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ESTIMATION OF IN VITRO CALCIUM RELEASING ACTIVITY OF FRUIT EXTRACTS OF ANNONAMURICATA L

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ABSTRACT

Within the kidney, physiochemical events including, growth, aggregation nucleation supersaturation and retention leads to formation of kidney stone. In the industrialized countries 10-12% of the people suffered with urinary stones.

Annona muricata (A.muricata) belongs to family of Annonaceae and it have long history of usage in the traditional medicine. A.muricata, also called as gunabana, soursop, graviola. It is a fruiting tree and an evergreen plant that is highly prevalent in tropical and subtropical regions and extensively the fruit of this plant is used to prepare shakes, beverages syrups, candies and ice-creams. Different parts of the A.Muricata contributed with different ethno medicinal activities world widely. Specially, indigenous communities of South America and Africa mostly use this plant in their folk medicine. Recent, investigations have reported antidiabetic, anticancer, antimalarial, anticonvulsant anti-arthritic, anti-parasitic and hepatoprotective activities. An in vitro study was designed to evaluate the calcium releasing effect from renal stones using the fruit extract of A.muricata. First of all, calcium releasing property was tested from various sources such as sea shell, cuttlefish bone and renal stone. Additionally, calcium realizing activity was tested at various concentration, ph level and temperature. This study showed that the tested fruit of extract A.muricata had a significant calcium realing effect from renal stone. This activity was dominant in high concentration, acidic medium and high temperature (42□).

Keywords: Annona muricata, renal stones

INTRODUCTION

Up to 5% of the population is affected by kidney stones, among this nearly 8-10% have the risk of passing kidney stone through the life time. High incidence was reported with highly industrialized and developed countries in the world with higher standards of living and it is also strongly associated with ethnicity, race, region and residence. Seasonal variation also influence to some extent. In men, high urinary calcium oxalate saturation occurred in summer and in women, occurred in early winter. Commonly men are affected twice than women. High incidence of men are recorded at a peak age of 30 however, women have bimodal age distribution of 35 – 55 years. One time kidney stone formation would result in approximately 50% risk in the formation of a second stone (Parmar, 2004). "Kidney stones are more common in certain parts of the dry zone and it is contributed with hardness of water" it is a widespread belief in Sri Lanka but there is no proven evidence, for formation of stones due water hardness. However, high consumption of fluoride may act as a mild promoter of formation of urinary stone by excretion of insoluble calcium fluoride and increasing oxalate excretion. If, the drinking water fluoride content rises above 3.5 to 4.9 ppm leads to 4.6 times

higher risk for the formation of urolithiasis. Commonly, Well water in the district of Ampara, Anuradhapura and Polannaruva has a fluoride content higher than 3ppm (Anuruddha, 2004).

Magnesium ammonium and calcium oxalate stones are the most common type of the kidney stones. There are several remedies used to treat urinary stones with age. In traditional medical system, plants are the major component in the remedies and experience the effectiveness the remedies. But there is no proven evidence through the clinical studies for the systemic pharmacological action expected from some plant material and drugs prepared by herbals. Moreover, pharmacotherapy actions of some herbs may reduce the risk of the reoccurrence of the stones. The use of plant products with claimed uses in the traditional systems of medicine assumes importance (Alok et al 2017). Calcium oxalate and magnesium ammonium phosphate are the most common type. Several remedies have been used to treat the urinary stones with different ages. In traditional system, plants are highly used for their remedies and have showed significant results after the use of plant remedies. But there is no well-established systemic pharmacological and clinical studies for some plants and herbal drugs. The risk of recurrence rate is reduced by pharmacotherapy. Therefore, usage of plant products in traditional medical system assumes importance (Aloka et al 2017). Numerous studies have mentioned the medicinal uses of A.muricata and worth of this plant in social life. "Soursop" is endless, slightly upright tree. Rises up to 5-6m height. Immature branches are rusty-hairy with smelly, oblong, elliptic slim obovate leaves, sharp at both ends 2-6cm wide and 6-20 cm long, usually evergreen, interspersed, shiny smooth on the upper surface, olive green, lighter underneath; borne single flowers appear any part of the trunk, branches or twigs. Its short stem is about 4-5cm length. Tri angular cone-shaped and stout. Outer scale plump, slightly spreading and greenish yellow. With these three sets, inner petals scale are lemon yellow in colour. More or less egg-shaped or heart in shaped. Irregular, Iopsided or bended shape occurres due to inappropriate carper growth, insect harm leads to non-uniform, Iopsided or curved shape. The dimensions of the fruit is about 10-30cm long and 15cm width weight nearly 4.5-6.8Kg. The fruit in combination is covered with crisscrossed, roughed-appear but tender inside. Non-edible bitter skin from which stick out few or many dumpy, or more lengthen and bended mushy, flexible "spines". Sharp end is easily broken when fruit is fully ripped. Unripen fruit skin is olive green and it turns to greenish yellow when it ripens. Ripen fruit is mushy to touch. Its inner part is cream colored and granulated and divide simply from the mass of milky white, stalky, juicy parts- mostly like flakes of fresh fish – enclosing the center part, soft, short central core. The smell of the pulp is somewhat pineapple like, but its musky, sub acid to acid in flavor. Most of covered segments are seedless. In each and every productive part, there is a single elliptic, even, firm black seed, nearly 1.25-2cm length; and large fruit may have nearly few dozens to 200 seeds. This plant is all most native to warmest tropical regions in North and South America including Amazon. A.muricata has become immigrant in most of the countries, nowadays it highly spreads in the tropical and subtropical regions worldwide. Including western part of Nigeria (Stephen et al 2006).

