. *Colloid Surfaces B*, **47**(2):160-164.

Bouanani, S., Henchiri, C., Migianugriffoni, E., et al. (2010). Pharmacological and toxicological effects of *Paronychia argentea* in experimental calcium oxalate nephrolithiasis in rats. *J Ethnopharmacol*, **129**(1):38-45.

Brancalion, A. P., Oliveira, R. B., Sousa, J. P., et al. (2012). Effect of hydroalcoholic extract from *Copaifera langsdorffii* leaves on urolithiasis induced in rats. *Urolithiasis*, **40**(5):475-481.

Brikowski, T. H., Lotan, Y., and Pearle, M. S. (2008). Climate-related increase in the prevalence of urolithiasis in the United States. *P Natl Acad Sci USA*, **105**(28):9841-9846.

Brundig, P., and Börner, R. H. (1987). Clinical results in the treatment of therapy-resistant calcium-stone formers with non-steroidal anti-inflammatory drugs. *Eur Urol*, **13**(1-2):49-56.

Busserolles, J., Gueux, E., Balasińska, B., et al. (2006). In vivo antioxidant activity of procyanidin-rich extracts from grape seed and pine (*Pinus maritima*) bark in rats. *Int J Vitam Nutri Res*, **76**(1):22.

Cabrera, C., Artacho, R., and Giménez, R. (2006). Beneficial Effects of Green Tea—A Review. *J Am Coll Nutr*, **25**(2):79-99.

Cass, A. S. (1995). Comparison of First Generation (Dornier HM3) and Second Generation (Medstone STS) Lithotriptors: Treatment Results With 13,864 Renal and Ureteral Calculi. *J Urology*, **153**(3):588-592.

Cermak, R. (2008). Effect of dietary flavonoids on pathways involved in drug metabolism. *Expert Opin Drug Met*, **4**(1):17-35.

Clayman, R. V. (2004). Modulators of Urinary Stone Formation. *Front Biosci*, **9**(2):1450-1482.

Coe, F. L., Evan, A., and Worcester, E. (2005). Kidney stone disease. *J Clin Invest*, **115**(10):2598-2608.

Dal, M. F., Mancini, M., Tavolini, I. M., et al. (2005). Cellular and molecular gateways to urolithiasis: a new insight. *Urol Int*, **74**(3):193-197.

Das, P., Kumar, K., Nambiraj, A., et al. (2017). Potential therapeutic activity of *Phlogacanthus thyrsiformis* Hardow (Mabb) flower extract and its biofabricated silver nanoparticles against chemically induced urolithiasis in male Wistar rats. *Int J Biol Macromol*, **103**:621-629.

Demir, A., Mercanoglu Taban, B., Aslan, M., et al. (2009). Antimicrobial effect of *Helichrysum plicatum* subsp plicatum. *Pharm Biol*, **47**:289–297.

Devbhuti, D., Gupta, J. K., Devbhuti, P., et al. (2008). Phytochemical and acute toxicity study on *Bryophyllum calycinum* Salisb. *Acta Pol Pharm*, **65**:501-504.

Divakar, K., Pawar, A. T., Chandrasekhar, S. B., et al. (2010). Protective effect of the hydro-alcoholic extract of *Rubia cordifolia* roots against ethylene glycol induced urolithiasis in rats. *Food Chem Toxicol*, **48**(4):1013-1018.

Dos, A., Ayhan, Z., Sumnu, G. (2005). Effects of different factors on sensory attributes, overall acceptance and preference of rooibos (*aspalathus linearis*) tea. *J Sens Stud*, **20**(3): 228–242.

Fasano, J. M., and Khan, S. R. (2001). Intratubular crystallization of calcium oxalate in the presence of membrane vesicles: an in vitro study. *Kidney Int*, **59**(1):169-178.

Fleisch, H. (1978). Inhibitors and promoters of stone formation. *Kidney Int*, **13**:361-371.

Frankel, E. N., Kanner, J., German, J. B., et al. (1993). Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet*, **341**(8843):454–457.

