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Calcium Chelation: Efficacy of Selected Antiurolithiatic Medicinal Plants

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Abstract

The present investigation focuses on the Calcium chelating property of the leaves of *Ocimum basilicum* (lamiaceae) and fruits of *Solanum torvum* (solanaceae) and *Embelia ribes* (myrsinaceae), three medicinal plants suggested by traditional healers of Kerala for the treatment of urolithiasis.

Keywords: Calcium, Medicinal plants, Kerala

Introduction

Medicinal knowledge in the Indian traditional system encoded for the treatment of various dreadful and life style diseases are not properly translated to the world population. Ayurvedic classics, *Charka Samhita*, *Sushruta Samhita* and*Ashtanga hridayam*elucidate the effect of various herbal formulations to treat many such diseases (Pandaey, 2013). Kidney stone disease continues to be a major health problem affecting nearly 12 percent of the world population in spite of various scientific advancements in the treatment strategies during the last few decades (Anderson.,1967). Complete recovery from this disease is rare because of its high rate of recurrence (Wilkinson, 2001). This scenario calls the need for alternative therapeutic measures for the effective management of urolithiasis. In this context, investigations on 'Pashanabeda' group of plants mentioned in ayurvedic literature have great relevance to develop suitable herbal drugs against urolithiasis.

During the progression of growth inhibition experiments on urinary stone constituents, we observed calcium sequestration property of the herbal extracts of selected members in Solanaceae, Lamiaceae and Myrsinaceae families. In traditional systems of medicines, several species of these three families were reported to have therapeutic properties against various diseases including urolithiasis. Besides, many pharmaceutically relevant bioactive compounds (terpinoids, flavonoids, phenolics, benzoquinones, alkaloids, saponins, tannins, steroids etc.) have also been isolated and characterized from many species of these three families.

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The major phytochemicals reported from the candidate medicinal plants of the present study are: linalool, eugeol, pinene, Ocimene [*Ocimum basilicum*], quercetin, rutin, chlorogenic acid, gallic acid, solanine, torvanol A [*Solanum torvum*]; embelin, embeliol,vilangin,embelinol [*Embelia ribes*]. As cited in the works of many earlier researchers [Vivianeet *al.,* (2016), Jayanmardiet *et al.,*(2002), Arthan *et al*., (2002),Alia *et al.,* (2012),Jaiswal (2012), Souravi and Rajasekharan (2014) and Sudhakaran ,2015)], the leaves of*Ocimum basilicum* and fruits of *Solanum torvum* and *Embelia ribes* have nutraceutical as well as therapeutical potential for the preparation of a variety of dietary products / folk medicine combinations. Hence, in the present study, we selected these three plants for detailed assessment of their calcium chelation property.

Even though calcium is an essential molecule for the normal growth and development of the body, its excess accumulation in various parts of the body may lead to various diseases. In general, such excess accumulation of calcium occurs as an age related metabolic disorder. However, its excess accumulation in arteries, renal tubules, joints and tendons, soft tissues in breasts and brain cellsof the human body very often aggravate various illness like urolithiasis, arteriosclerosis, Gout, Breast calculi, salivary gland stones, testicular microliths, gall stones, dental pulp stones and Alzheimers (Bruce *et al*., 2008; Gerald *et al*.,2003; Sonia et al.,2009; Dalinka *et al.,*1982;Shanmugasundharam *et al.* 2007,Lim *et al.,* 2012; Zeng *et al.*, 2012; Diana and George ,2012,2013). The role of phytoactive molecules that can regularize calcium metabolism by maintaining calcium homeostasis in different cells and organs of the body have not been subjected to detailed investigations. A perusal of literature revealed chelation therapy as an alternative therapeutic strategy suggested for ameliorating heavy metal toxicity (Laurie, 2003; Yang *etal.* 2014). However, this therapy has limited acceptance among medical experts due to harmful effects of Fe-EDTA chelation on kidney health. In the above context, renal protective phytochelators have tremendous scope to replace currently used Fe-EDTA. Moreover, studies conducted by Hedge *et al*. (2009) substantiated the effectiveness of certain biomolecules like clioquinol, curcumin, epigallocatechin as effective metal chelators. Similarly, Lactic acid and Citric acid molecules have also been reported to have calcium chelating property (Patel and Vadalia 2011).