According to siddha medicine the sign and symptoms of renal stone is compare with Kalladaippu noii such as pain in the tip of the penis. Sudden obstruction of the urine while voiding, lower back pain, blood pass with urine. There are mainly two types of classification of Kalladaippu, according to the first classification it has four types, Vazhi Kalladaippu, Azhal

Kalladaippu, Iya Kallaippu and Mukkutra Kalladaippu. According to second classification there are 5 types of Kalladaippu, Vazhi Kalladaippu, Azhal Kalladaippu, Iya Kalladaippu, Sukkila Kalladaippu and Sakkara Kalladaippu. This condition can be treated by internal medicine and surgery (Kuppusami, 2007).

Background and justification

Researcher, identified the natural fruit extract of A.muricata have the ability to dissolve calcium which is the most common component of urinary stone. This study may lead to advance in the treatment of calcium stones. Swintariet al., (2017) has mentioned that ethanolic extract of leaves of A.muricata L. leaves combined with Centella asiatica L. improved their effectiveness in removal of calcium kidney stones. However, no studies have been reported on calcium assimilation effect of A. muricata fruit. Therefore, it will be remarkable to study the calcium releasing effect of the fruit of A. muricata plant to fulfill the current time research gap.

Objectives

General objectives

To Estimate the in vitro calcium releasing activity of A. muricata fruit on different calcium sources.

Specific objectives

To evaluate the optimum concentration of the A. muricata L. extract which have the highest calcium realizing activity.

To evaluate the optimum pH which shows the highest calcium realizing activity

To identify the effect of the temperature on calcium realizing activity of A. muricata fruit extract.

LITERATURE REVIEW

Taxonomical description of the plant

Botanical name : Annona muricata L.
Family name : Annonaceae
Tamil name : Mutseethappalma
English name : Sour soap
Sinhala name : Katuanoda

Distribution

A.muricata also known as gunabana, sour soap, graviola, sirsak and paw-paw, belongs to Annonaceae family, which has approximately 130 genera and 2300 species, A.muricata is native to the warmest tropical areas in North and South America. Nowadays, widely distributed in subtropical parts of the world including Nigeria, Malaysia and India (Sejal et al 2015).

Botanical description

Morphology

A.muricata is an olive green, earthly, upright plant growing upto 5-8m (Sejal et al 2015).

Leaves

Characteristic unclosed, rounded canopy with large, shiny, olive green leaves (Sejal et al 2015).

Flowers

A. muricata has an enlarged single yellow flowers on wooded stem. Flowers are large and separately situated, yellow or yellowish green in colour. Three outer scales are mostly ovate with heart-shaped base, inner 3 set of scale big elliptical and rounded (Sejal et al 2015).

Fruits

Fruit of this plant is large, ovate or heart-shaped and green in colour, often irregular asymmetrical composite fruit, from which many fruits lets are originating and may weigh more than 4kg weight. The diameter vary 15-20cm. Inside the fruit, is a white fibrous juicy parts covered with an elongated receptacle. In every productive part there is single ovoid, even hard, black seed about 1.25-2cm length. A fruit may have about 5 to 200 or more seeds. There is a reticulated rough appearance with small spines. Inside is a cream, granulated and simply divided white mass, pulpy juicy parts which is surrounded in a middle mushy core (Sejal et al 2015).