Gadge, N. B., and Jalalpure, S. S. (2012). Curative treatment with extracts of *Bombax ceiba* fruit reduces risk of calcium oxalate urolithiasis in rats. *Pharm Biol*, **50**(3):310-317.

Gambaro, G., Vezzoli, G., Casari, G., et al. (2004). Genetics of hypercalciuria and calcium nephrolithiasis: from the rare monogenic to the common polygenic forms. *Am J Kidney Dis*, **44**(6):963-986.

Ganzera, M., Zhao, J., and Khan, I. A. (2002). *Hypericum perforatum*—Chemical profiling and quantitative results of St. John's Wort products by an improved high-performance liquid chromatography method. *J Pharm Sci,* **91**(3):623-630.

Getz, G. S., and Reardon, C. A. (2004). Paraoxonase, a cardioprotective enzyme: continuing issues. *Curr Opin Lipidol*, **15**(3):261.

Ghodasara, J., Pawar, A., Deshmukh, C., et al. (2011). Inhibitory effect of rutin and curcumin on experimentally-induced calcium oxalate urolithiasis in rats. *Pharm Res*, **2**(6):388-392.

Grases, F., Prieto, R. I., Sanchis, P., et al. (2009). Phytotherapy and renal stones: the role of antioxidants: A pilot study in Wistar rats. *Urolithiasis*, **37**(1):35-40.

Hosseinzadeh, H., Khooei, A. R., Khashayarmanesh, Z., et al. (2010). Antiurolithiatic activity of *Pinus eldarica* medw: fruits aqueous extract in rats. *Urol J*, **7**(4):232-237.

Hughes, S. D., Ketheesan, N., and Haleagrahara, N. (2017). The Therapeutic Potential of Plant Flavonoids on Rheumatoid Arthritis. *Crit Rev Food Sci*, **57**(17):3601-3613.

ilbey, Y. O., Ozbek, E., Simsek, A., et al. (2009). Effects of pomegranate juice on hyperoxaluria-induced oxidative stress in the rat kidneys. *Renal Failure*, **31**(6):522-531.

Itoh. Y., Yasui, T., Okada, A., et al. (2005). Preventive effects of green tea on renal stone formation and the role of oxidative stress in nephrolithiasis. *J Urology*, **173**(1):271-275.

Izzo, A. A., and Ernst, E. (2009). Interactions Between Herbal Medicines and Prescribed Drugs. *Drugs*, **69**(13):1777-1798.

Janbaz, K. H., Saeed, S. A., and Gilani, A. H. (2002). Protective effect of rutin on paracetamol- and CCl_4-induced hepatotoxicity in rodents. *Fitoterapia*, **73**(7-8):557-653.

Kajander, E. O., Ciftcioglu, N., Aho, K., et al. (2003). Characteristics of nanobacteria and their possible role in stone formation.*Urolithiasis*, **31**(2):47-54.

Kamboj, A., and Saluja, A. K. (2009). *Bryophyllum pinnatum* (Lam.) Kurz.: phytochemical and pharmacological profile: a review. *Pharma Rev,* **3**(6):364-374.

Kamiya, K., Yoshioka, K., Saiki, Y., et al. (1997). Triterpenoids and flavonoids from Paeonia lactiflora. *Phytochemistry*, **44**(1):141-144.

Kapoor, L. D. (1990). Handbook of Ayurvedic Medicinal Plants. Boca Raton: CRC Press.

Kaviarasan, S., Vijayalakshmi, K., Anuradha, C. V. (2004). Polyphenol-rich extract of fenugreek seeds protects erythrocytes from oxidative damage. *Plant Foods Hum Nutr,* **59**:143–147.

Kawai, Y., Nakao, T., Kunimura, N., et al. (2006). Relationship of intracellular calcium and oxygen radicals to Cisplatin-related renal cell injury. *J Pharmacol Sci*, **100**(1):65-72.

Khalili, M., Jalali, M. R., and Mirzaeiazandaryani, M. (2012). Effect of hydroalcoholic extract of *Hypericum perforatum L*. leaves on ethylene glycol-induced kidney calculi in rats. *Urol J*, **9**(2):472-479.