The present investigation focuses on the Calcium chelating property of the leaves of *Ocimum basilicum* (lamiaceae) and fruits of *Solanum torvum* (solanaceae) and *Embelia ribes* (myrsinaceae), three medicinal plants suggested by traditional healers of Kerala for the treatment of urolithiasis.

It is expected that studies on chelation of Calcium ions by plant derived compounds can help to develop novel treatment strategies against urolithiasis, arteriosclerosis, gout and alzheimers.

Material and Methods

Chemicals used for the study

All the chemicals utilized for the experiment like Ethylenediaminetetraacetic acid(EDTA), Erichrome black T indicator, $ZnSO_4.7H_2O$,Ammonia Solution(30%), Ammonium Chloride, $CaCl_2 .2H_2O$ are all analytical grade and are purchased from Merck. The solutions are prepared using Milli Q water.

Preparation of plant extract

The leaves of*Ocimum basilicum* L.(Lamiaceae)(Figure 1.) and fruits of *Solanum torvum* Sw. (Solanaceae)(Figure 2.) were collected from the experimental garden at Kadakasseril, Karunya Lane, Vadavathoor, Kottayam district, Kerala. Fruits of *Embelia ribes* Burm f. (Myrsinaceae) (Figure 3.) were collected from M.S.Swaminathan Research Foundation (MSSRF) , Wayanad district ,Kerala. The plant parts were washed thoroughly in tap water, shade dried, powdered and kept in air tight containers for further analysis/ preparation of appropriate plant extracts.

About 10 g. of each plant materials were taken in separate air tight glass containers and soaked in 90% Ethanol, sealed and kept for 2-4 days. The ethanol extracts of all four plant specimens were filtered and then centrifuged at 4000 rpm for 5 minutes. The supernatant solution of all samples was made up to 100 ml using 90% ethanol. These stock solutions were stored in sealed containers.

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EDTA titration technique to check calcium chelation property

This titration is a complexometric titration. Here calcium can easily form complex with very large molecule Ethylenediaminetetraacetic acid (EDTA) (Hussain., 2007).So the amount of calcium in a given solution can be estimated directly by titrating against standard EDTA solution using Erichrome black T indicator.

Preparation of chemicals for titration

Standard EDTA(0.5M) was prepared using milli Q water. $ZnSO_4.7H_2O$ (0.5M) was prepared in 100 ml volumetric flask for calculating the molarity of EDTA. An ammonia buffer with pH 10 is also prepared.

Preparation of test solution for titration

A control is made with 50ml 0.5 M $CaCl_2$ solution. A test solution with different concentration of plant extracts were prepared as following:

T1- 2ml plant extract+ 50ml 0.5 M $CaCl_2$
T2- 4ml plant extract+ 50ml 0.5 M $CaCl_2$
T3- 5ml plant extract+ 50ml 0.5 M $CaCl_2$
T4- 10ml plant extract+ 50ml 0.5 M $CaCl_2$
T5- 15ml plant extract+ 50ml 0.5 M $CaCl_2$
T6- 20ml plant extract+ 50ml 0.5 M $CaCl_2$

The treatments werekept as such for 2-3 days. Meanwhile some precipitations were observed which werecentrifuged after 3 days of incubation. The supernatant solutionswereused for titration.

Chelation experiment:

The EDTA solutionwas taken in the burette. Exactly 20 ml of the $ZnSO_4.7H_2O$ was taken in a clean conical flask. 2 ml of Ammonia buffer is carefully added to the $ZnSO_4.7H_2O$ and a pinch of Erichrome black T indicator was also added to the solution. This was titrated against EDTA accurately and the end point is marked when a vine red colour changed to blue. The experiment was repeated for 3 times and concordant value of EDTA was noted.