Chemical Composition

Parts of A.muricata plants have rice phytochemical constituent such as Alkaloid, Megastigmanes, Flavonoltriglycosides, Phenolics, Cyclopeptides, Essential oil. However, members of Annona species including A.muricata have rich amount of annonaceousacetogenin compounds. Beyond that, major minerals such as Na, K,Ca,Fe, Mg and Fe are also present in the plant. Consumption of A.muricata fruit may provide essential nutrients and elements to the body (Sejal et al 2015).

METHODOLOGY

Collection of calcium sources

Renal stones, cattle fish bone and sea shells were used as calcium sources. Renal stones which was surgically removed from patients with renal calculi, collected from the Batticaloa general Hospital and Badulla general Hospital. Renal stones were washed by distilled water and was allowed to air dry. Cattle fish bone was collected from local market of Trincomalee and washed by distilled water and allow to air dry. Sea shells collected from Nilaveli coastal area, Trincomalee and wasted by distilled water and allow to air dry.

4.2 Preparation of plant juice:

Ripen Fruits of A. muricata were collected from local market of central province. Skin of the fruits peeled off and the seeds were separated from the pulp. Extracts of pulp were obtained separately. Resulting extract were freeze dried. Solvent free extract was stored in an air tight container at -40oC until further use.

The study was conducted in 4 Phases.

Phase I

Preparation of the fruit juice extract:

30g Dried freeze powder of A. muricata were obtained for each testing and dissolved in 30ml of distilled water. It was centrifugedunder the 10000 rpm for 10 minutesand the supernatant wasseparated in three beakers.

Estimation of calcium realizing capacity on various calcium sources:

Renal stone (2.472g), Cattle fish bone (2.079g) and sea shells (5.272g) were taken and soaked separatelyinto the 25ml of Ripen Fruit extract of A. Muricata for an hour. Meantime ten test tubes were taken and labelled properly. 0.5 ml of calcium buffer and 0.5ml of calcium die were added into the each test tubes. Each tubes were had duplicate settings. After one hour 10µl of soaked supernatant of Ripen Fruit extract ofA. Muricata was added into the Calcium buffer and Calcium die contained test tubes. The mixturewere blended by using vertex mixture. Finally the reading was taken by using spectrophotometer in 570nm wave length. $CaCO_3$ was used as standard. The assay was carried out as duplicate.

Phase II

Preparation of the fruit juice extract:

100mg Dried freeze powder of A. muricata were obtained for each testing and dissolved in 100ml of distilled water. It was centrifuged under the 10000 rpm for

10 minutes and the supernatant was separated in three beakers. 25%, 50%, 75%, 100% concentrated fruit juice were prepared. The assay was carried out six times with different sample (Sample A, B, C, D, E, F).

Evaluate the optimum concentration of the Annonamuricata L. extract which have the highest calcium releasing activity.

Fruit of A

3.008g, 3.102g, 2.982g, 3.007g of renal stone pieces were immersedin the 100%, 75%, 50%, and 25% concentration of Annona muricata freeze dry powder extract respectively.

Fruit of B

3.476g, 3.682g, 3.288g, 3.467g of renal stone pieces were immersed in the 100%, 75%, 50%, and 25% concentration of Annona muricata freeze dry powder extract respectively

Fruit of C

3.362g, 3.157g, 3.244g, 3.178g of renal stone pieces were immersed in the 100%, 75%, 50%, and 25% concentration of Annona muricata freeze dry powder extract respectively

Fruit of D

3.689g, 3.608g, 3.702g, 3.717g of renal stone pieces were immersed in the 100%, 75%, 50%, and 25% concentration of Annona muricata freeze dry powder extract respectively

Fruit of E

2.984g, 3.102g, 3.009g, 3.137g of renal stone pieces were immersed in the 100%, 75%, 50%, and 25% concentration of Annona muricata freeze dry powder extract respectively

Fruit of F

3.326g, 3.217g, 3.028g, 3.128g of renal stone pieces were immersed in the 100%, 75%, 50%, and 25% concentration of Annona muricata freeze dry powder extract respectively

Meantime 50 test tubes were taken and labelled properly. 0.5 ml of calcium buffer and 0.5ml of calcium die were added into the each test tubes. Each tubes were had duplicate settings. After one hour 10µl of soaked supernatant of Ripen Fruit extract ofA. Muricata was added into the Calcium buffer and Calcium die contained test tubes. The mixture were blended by using vertex mixture. Finally the reading was taken by using spectrophotometer in 570nm wave length. CaCO3 was used as standard. The assay was carried out as duplicate. The same process were repeated after two hours too.