Khan, A. U, Gilani, A. H., and Najeeb-ur-Rehman. (2011). Pharmacological studies on *Hypericum perforatum* fractions and constituents. *Pharm Biol*, **49**(1):46-56.

Khan, S. R. (2011). Crystal/cell interaction and nephrolithiasis. *Arch Ital Urol Androl*, **83**(1):1-5.

Khan, S. R. (2005). Hyperoxaluria-induced oxidative stress and antioxidants for renal protection. *Urolithiasis*, **33**(5):349-357.

Khan, S. R. (2013). Reactive oxygen species as the molecular modulators of calcium oxalate kidney stone formation: evidence from clinical and experimental investigations. *J Urology*, **189**(3):803-811.

Khan, S. R. (2006). Renal tubular damage/dysfunction: key to the formation of kidney stones. *Urolithiasis*, **34**(2):86-91.

Khare, C. P. (2007). Indian Medicinal Plants: An Illustrated Dictionary, p. 22. 1st ed. LLC, Springer Science + Business Media, Berin.

Kishimoto, T., Yamamoto, K., Sugimoto, T., et al. (1986). Side effects of extracorporeal shock-wave exposure in patients treated by extracorporeal shock-wave lithotripsy for upper urinary tract stone. *Eur Urol*, **12**(5):308-313.

Kohri, K., Nomura, S., Kitamura, Y., et al. (1993). Structure and expression of the mRNA encoding urinary stone protein (osteopontin). *J Biol Chem*, **268**(20):15180-15184.

Laroubi, A., Touhami, M., Farouk, L., et al. (2007). Prophylaxis effect of *Trigonella foenum graecum* L. seeds on renal stone formation in rats. *Phytother Res*, **21**(10):921-925.

Leal, J. J., and Finlayson, B. (1977). Adsorption of naturally occurring polymers onto calcium oxalate crystal surfaces. *Invest Urol,* **14**(4):278-283.

Li, X., Wang, W., Yu, S., et al. (2017). Inhibitory effect of an aqueous extract of *Radix Paeoniae Alba* on calcium oxalate nephrolithiasis in a rat model. *Renal Failure*, **39**(1):120-129.

Liang, Q., Li, X., Zhou, W., et al. (2016). An Explanation of the Underlying Mechanisms for the In Vitro and In Vivo Antiurolithic Activity of *Glechoma longituba*. *Oxid Med Cell Longev*, (1):3134919.

Liao, L. L., and Richardson, K. E. (1972). The metabolism of oxalate precursors in isolated perfused rat livers. *Arch Biochem Biophys*, **153**(2):438-448.

Lin, W. C., Lai, M. T., Chen, H. Y., et al. (2012). Protective effect of *Flos carthami* extract against ethylene glycol-induced urolithiasis in rats. *Urolithiasis*, **40**(6):655-661.

Lu, Y., Hu, Y. L., Kong, X. F., et al. (2008). Selection of component drug in activating blood flow and removing blood stasis of Chinese herbal medicinal formula for dairy cow mastitis by hemorheological method. *J Ethnopharmacol*, **116**(2):313-317.

Maeda, K., Kuzuya, M., Cheng, X. W., et al. (2003). Green tea catechins inhibit the cultured smooth muscle cell invasion through the basement barrier. *Atherosclerosis*, **166**(1):23-30.

Maheshwari, R. K., Singh, A. K., Gaddipati, J., et al. (2006). Multiple biological activities of curcumin: a short review. *Life Sci,* **78**(18):2081-2087.

Menon, M., and Resnick, M. I. (2002). **In**: Campbell's Urology 8th ed, Urinary lithiasis: Etiology, diagnoses, and medical management, pp. 3229– 3305. Walsh PC, Retik AB, Vaughan ED, Wein AJ. Eds. WB Saunders, Philadelphia.

