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Original Article

In vitro antilithiatic properties of five medicinal plants towards calcium phosphate mineralization

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Abstract

Urolithiasis is a disease afflicting mankind since centuries and is a universal health problem even today. Many plants are being used as a traditional medicine for the treatment of urolithiasis. In recent years there has been a resurgence of interest in such medicinal plants. Five plants *Cocos nucifera, Achyranthes aspera, Zingiber officinale, Terminalia arjuna* and *Carum copticum* were selected based on their use since ancient time for urolithiasis treatment. The extract of these plants were analyzed and compared for their anticalcifying ability using homogenous system of mineralization. Their effect was studied on initial mineral phase formation, growth and demineralization of preformed mineral phase. Of all five plants, *Terminalia arjuna* showed the maximum inhibitory potency followed by *Cocos nucifera* and *Carum copticum* which possess moderate ability to inhibit mineralization, followed by *Achyranthes aspera* and *Zingiber officinale*. Although *Achyranthes aspera* showed about 90% inhibitory potency towards initial mineral phase formation, its ability to inhibit growth and cause demineralization of preformed mineral phase was negligible. The exact mechanism by which these plants inhibit mineralization is not understood, but it could be suggested that these plants have biomolecules possessing the activity to inhibit mineralization. There are more than one biomolecules responsible for this action. In *Achyranthes aspera, Terminalia arjuna* and *Carum copticum* biomolecules having molecular weight more than and less than 10 kD possess comparable inhibitory potential. On the other hand in *Cocos nucifera* and *Zingiber officinale* the biomolecule(s) having molecular weight less than 10 kD have higher inhibitory potency.

Keywords: Antilithiatic, *Cocos nucifera, Achyranthes aspera, Zingiber officinale, Terminalia arjuna, Carum copticum*

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1. Introduction

Urolithiasis, the formation of calculi in urinary tract is a disease afflicting mankind and continues to pose a universal health problem, more or less throughout the world even today. Among all the major types of crystalline substances involved in renal stone formation, calcium oxalate and calcium phosphate renal stones are the predominant type in Europe, North America, Australia, South Africa and India.

Although calcium oxalate is the major component of most renal stones, 29% of all stones contain calcium phosphate, mostly in the form of hydroxyapetite [1]. It has been proposed that hydroxyapatite forms the nidus for all calcium-based stones. The most important phosphate-containing calculi involved in urinary stone disease are carbonate apatite, brushite, and struvite. Calcium and phosphate are present in urinary stones as either apatite (the principal constituent of bones and teeth) or brushite (calcium monohydrogen phosphate) [2].

The formation of such concretion encompasses several physico-chemical events beginning with crystal nucleation, growth, aggregation and ending by retention within urinary tract. The mechanisms governing the induction of all these processes remain speculative. One of the important phenomena that characterize urolithiasis is its high recurrence. Developments of modern techniques have revolutionized surgical management of the problem, yet not much progress has been made towards its medical management. Therefore, it is worthwhile to look for an alternative to these conventional methods by using medicinal plants or phytotherapy.

A large number of Indian medicinal plants are being routinely used by practitioners of Ayurvedic system of medicine in the treatment of urinary stone disease. Interestingly, the areas having high consumption of these plant products, reported a very low incidence of urolithiasis. The prevalence of urolithiasis has showed considerable differences in different geographic areas of Italy; the rate is higher in southern Italy and much lower in northern Italy (about half of the south) [3]. There is a wide difference in the eating habits of north and south Italy, so it is possible that northern Italy have a particular ingredient in their food which is absent in southern Italy and that is responsible for the difference in occurrence of urinary calculi. In addition, two distinct stone belts having a high incidence of urinary calculi have been identified in northern and central India. Dietary patterns have been thought to play an important role for varied incidence of urinary calculi in the specific regions [4].

In recent years there has been a resurgence of interest in medicinal plants that are effective, safe and culturally acceptable. However, only few studies have been reported to assess their beneficial effects in treating urinary calculi. Hence, we propose to conduct detailed studies on some of the traditional medicinal plants helpful in reducing ailments of urolithiasis using *in vitro* homogeneous system of mineralization.