Strength of $ZnSO_4.7H_2O$ were calculated from the equation:

$$\text{strength of solution} = \frac{\text{weight taken} \times 10}{\text{molecular mass}}$$

So the strength of standard EDTA was calculated using the equation:

$M_1V1 = M_2V_2$

The estimation of calcium content in the control as well as the treatments were carried out using EDTA titration method. The standard EDTA solution was taken in the burette and exactly 10ml of the solutions were taken from control and treatments separately and titrated against EDTA solution after adding 2ml of Ammonia buffer and a pinch of Erichrome black T indicator. The end point is marked when wine red coloured solution changed to blue colour. The experiment was repeated for each of the treatments and control for 3 times and concordant values were noted accurately.

Strength of calcium in the solutions were calculated using the formula:

$M_1V1 = M_2V_2$

The amount of Calcium present in given solutions were calculated by the formula:

$$\text{Amount of Calcium} = \frac{\text{Strenghth of Ca ion in the solution} \times \text{Atomic mass of Ca}}{10}$$

The calcium chelation efficacy (% efficiency of calcium chelation) of the candidate medicinal plant extracts (*O.basilicum*,*S. americanum, E,ribes*) was calculated from the estimated strength of calcium in different treatments and its control by the equation:

$$\frac{\text{Amount of Calcium in Control} - \text{Amount of Calcium in treatment} \times 100}{\text{Amount of Calcium in Control}}$$

Results and Discussion

EDTA Standardization

The strength of EDTA solution prepared was accurately calculated by titrating against $ZnSO_4.7H_2O$ as 0.55 M. and this value was used to calculate the amount of calcium in control and treatments.

Estimation of Calcium Chelation in selected medicinal plants

In the control the amount of calcium was estimated by using the Standard EDTA(0.55 M) by employing complexometrc titration.Amount of Ca present in 50 ml $CaCl_2$ was obtained as 1.1406 g.Similarly amount of Ca^{++} ions in different concentrations of test solutions using*Ocimum basilicum. Solanum torvum and Embelia ribes* plant extracts were calculated and are depicted in the table 1 and Figure 1 respectively.A clear picture about the potent calcium chelation effect

of the leaf, fruit extracts of selected aniturolithiatic plants can be understood from the results shown in the graph.

The leaf extracts of *Ocimum basilicum* L. manifests the most capability to chelate calcium as the amount of calcium in the solution has chelated to almost half the initial concentration i.e. 0.551g. It is also noted thatitscalcium chelation property remains stable and independent of the concentration of plant extracts after T3. Phytochemical studies on*O. basilicum* reported the presence of menthone, estragol, isoneomenthol, menthol, pulegone, Limonene, α-Cadinol , chicoric acid, rosmarinic acid, caftaric acid(Figure.5)(Syedea *et al.,*2012; Javarmandi 2002, Junguin Lee.,2009). These chemical components are rich in carbonyl carbon and hydroxyl groups that are proved to be efficient to chelate metal ions with their lone pairs of electron.

As revealed in this study, the fruit extract of *Solanum torvum* Sw. also shows calcium chelating property and its effectiveness is dose dependent. Bioactive compounds such as rutin, neochlorogenic acid, solagenin, isoquercitrin, quercetin, kaemferol, torvanol A (Figure 6.) (Jaiswal., 2012; Arthan.,2002, Zubaida *et al*., 2013) have been reported from this medicinal plant. However, further studies are needed to substantiate the comparative efficacy of thesephytochemicals to sequester Ca^{++} ions under ideal experimental conditions.