Miao, H., Plessis, J. D., Preez, J. D., et al. (2008). Transport of aspalathin, a Rooibos tea flavonoid, across the skin and intestinal epithelium. *Phytother Res*, **22**(5):699-704.

Mo, L., Huang, H. Y., Zhu, X. H., et al. Tamm-Horsfall protein is a critical renal defense factor protecting against calcium oxalate crystal formation. *J Urology*, **175**(1):1159-1166.

Moe, O. W. (2006). Kidney stones: pathophysiology and medical management. *Lancet*, **367**(9507):333-344.

Naghii, M. R., Eskandari, E., Mofid, M., et al. (2014). Antioxidant therapy prevents ethylene glycol-induced renal calcium oxalate crystal deposition in Wistar rats. *Int Urol Nephrol*, **46**(6):1231-1238.

Niimi, K., Yasui, T., Hirose, M., et al. (2012). Mitochondrial permeability transition pore opening induces the initial process of renal calcium crystallization. *Free Radical Bio Med*, **52**(7):1207-1217.

Nogata, Y., Ohta, H., Ishii, T., et al. (2010). Isolation of eriocitrin (eriodictyol 7-O-rutinoside) as an arachidonate lipoxygenase inhibitor from Lumie fruit (*Citrus lumia*) and its distribution in Citrus species. *J Sci Food Agr*, **87**(1):82-89.

Nojavan, S., Khalilian, F., Kiaie, F. M., et al. (2008). Extraction and quantitative determination of ascorbic acid during different maturity stages of *Rosa canina L*. fruit. *J Food Compos Anal*, **21**(4):300-305.

Okwu, D. E., and Nnamdi, F. U. (2011). Two novel flavonoids from *Bryophyllum pinnatum* and their antimicrobial activity. *J Chem Pharm Res*, **3**:1-10.

Onaran, M., Orhan, N., Farahvash, A., et al. (2016). Successful Treatment of Sodium Oxalate Induced Urolithiasis with *Helichrysum* Flowers. *J Ethnopharmacol*, **186**:322-328.

Orhan, D. D., Hartevioglu, A., Kupeli, E., et al. (2007). In vivo anti-inflammatory and antinociceptive activity of the crude extract and fractions from *Rosa canina L.* fruits. *J Ethnopharmacol*, **112**(2):394-400.

Poonguzhali, P. K., and Chegu, H. (1994). The influence of banana stem extract on urinary risk factors for stones in normal and hyperoxaluric rats. *Brit J Urol*, **74**(1):23-25.

Prabhu, V. V., Sathyamurthy, D., Ramasamy, A., et al. (2016). Evaluation of protective effects of diosmin (a citrus flavonoid) in chemical-induced urolithiasis in experimental rats. *Pharm Biol*, **54**(7-9):1513-1521.

Rathod, N. R., Biswas, D., Chitme, H. R., et al. (2012). Anti-urolithiatic effects of *Punica granatum* in male rats. *J Ethnopharmacol*, **140**(2):234-238.

Saddiqe, Z., and Naeem, I. A. (2010). A review of the antibacterial activity of *Hypericum perforatum L. J Ethnopharmacology*, **131**(3):511-521.

Schepers, M. S. J., Ballegooijen, E. S. V., Bangma, C., et al. (2005). Oxalate is toxic to renal tubular cells only at supraphysiologic concentrations. *Kidney int*, **68**(4):1660-1669.

Seshadri, V., Batta, A. K., and Rangaswamy, S. (1971). Phenolic components of *Bombax malabaricum* (Root-Bark). *Curr Sci India,,* **40**:630–631.

Sezik, E., Aslan, M., Yesilada, E., Ito, S. (2005). Hypoglycaemic activity of *Gentiana olivieri* and isolation of the active constituent through bioassay-directed fractionation techniques. *Life Sci*, **76**: 1223–1238.

Sezik, E., Yesilada, E., Honda, G., et al. (2001). Traditional medicine in Turkey X Folk medicine in Central Anatolia. *J Ethnopharmacol*, **75**:95–115.

