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EVALUATION OF SELECTED MEDICINAL PLANTS FOR ANTIUROLITHIATIC ACTIVITY

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ABSTRACT

Keywords:

Allium sativum, *Punica granatum*,
Antiuro lithiatic activity, Titrimetry,
Colorimetry

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The main aim of our study is to evaluate the antiuro lithiatic activity of pomegranate (*punica granatum*, Family: Puniaceae) and garlic (*Allium sativum*, Family: Liliaceae). For this, we have subjected the seeds of pomegranate and garlic bulbs to maceration using 95% of ethanol, ethyl acetate, petroleum ether and water at a ratio of 1:4 for 24 hours, followed by reflux over a water bath for 3 hours and the obtained extract was concentrated under vacuum. The prepared extracts were then subjected to titrimetry and colorimetry for the estimation of calcium and phosphate respectively. By comparing the result of different extracts, we have found that ethanol extract of garlic has showed better result in inhibiting precipitation of calcium and phosphate. But individual isolation and further studies are to be carried out to confirm this activity.

INTRODUCTION: Urolithiasis is a condition that is characterized by formation of calculi in urinary tract. Stone disease is a multifactorial disease, ingestion of excessive amounts of purines, oxalates, calcium phosphate, sodium and other elements often results in excessive excretion of these components in urine thus increase in the risk of calculi formation¹.

Medical conditions that are associated with increased risk of kidney stone formation include hyperparathyroidism, hyperthyroidism, gout, systic fibrosis². Symptoms for urolithiasis include abnormal urine colour, blood in urine, fever, nausea and vomiting³.

Though some synthetic drugs are available in market for treating urolithiasis, herbal medicines have their own emphasis. In the present scenario, the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal value.

By knowing the importance of herbal medicine, we have chosen pomegranate and garlic to evaluate antiuro lithiatic activity.

Punica granatum (Family: Puniaceae) is a tree that grows best in well drained ordinary soil. The edible fruit is a berry and is between a lemon and a grapefruit in size, 5-12 cm in diameter with a rounded hexagonal shape, and has thick reddish skin. The exact number of seeds in a pomegranate can vary from 200 to about 1400 seeds, contrary to some beliefs that all pomegranates have exactly the same number of seeds.

Each seed has a surrounding water-laden pulp-the edible aril-ranging in color from white to deep red or purple. The seeds are embedded in a white, spongy, astringent pulp. juice of pomegranate contains 85.4% water, 10.6% total sugars, 1.4% pectins, 0.2-1.0% polyphenols⁴.

Allium sativum (Family: Liliaceae) is cultivated in well drained moderately clay loamy soil.

Garlic bulbs contain 29% of carbohydrates, about 56% of proteins (albumin), 0.1% of fat, mucilage, and 0.06 to 0.1% of volatile oil. It also contains phosphorus, iron and copper. Volatile oil of the drug is the chief active constituent, and contains allyl propyl disulphide, diallyl disulphide alliin and alliin.

Alliin by action of enzyme allinylase is converted into alliin. Garlic oil is yellow in colour and has specific gravity of 1.046. It is optically inactive⁵

MATERIALS AND EQUIPMENTS:

Plant material used:

- Pomegranate (*Punica grantum*)
- Garlic (*Allium sativum*)

Apparatus used:

- El Colorimeter with matched glass cuvettes
- Type A glass Burette
- Conical flasks
- Beakers
- Digital pH meter

TABLE 1: CHEMICALS USED

S. No.	Chemical Name	Manufactured By
1.	Ethanol	Thermo electron LLS India pvt. Ltd
2.	Sodium Oxalate	Qualikems fine chem Pvt. Ltd
3.	TRIS – Buffer pH-7.4	Thermo electron LLS India pvt. Ltd
4.	Calcium chloride	Qualikems fine chem Pvt. Ltd
5.	Sodium chloride	Qualikems fine chem Pvt. Ltd
6.	Di sodium hydrogen phosphate	Thermo electron LLS India pvt. Ltd
7.	Ethyl acetate	Qualikems fine chem Pvt. Ltd
8.	Petroleum ether	Thermo electron LLS India pvt. Ltd

Reagents preparation:

0.1 M TRIS buffer: 0.1 M TRIS buffer was prepared by using solution A and solution B.

Solution A: 0.4 M TRIS 48.4 g of tris (trihydroxymethyl) amino methane was accurately weighed and dissolved in few ml of water and finally made upto 1000 ml with water).