Five plants *Cocos nucifera* (L.) (Arecaceae), *Achyranthes aspera* (L.) (Amaranthaceae), *Zingiber officinale* (Rosc.) (Zingiberaceae), *Terminalia arjuna* (Roxb.) Wight & Arn f. (Combretaceae) and Carum copticum (L.) Benth. & Hook. f. (Apiaceae) were selected based on their use since ancient times for renal stones treatment. The extract of these plants were analyzed and compared for their ability to inhibit or stimulate mineralization under *in vitro* physiological conditions.

2. Materials and Methods:

Plant materials

All the plant materials were collected from local market and nearby fields, identified and then authenticated by microscopical and physiochemical data. Roots of *Achyranthes aspera* and *Zingiber officinale,* bark of *Terminalia arjuna* and seeds of *Carum copticum* were dried, powdered and stored before extraction. Milky water from fresh *Cocos nucifera* was taken and stored at -20^{o}C.

Preparation of extracts

The effective part of three plants viz. *Achyranthes aspera, Zingiber officinale* and *Carum copticum* is weighed and soaked in double-distilled water (10%w/v) overnight at 4°C. The extract so obtained was filtered through muslin cloth and subjected to centrifugation at 5000 rpm for 30 min at 4°C in a cold centrifuge. The supernatant thus obtained is referred to as aqueous extract. *Cocos nucifera* extract was prepared by centrifugation of its milky water at 5000 rpm for 30 min at 4°C in a cold centrifuge. Decoction of *Terminalia arjuna* was done by boiling 10%w/v aqueous extract till its volume is halved. These extracts were employed to study their effect on initial, growth and demineralization of calcium and phosphate ions precipitation.

Homogeneous system of mineral phase formation

Mineralization system already standardized in our laboratory was used to study the extent of *in vitro* mineral phase formation in the absence of any matrix [5]. This *in vitro* homogeneous assay system was modified by replacing 17.5 mM barbital buffer with1.0 M Tris buffer (pH 7.4) [6]. The homogenous assay system was made with a final volume of 1 or 5 ml. The $Ca^{2+}$and HPO_4^{2-} ions concentration in above samples represented the extent of precipitation of these ions in the presence and absence of plant extract(s), which has occurred in the form of mineral phase. Calcium and phosphate ion concentrations in the sample were estimated.

Phytochemical screening

Various chemical tests were carried out on the aqueous extract using standard procedures to identify the constituents such as tannins, saponins, terpenoids, flavonoids and alkaloids.

Data analysis

The data are represented as the mean ± standard deviation of five replicates.

The results showed that all the five plants are showing inhibitory effect towards mineralization. *Achyranthes aspera* (84.9% for Ca^{2+} and 76.7% for HPO_4^{2-}) shows