Sharmistha, C., and Jogen, C. K. (2012). Antihyperglycaemic effect of flower of P*hlogacanthus Thyrsiflorus Nees* on streptozotocin induced diabetic mice. *Asian Pac J Trop Biomed*, **2**(3):1357-1361.

Silva, J., Rigaud, J., Cheynier, V., et al. (1991). Procyanidin dimmers and trimers from grape seeds. *Phytochemistry*, **30**:1259–1264.

Soundararajan, P., Mahesh, R., Ramesh, T., et al. (2006). Effect of Aerva lanata on calcium oxalate urolithiasis in rats. *Indian J Exp Biol*, **44**(12):981-986.

Sridharan, B., Michael, S. T., Arya, R., et al. (2015). Beneficial effect of *Citrus limon* peel aqueous methanol extract on experimentally induced urolithic rats. *Pharm Biol*, **54**(5):759-769.

Sumathi, R., Jayanthi, S., Kalpanadevi, V., et al. (1993). Effect of DL alpha-lipoic acid on tissue lipid peroxidation and antioxidant systems in normal and glycollate treated rats. *Pharmacol Res*, **27**(27):309-318.

Sun, M., Zhang, J. J., Shan, J. Z., et al. (2009). Clinical observation of Danhong injection (herbal TCM product from *Radix Salviae miltiorrhizae* and *Flos Carthami tinctorii*) in the treatment of traumatic intracranial hematoma. *Phytomed Int J Phytother Phytopharmacol*, **16**(8):683-689.

Tardif, J. C., Côté, G., Lespérance, J., et al. (1997). Probucol and multivitamins in the prevention of restenosis after coronary angioplasty. *New Engl J Med*, **337**(6):365-372.

Tavichakorntrakool, R., Prasongwattana, V., Sungkeeree, S., et al. (2012). Extensive characterizations of bacteria isolated from catheterized urine and stone matrices in patients with nephrolithiasis. *Nephrol, dial, transpl,* **27**(11):4125-4130.

Tayefi-Nasrabadi, H., Sadigh-Eteghad, S., and Aghdam, Z. (2012). The effects of the hydroalcohol extract of *Rosa canina L*. fruit on experimentally nephrolithiasic Wistar rats. *Phytother Res,* **26**(1):78–85.

Thamilselvan, S., Khan, S. R., and Menon, M. (2003). Oxalate and calcium oxalate mediated free radical toxicity in renal epithelial cells: effect of antioxidants. *Urolithiasis*, **31**(1):3-9.

Thirunavukkarasu, V., Anuradha, C. V., and Viswanathan, P. (2003). Protective effect of fenugreek (T*rigonella foenum graecum*) seeds in experimental ethanol toxicity. *Phytother Res*, **17**(7):737.

Tiselius, H. G. (2003). Epidemiology and medical management of stone disease. *BJU Int*, **91**(8):758-767.

Tracy, C. R., Henning, J. R., Newton, M. R., et al. (2014). Oxidative stress and nephrolithiasis: a comparative pilot study evaluating the effect of pomegranate extract on stone risk factors and elevated oxidative stress levels of recurrent stone formers and controls. *Urolithiasis*, **42**(5):401-408.

Tsujihata, M., Yoshioka, I., Tsujimura, A., et al. (2011). Why does atorvastatin inhibit renal crystal retention?. *Urolithiasis*, **39**(5):379-383.

Tugcu, V., Kemahli, E., Ozbek, E., et al. (2008). Protective effect of a potent antioxidant, pomegranate juice, in the kidney of rats with nephrolithiasis induced by ethylene glycol. *J Endourol*, **22**(12):2723-2731.

Umekawa, T., Iguchi, M., Uemura, H., et al. (2006). Oxalate ions and calcium oxalate crystal-induced up-regulation of osteopontin and monocyte chemoattractant protein-1 in renal fibroblasts. *BJU Int*, **98**(3):656-660.

Wang, S., Peng, D., Ning, Z., et al. (2016). Oligomeric proanthocyanidins protect against HK-2 cell injury induced by oxalate and calcium oxalate monohydrate crystals. *Urolithiasis*, **44**(3):203-210.