Dose dependent calcium chelation property was also observed in the fruit extract of*Embeliaribes*. However, its overall effectiveness is less when compared to that of *Solanum torvum*and *Ocimumbasilicum*. The fruits of *E.ribes* are rich in benzoquinones like embelin, embelinol, vilangin (Figure 7.) (Souravi and Rajasekharan, 2014).It is to be noted thatphytochemicals mentioned in this study from *S.torvum* and *E.ribes*are rich in functional groups like carbonyl group along with hydroxyl group. These functional moieties are capable of attracting the positive calcium ions and chelate or complex with calcium using the lone pairs in oxygen of carbonyl carbon and replacing hydrogen in hydroxyl group and chances for hydrogen bonds are also high. Thus the specific structural details of the phytochemicals of the candidate medicinal plants suggest the possible mechanism of calcium chelation as evinced in the present study. The Fe-EDTA chelators which are efficient in Ca^{++} chelation is mechanized by the attraction between negative functional groups and positive calcium ions hence our assumption proves the scientific ground for Ca^{++} chelation.

Conclusion

To conclude thefindings, the leaf extract of *Ocimum basilicum*(lamiaceae) showed the maximum calcium chelation property followed by the fruit extracts of *Solanum torvum*(Solanaceae) and *Embelia ribes* (Myrsinaceae). Bioactivity guided fractionsand structural elucidation of the compounds responsible for the calcium sequestration effect from these plants would be helpful to develop suitableherbal drugs for treatingcalcium accumulating diseases.

Acknowledgements

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Fig. 1: *Ocimum basilicum* **Fig. 2: *Solanum torvum* Sw.** **Fig. 3: *Embelia ribes***

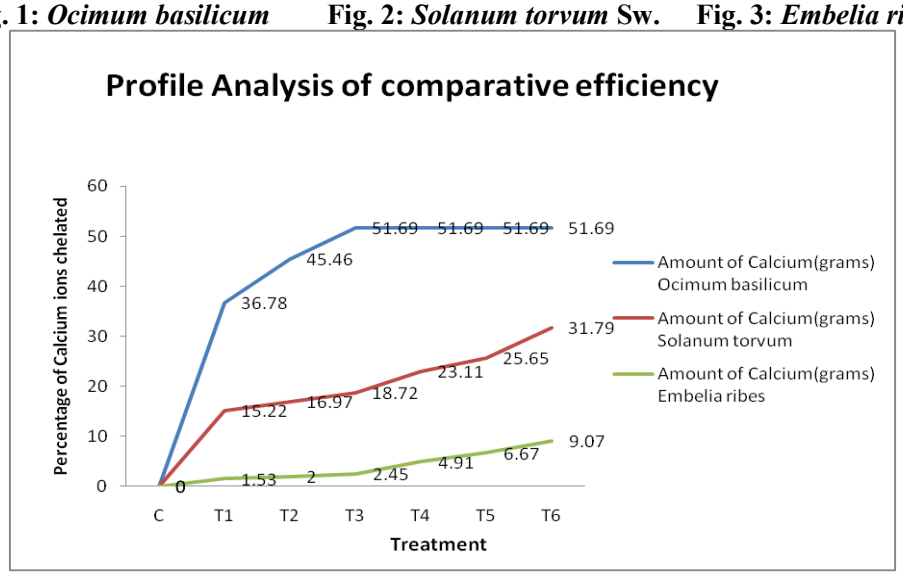

Fig. 4: Percentage efficiency of Ca chelation by *O. basilicum, E. ribes and S . torvum* extracts in relation to control