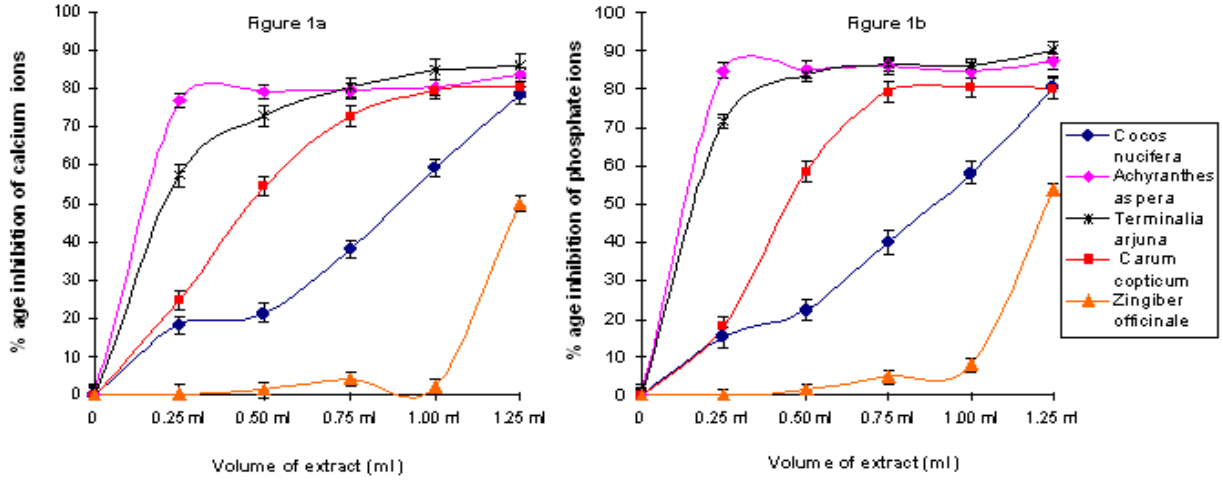

Figure 1: Effect of varied volumes of different plants extract on initial mineral phase formation. Figure 1a Percentage inhibition of calcium ions by different plants extracts. Figure 1b Percentage inhibition of phosphate ions by different plants extracts. (Values are mean ± SD of 5 determinations).

maximum inhibition towards mineralization with as less as 0.25 ml of extract followed by Terminalia arjuna but *Cocos nucifera* and *Zingiber officinale* shows very less percentage inhibition of

both calcium and phosphate ions at this volume of plant extract. However with increase in volume of plant extract the inhibition of mineralization by *Achyranthes aspera* remains almost constant and marginally increased upto 83.8% and 87.4% for Ca^{2+} and HPO_4^{2-} ion respectively at 1.25 ml, while in the case of other plants like *Cocos nucifera, Terminalia arjuna, Carum copticum* and *Zingiber officinale* with increase in volume of plant extract, their ability to inhibit mineralization increased considerably. *Terminalia arjuna* have the maximum inhibitory potency towards mineralization during initial mineral phase formation, which is about 86.11% and 90.51% for Ca^{2+} and HPO_4^{2-} ion respectively.

The aqueous extract of all five plants was qualitatively screened for proteins and various phytochemicals viz. tannins, saponins, terpenoids, flavonoids and alkaloids as depicted in Table 1. All plants showed the presence of proteins and terpenoids. Tannins were found to be present in *Achyranthes aspera*, *Terminalia arjuna and Carum copticum*. All plants showed the presence of saponins except *Carum copticum*. But presence of phytochemical flavonoids was shown by two plants *Terminalia arjuna and Carum copticum*.

Discussion

When the homogeneous system of *in vitro* mineralization was employed under physiological conditions of temperature, pH and ionic strength of the media, the Ca^{2+} and HPO_4^{2-} ions got precipitated as solid mineral phase as hydroxyapetite ($Ca_{10}(PO_4)_6(OH)_2$) in nature. Using such a system, the effect of all the five plants on the initial mineral phase formation was investigated. Usually the plants extract possessing antilithiotropic properties exert their action on the body by altering ionic composition of urine [7]. The exact mode of action by which the ionic strength gets altered is not known. Possibly in *in vitro* system the plants extract alter ionic concentration of Ca^{2+} and HPO_4^{2-} may be by stereospecifically regulating mineralization of calcium containing crystals, as most aspartic acid rich proteins like uropontin acts *in vitro* [8] and thus decrease their precipitation.

The inhibitory human proteins preventing further growth of calcium stones in kidneys do so by binding to the surface of calcium crystals and thus preventing further aggregation of calcium salt precipitates [9]. We can hypothetically

Table 1: Qualitative analysis of the tannins, saponins, terpenoids, flavonoids, alkaloids and proteins present in plant extracts

Plants	tannins	saponins	terpenoids	flavonoids	alkaloids	proteins
Cocos nucifera	-	+	+	-	-	+
Achyranthes aspera	+	+	-	+	+	+
Zingiber officinale	-	+	+	+	+	+
Terminalia arjuna	+	+	+	+	-	+
Carum copticum	+	-	-	+	-	+

predict that plants extract under experimentation contains such biomolecule(s) which not only prevents initial nucleation of calcium phosphate precipitation but also have such biomolecule(s) which binds to or get absorbed at mineral phase surface of already formed crystals and thereby block the growth sites.