Wang, X., Krambeck, A. E., Jr, W. J., et al. (2014). Distinguishing characteristics of idiopathic calcium oxalate kidney stone formers with low amounts of Randall's plaque. *Clin J Am Soc Nephrol*, **9**(10):1757-1763.

Wesson, J. A., Worcester, E. M., Wiessner, J. H., et al. (1998). Control of calcium oxalate structure and cell adherence by urinary macromolecules. *Kidney Int*, **53**(4):952-957.

Woottisin, S., Hossain, R. Z., Yachantha, C., et al. (2011). Effects of *Orthosiphon grandiflorus, Hibiscus sabdariffa* and *Phyllanthus amarus* Extracts on Risk Factors for Urinary Calcium Oxalate Stones in Rats. *J Urology*, **185**(1):323-328.

Wu, H., Lozano, and G. (1994). NF-kappa B activation of p53. A potential mechanism for suppressing cell growth in response to stress. *J Biol Chem*, **269**(31):20067.

Xiang, S., Zhou, J., Li, J., et al. (2015). Antilithic effects of extracts from different polarity fractions of *Desmodium styracifolium* on experimentally induced urolithiasis in rats. *Urolithiasis*, **43**(5):433-439.

Yadav, M., Gulkari, V. D., and Wanjari, M. M. (2016). *Bryophyllum pinnatum* Leaf Extracts Prevent Formation of Renal Calculi in Lithiatic Rats. *Anc Sci Life*, **36**(2):90-97.

Yuen J W M, Gohel M D I, Poon N W, et al. The initial and subsequent inflammatory events during calcium oxalate lithiasis. *Clinica Chimica Acta*, 2010, 411(15–16):1018-1026.

Zhai, W., Zheng, J., Yao, X., et al. (2013). Catechin prevents the calcium oxalate monohydrate induced renal calcium crystallization in NRK-52E cells and the ethylene glycol induced renal stone formation in rat. *Bmc Complem Altern M*, **13**(1):228.

Zhang, H., Li, N., Li, K., et al. (2014). Protective effect of *Urtica dioica* methanol extract against experimentally induced urinary calculi in rats. *Mol Med Rep*, **10**(6):3157-3162.

Zhao, M., Duan, J. A., and Che, C. T. (2007). Isoflavanones and their O-glycosides from *Desmodium styracifolium*. *Phytochemistry*, **68**(10):1471-1479.

Zhou, J., Jin, J., Li, X., et al. (2017). Total flavonoids of *Desmodium styracifolium* attenuates the formation of hydroxy-L-proline-induced calcium oxalate urolithiasis in rats. *Urolithiasis*, 1-11.

Table 1. The flavonoid-rich plant extracts which have the potential to be applied in the prevention of urinary stone disease.

Identified flavonoids in plant extracts needing to be given to animal models of urinary stone disease in the following studies	**The flavonoid-rich plant extracts requiring for further qualitative analysis**
Quercitrin, afzelin, quercetin-3, luteolin, gossypetin, kaempferol-3-O-rutinoside, kaempferol-3-O-glucoside, vicenin 1, vicenin 2, schaftoside, isoschaftoside, isovitexin, carthamin, neocarthamine , kaempferol-3-O-rhamnoglucoside, helichrysins A and B, kaempferol, apigenin, naringenin, isoastragalin, isosalopurposide, hyperoside, isoquercitrin, *trans*- and *cis*-tiliroside, hesperidin, eriocitrin, mangiferin, kaempferol-3,7-di-O–glucoside, kaemepferol-3-O-rhamnoside	The *Pinus eldarica* fruit extracts, extracts from the leaves of *Bryophyllum pinnatum*, *Hibiscus sabdariffa* and *Phyllanthus amarus*, extracts from the flowers of *Phlogacanthus thyrsiformis*, extracts from the seeds of *Trigonella foenum graecum*, OPCs