Phase III

Preparation of the fruit juice extract:

100mg Dried freeze powder of A. muricata were obtained for each testing and dissolved in 100ml of distilled water. It was centrifuged under the 10000 rpm for 10 minutes and the supernatant was separated in three beakers. 25%, 50%, 75%, 100% concentrated fruit juice were prepared. The assay was carried out six times with different sample (Sample A, B, C, D, E, F). The initial pH level of the extract were recorded. The initial pH of the extract was taken as acid medium. 3mg of NaOH were added to prepare the neutral pH and 6mg of NaOH were added to prepare the alkaline pH.

Evaluate the optimum pH of the Annonamuricata L. extract which have the highest calcium releasing activity.

Fruit of A

3.008g, 3.103g, and 3.028 g of renal stones were immersed in the acid, neutral, and alkalinemedium extract respectively.

Fruit of B

3.476g, 3.265g, and 3.476 g of renal stones were immersed in the acid, neutral, and alkaline medium extract respectively.

Fruit of C

3.362g, 3.404g, and 1.325g of renal stones were immersed in the acid, neutral, and alkaline medium extract respectively.

Fruit of D

3.689g, 3.521g, and 1.528 g of renal stones were immersed in the acid, neutral, and alkaline medium extract respectively.

Fruit of E

3.984g, 4.108g, and 4.064 g of renal stones were immersed in the acid, neutral, and alkaline medium extract respectively.

Fruit of F

3.326g, 3.357g, and 3.409g of renal stones were immersed in the acid, neutral, and alkaline medium extract respectively.

The above mentioned renal stones were immersed in respective extract for one hour. Meantime 50 test tubes were taken and labelled properly. 0.5 ml of calcium buffer and 0.5ml of calcium die were added into the each test tubes. Each tubes were had duplicate settings. After one hour 10µl of soaked supernatant of Ripen Fruit extract ofA. muricatewas added into the Calcium buffer and Calcium die contained test tubes. The mixture were blended by using vertex mixture. Finally the reading was taken by using spectrophotometer in 570nm wave length. $CaCO_3$ was used as standard. The assay was carried out as duplicate. The same process were repeated after two hours too.

Phase IV

Preparation of the fruit juice extract:

150mg Dried freeze powder of A. muricata were obtained for each testing and dissolved in 150ml of distilled water. It was centrifuged under the 10000 rpm for 10 minutes and the supernatant was separated in three beakers as 50 ml in a beaker and labeled properly.The temperature of 32°C, 37°C,42°C were set up in different water bath and the all tubes were kept in respective water bath for 10 minutes. The process was repeated six times with different samples.

Evaluate the optimum temperature of the Annonamuricata L. extract which have the highest calcium releasing activity.

Fruit of A

3.147g, 3.174g, and 3.123 g of renal stones were immersed in the 32°C, 37°C, 42°C temperature respectively.

Fruit of B

3.753g, 3.582g, and 3.561 g of renal stones were immersed in the 32°C, 37°C, 42°C temperature respectively.

Fruit of C

3.597g, 3.596g, and 3.547g of renal stones were immersed in the 32°C, 37°C, 42°C temperature respectively.

Fruit of D

3.881g, 3.951g, and 3.956g of renal stones were immersed in the 32°C, 37°C, 42°C temperature respectively.

Fruit of D

3.124g, 3.174g, and 3.139g of renal stones were immersed in the 32°C, 37°C, 42°C temperature respectively.

Fruit of E

3.643g, 3.645g, and 3.718g of renal stones were immersed in the 32°C, 37°C, 42°C temperature respectively.

Fruit of F

3.998g, 4.001g, and 4.123 g of renal stones were immersed in the 32°C, 37°C, 42°C temperature respectively.