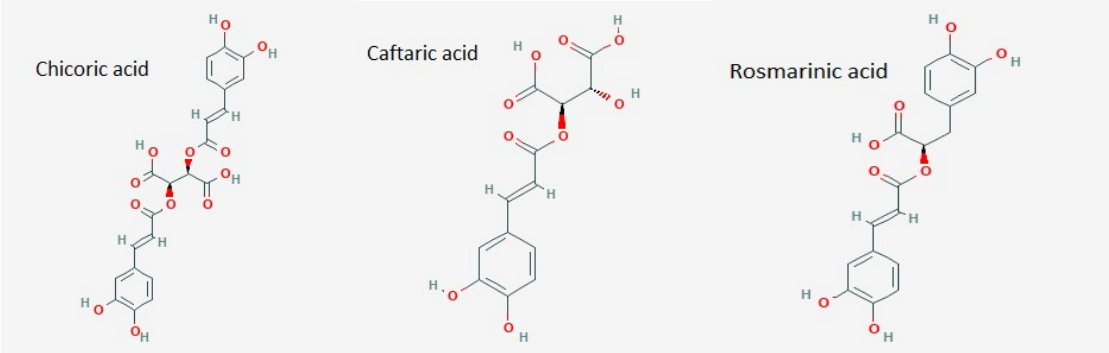

Fig. 5: Phytochemicals in *Ocimum basilicum*

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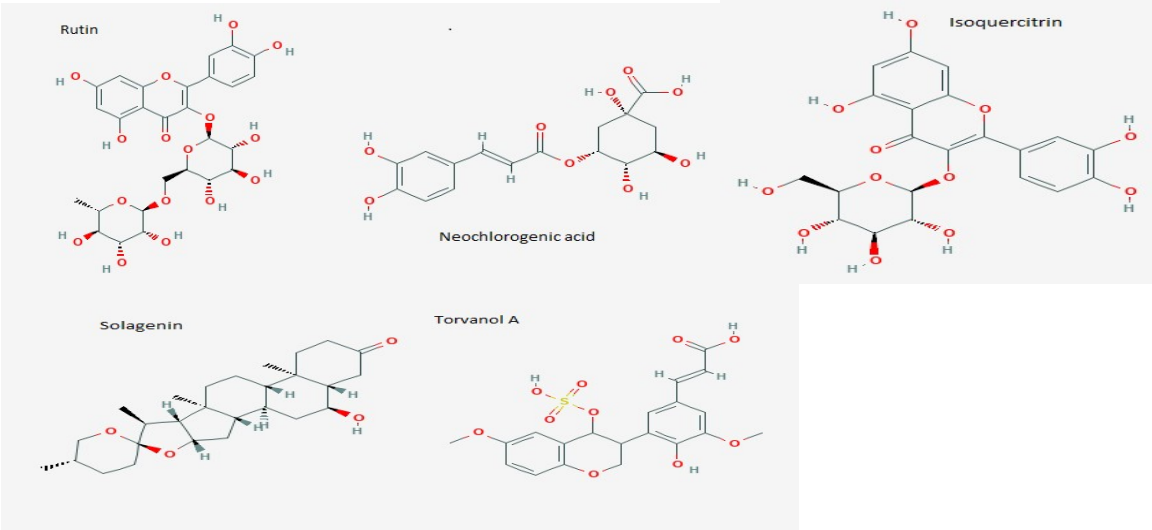

Fig. 6: Phytochemicals in *Solanum torvum*

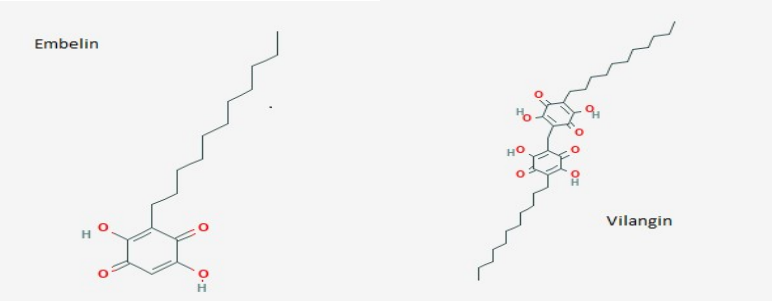

Fig. 7: Phytochemicals in *Embelia ribes*

Table 1: Amount of calcium in differet concentration of test solutions in *O. basilicum, E. ribes* and *S. torvum*

Treatments	Amount of Calcium(grams)		
	Ocimum basilicum	***Solanum torvum***	***Embelia ribes***
C	1.1406	1.1406	1.1406
T1	0.551	0.927	1.1127
T2	0.551	0.877	1.0845
T3	0.551	0.848	1.0645
T4	0.551	0.778	1.0371

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