OPCs: Oligomeric proanthocyanidins

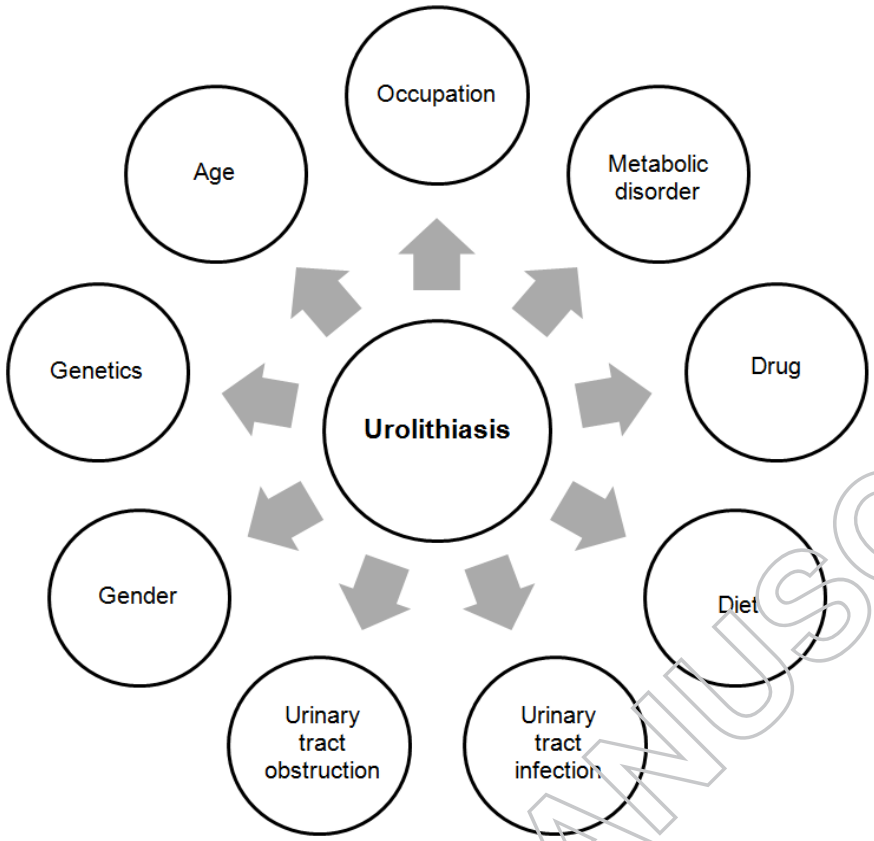

Figure. 1 Multiple risk factors relating with urolithiasis (Gambaro 2004).

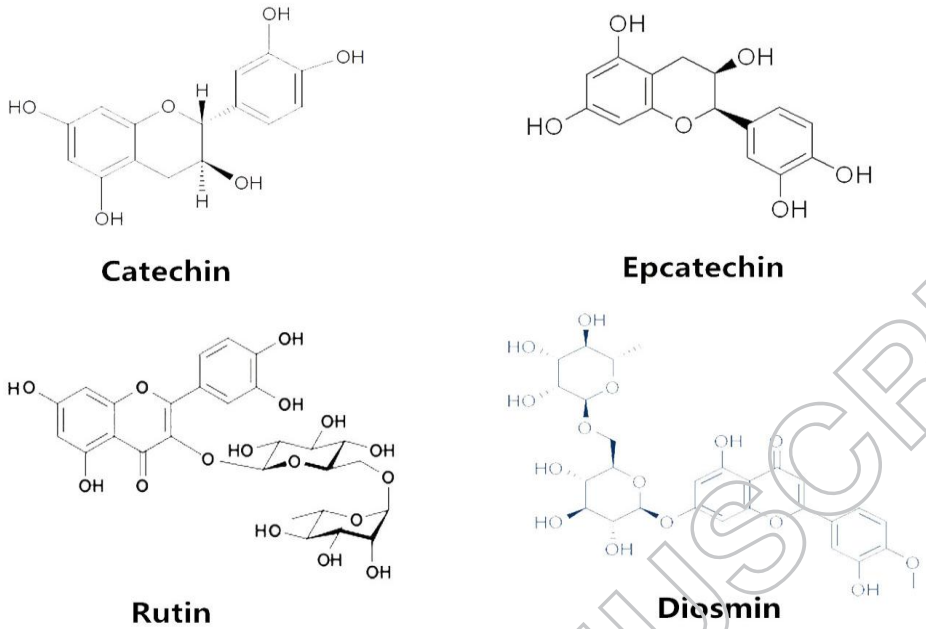

Figure. 2 The chemical structures of flavonoids endowed with anti-urolithiasis activity.