The above mentioned renal stones were immersed in respective temperature extracts for one hour. Meantime 38 test tubes were taken and labelled properly. 0.5 ml of calcium buffer and 0.5ml of calcium

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were added into the each test tubes. Each tubes were had duplicate settings. After one hour 10µl of soaked supernatant of Ripen Fruit extract of A. Muricata was added into the Calcium buffer and Calcium die contained test tubes. The mixture were blended by using vertex mixture. Finally the reading was taken by using spectrophotometer in 570nm wave length. CaCO3 was used as standard. The assay was carried out as duplicate. The same process were repeated after two hours too.

RESULTS

Evaluate Optimum concentration of the Annonamuricata L. extract which have the highest calcium realizing activity.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Grops	.980	3	.327	16.336	.000
Within Groups	.400	20	.020		
Total	1.380	23			

P value is less than 0.05. Therefore it is indicate statistically significant in all variable concentration after the second hour.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
100% con.	6	1.025667	.0975329	.0398176	.923312	1.128021	.9535	1.2185
75% con.	6	.868167	.0972608	.0397066	.766098	.970236	.7375	1.0075
50% con.	6	.630083	.1636100	.0667935	.458385	.801782	.5020	.9370
25% con.	6	.506583	.1850868	.0755614	.312347	.700820	.3890	.8765
Total	24	.757625	.2449593	.0500021	.654188	.861062	.3890	1.2185

The mean in the 100% concentration is highest (1.025667) when compared with other concentration.

After the second hour

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1.462	3	.487	17.973	.000
Within Groups	.542	20	.027		
Total	2.004	23			

P value is less than 0.05 therefore it's indicate statistically significant in all variable concentration after the second hour.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
100% con	6	1.186667	.1349195	.0550806	1.045077	1.328256	.9945	1.4020
75% con	6	.919167	.1158593	.0472993	.797580	1.040753	.8040	1.1150
50% conc	6	.662583	.1684614	.0687741	.485794	.839373	.5170	.9830
25% con	6	.546333	.2201013	.0898560	.315351	.777315	.4250	.9900
Total	24	.828688	.2951899	.0602554	.704040	.953335	.4250	1.4020

The mean in the 100% concentration is highest (1.186667) than the other concentration.

When compare with the means of two tables, mean of 100% concentration when compare with other three concentration is highest. Among that the mean of 100% concentration of second hour is the highest (1.186667)) according to these the calcium releasing activity of maximum in the 100% concentrated solvent after second hour.

5.2 Evaluate the optimum pH in which shows the highest calcium realizing activity is high.

After one hour

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.477	2	.238	22.481	.000
Within Groups	.159	15	.011		
Total	.636	17			

P value is less than 0.05. Therefore it's indicate it was statistically significant in all the three medium after one hour.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Acidic	6	1.025667	.0975329	.0398176	.923312	1.128021	.9535	1.2185
Neutral	6	.780250	.0956372	.0390437	.679885	.880615	.6635	.9370
Alkaline	6	.631000	.1146120	.0467901	.510722	.751278	.5280	.8185
Total	18	.812306	.1933465	.0455722	.716157	.908454	.5280	1.2185

The mean in the acidic medium is highest (1.025667) when compared with alkaline and neutral medium.

After second hour

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.784	2	.392	27.851	.000
Within Groups	.211	15	.014		
Total	.995	17			

P value is less than 0.05 therefore it's indicate it was statistically significant in all the three medium after two hour.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Acidic	6	1.186583	.1349038	.0550743	1.045010	1.328156	.9945	1.4020
Neutral	6	.818917	.1075353	.0439011	.706065	.931768	.7070	.9910
Alkaline	6	.695250	.1115727	.0455494	.578162	.812338	.5595	.8425
Total	18	.900250	.2419064	.0570179	.779953	1.020547	.5595	1.4020

The mean in the acidic medium is highest (1.186583) when compared with alkaline and neutral medium.

When compare with the means of two tables, mean of acidic medium when compare with other two medium is highest. Among that the mean of acidic medium of second hour is the highest (1.186583) according to these the calcium releasing activity is maximum in the acidic medium after the second hour.

Effect of the temperature on calcium realizing activity of Annonamuricata L fruit extract.

After one hour

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.516	2	.258	15.160	.000
Within Groups	.255	15	.017		
Total	.771	17			

P value is less than 0.05 therefore it is indicate statistically significant in all variable Temperature after the first hour.