As compared to the inhibition of initial mineral phase formation, much higher concentration of crude extract was required to inhibit the growth of the preformed mineral phase. This observation can be explained by postulating that the inhibitory biomolecule(s) acts by binding to the surface of crystals. As we go from initial precipitation to the subsequent growth of the preformed mineral phase the ratio of calcium phosphate precipitation to inhibitory biomolecule(s) present in the plants extract decreases as compared to initial mineral phase formation and it is logical to expect much lower inhibition of the growth of mineral phase as compared to initial precipitation. Demineralization is a process of releasing Ca^{2+} and HPO_4^{2-} ions from its bound precipitated form to free state. Here, again *Terminalia arjuna* showed maximum dissolution of preformed calcium phosphate into the aqueous phase.

The aqueous extract of plants was tested for the presence of various phytochemicals. It can be deduced that fraction constitutes saponins, terpenoids, flavonoids and alkaloids (molecular weight range approx. 200-5000 daltons). Tannins are phytochemicals with molecular weight ranging from 500-20,000 daltons, so these compounds were possibly present in both fraction depending on its type and molecular weight. As stated above less *Cocos nucifera* and *Zingiber officinale* possess higher inhibitory potency and phytochemical screening revealed presence of saponins and terpenoids in these plants extract. The inhibitory potency by these extracts can be tentatively attributed to presence of antilithiatic properties of saponins. [10]. Additionally saponins can also disaggregate suspensions of mucoproteins, which are actually promoters of crystallization [7].

Since we are dealing with *in vitro* study which is a static system, the observed effect would be obviously many folds more during dynamic *in vivo* system where there is a continuous draining of water by efficient urinary system of our body. Thus it could be suggested that out of five plants studied, *Terminalia arjuna* showed the maximum inhibitory potency followed by *Cocos nucifera* and *Carum copticum* which possess moderate ability to inhibit mineralization, followed by *Achyranthes aspera* and *Zingiber officinale*. Although *Achyranthes aspera* showed about 90% inhibitory potency towards initial mineral phase formation, its ability to inhibit growth and demineralization of preformed mineral phase was negligible.

References

[1] Beshensky AM, Wesson JA, Worcester EM, Sorokina EJ, Snyder CJ, Kleinman JG. Effects of urinary macromolecules on hydroxyapatite crystal formation. J Am Soc Nephrol 2001;12:2108.

[2] Coe FL, Evan A, Worcester E. Kidney stone disease. J Clin Invest 2005;115(10):2598.

[3] Serio A, Fraioli Epidemiology of nephrolithiasis. A. Nephron 1999;81:26.

[4] Grases F, Costa-Bauza A, Prieto RM. Key Aspects of Myo-Inositol Hexaphosphate (Phytate) and Pathological Calcifications. Nutr J 2006;5:23.

[5] Kabra SG, Kabra V, Banerji P, Jain LK, Bhargava A, Chaturvedi RP. In vitro calculogenesis: methods to develop concretions of desired chemical composition. Ind J Exp Biol 1978;16:212.

[6] Garimella TS, Jolly CI, Narayanan S. In vitro studies on antilithiatic activity of seeds of Dolichos biflorus Linn. and rhizomes of Bergenia ligulata Wall Phytother Res 2001;15:351.

[7] Gurocak S, Kupeli B. Consumption of historical and current phytotherapeutic agents for urolithiasis: a critical review. J Urol 2006;176:450.

[8] Shiraga H, Min W, Van Dusen WJ, Clayman MD, Miner D, Terrell CH, Sherbotie JR, Foreman JW, Przysiecki C, Neilson EG, Hoyer JR. Inhibition of calcium oxalate crystal growth in vitro by uropontin: another member of the aspartic acid-rich protein superfamily. Proc Natl Acad Sci 1992;89:426.

[9] Grover PK, Moritz RL, Simpson RJ, Ryall RL. Inhibition of growth and aggregation of calcium oxalate crystals in vitro--a comparison of four human proteins. Eur J Biochem 1998;253(3):637.

[10] Grases F, Ramis M, Villacampa AI, Costa-Bauza A. Urol Int 1999;62(4):201. Urolithiasis and phytotherapy. Int Urol Nephrol 1994;26(5):507.