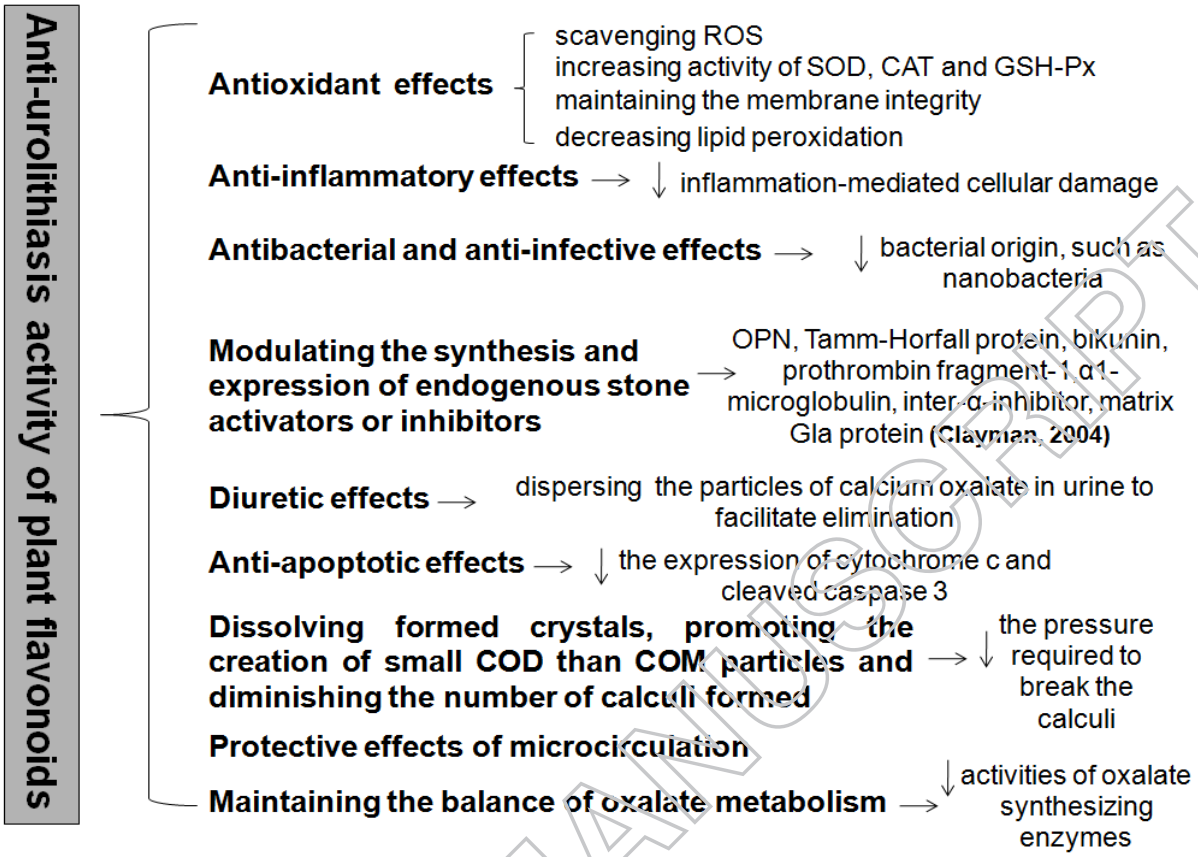

Figure. 3 Anti-urolithiasis activity of plant flavonoids. ↓: Reducing effect.