	N	Mean	Std. Deviation	Std. Error	995% Confidence interval for Mean		Mini mum	Maxi mum
					Lower Bound	Upper Bound		
42°C	6	1.358583	.0672498	.0274546	1.288009	1.429158	1.2775	1.4290
37°C	6	1.025667	.0975329	.0398176	.923312	1.128021	.9535	1.2185
32°C	6	.977917	.1924181	.0785544	.775986	1.179847	.8245	1.3535
Total	18	1.120722	.2130094	.0502068	1.014795	1.226649	.8245	1.4290

The mean in the 42°C is highest (1.358583) when compared with other temperature.

After second hour

	Sum of squares	Df	Mean Square	F	Sig.
Between groups	.536	2	.268	18.789	.000
Within Groups	.214	15	.014		
Total	.751	17			

P value is less than 0.05 therefore it is indicate statistically significant in all variable Temperature after the second hour.

	N	Mean	Std. Deviation	Std. Error	95% confidence interval for mean		Minimu m	Maxim um
					Lower Bound	Upper Bound		
42°C	6	1..498083	.0755013	.0308233	1..418850	1..577317	1.4250	1.6225
37°C	6	1.186583	.1349038	.0550743	1.045010	1.328156	.9945	1.4020
32°C	6	1.094667	.1375811	.0561673	.950284	1.239049	.9080	1.3080
Total	18	1.259778	.2101249	.0495269	1.155285	1.364270	.9080	1.6225

The mean in the 42°C is highest (1.498083) when compared with other temperature.

When compare with the means of two tables, mean of 42°C when compare with other various temperature is highest. Among that the mean of 42°C of second hour is the highest (1.498083) according to these the calcium releasing activity is maximum in the 42°C after the second hour.

DISCUSSION

Urology field of modern medical system reached tremendous advance level but there is still question for the satisfactory antilithiotic drugs with the capable of prevent the stone formation and recurrent re occurrence or dissolve the stones. Therefore, physician have to depend on the other alternative medical systems such as Ayurveda, Siddha and Unani. Herbal preparations are playing the major role of different alternative medical systems. A.muricata is a tropical fruit that grows widely in sri Lanka and it was used to eat as fruit in SriLanka A. muricata fruit extract shows acidity which is helps to realizing calcium from renal stone. This plant was used in kidney disease that was

mentioned in few websites such as "Ayurveda sri Lankan" and parts of the this plant also have the capacity of removing renal stones. Swintariet al., (2017) has concluded that A.muricata leaves' ethanolic extract combined with Centella asiatica L. improved their effectiveness in removal of calcium kidney stones. Therefore, the present study also focused to a fruit extract of A. muricataand aimed to elucidate Calcium releasing against renal stones.

In this invitro study, our data showed that calcium was released from the renal stones concentration, pH, Temperature and time dependent manner. The calcium releasing activity is highest in the high concentration, high temperature and acidic medium. With these time also influenced to calcium releasing activity. This study was showed that extract could release calcium directly from renal stones. 100% concentration of the fruit extract was showed 1.025667 mean value, 75% concentrated extract was showed 0.868167 mean value, 50% concentration extract was showed 0.630083 and 25%% concentration was showed 0.506583 mean value after one hour. 1.186667, 0.919167, 0.662583, and 0.546333 mean values wereshowed after second hour respectively.

Acidic medium was showed 1.025667 mean value, neutral medium was showed 0.78025 mean value and alkaline medium shows 0.631 mean value after first one hour. 1.186583, 0.818917 and 0.695250 mean values were showed after the second hour respectively. 42°C temperature was showed 1.358583 mean value, 37°C temperature was showed 1.025667 mean value and 32°C temperature was showed 0.977917 mean value. 1.498083, 1.186583 and 1.094667 mean values were showed after the second hour respectively. Zeynepet al., (2014) was reported high acid medium leads to reabsorption of renal citrate, therefore reducing its elimination. The study was showed extract of fruit was had acidity (pH 3-4). Therefore, which facilitating reabsorption of citrated and realizing of calcium from the calcium citrate renal stone.

In summary, this work is the first to clearly demonstrate that A. muricatafruit extract releasing the calcium from the renal stones in vitro. And fruit extract of A.muricata could be used to treat patients with nephrolithiasis.

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