Solution B: 0.4 M hydrochloric acid (33.6ml of concentrated hydrochloric acid was measured and added in few ml of water and finally made upto 1000ml with water).

A working solution was made by adding 25ml of solution A and 20.7 ml of solution B, made up to 100 ml by using water & then the pH was adjusted to 7.4.⁶

Sodium chloride preparation: It was prepared by dissolving 5.85 g of sodium chloride in 10 ml of water.

Sodium oxalate preparation: It was prepared by dissolving 1.3 g of sodium oxalate in 10 ml of water.

Preparation of extracts:

1. **Pomegranate extract:** The seeds of pomegranate are subjected to maceration using 95% of ethanol, ethyl acetate, petroleum ether and water at a ratio of 1: 4 for 24 hours, followed by reflux over a water bath for 3 hours and the obtained extract was concentrated under vacuum.
2. **Garlic extract:** The garlic bulbs are subjected to maceration using 95% of ethanol, petroleum ether, ethyl acetate and water at a ratio of 1: 4 for 24 hours, followed by reflux over a water bath for 3 hours and the obtained extract was concentrated under vacuum.

Methodology:

Plant authentication: The plants were collected from Kantepudi, sattenapalli and authenticated by Mrs.Ch. Jhansi vani, Assistant Professor, A.C. College, Guntur.

Methods:

Estimation of calcium: Two sets of test tubes (A₁, A₂) were taken. To these test tubes 2ml of TRIS-HCl buffer, 1ml of sodium chloride, 1ml of calcium chloride di hydrate, and 1ml of sodium oxalate were added. Now 2ml of extract (Ethanol, Ethyl acetate, Petroleum ether, Water) and vehicle under investigation was added. Amount of calcium was estimated by titrimetry using disodium edetate as titrant and solochrome black T as an indicator.

Formula:

Amount= (titre volume × equivalent factor × actual molarity) / (wt. taken × expected molarity)

Estimation of phosphate: Two sets of test tubes (B₁, B₂) were taken. To these test tubes 2ml of TRIS-HCl buffer, 1ml of sodium chloride, 1ml of Calcium chloride di hydrate, and 1ml of disodium hydrogen phosphate were added. Now 2ml of extract (Ethanol, Ethyl acetate, Petroleum ether, Water) and vehicle under investigation was added. Amount of phosphate present was estimated by colorimetric analysis at 530 nm against blank.

Results of pomegranate:**TABLE 2: PERCENTAGE INHIBITION USING ETHYL ACETATE EXTRACT OF *PUNICA GRANATUM***

Ethyl acetate extract (0.3mg/ml)	Amount of Precipitates (mg)		% of inhibition	
	Calcium	Phosphate	Calcium	Phosphate
Control	610	563	-	-
Test	510	521	16.3	7.4

TABLE 3: PERCENTAGE INHIBITION USING PETROLEUM ETHER EXTRACT OF *PUNICA GRANATUM*

Petroleum ether extract (0.3mg/ml)	Amount of Precipitates (mg)		% of inhibition	
	Calcium	Phosphate	Calcium	Phosphate
Control	43	503	-	-
Test	370	462	13.9	8.1

TABLE 4: PERCENTAGE INHIBITION USING ETHANOL EXTRACT OF *PUNICA GRANATUM*

Ethanol extract (0.3mg/ml)	Amount of Precipitates (mg)		% of inhibition	
	Calcium	Phosphate	Calcium	Phosphate
Control	520	437	-	-
Test	410	391	21.1	10.5

TABLE 5: PERCENTAGE INHIBITION USING AQUEOUS EXTRACT OF *PUNICA GRANATUM*

Aqueous extract (0.3mg/ml)	Amount of Precipitates (mg)		% of inhibition	
	Calcium	Phosphate	Calcium	Phosphate
Control	420	480	-	-
Test	360	444	14.2	7.5

Results of Garlic:**TABLE 6: PERCENTAGE INHIBITION USING ETHYL ACETATE EXTRACT OF *ALLIUM SATIVUM***

Ethyl acetate extract (0.3mg/ml)	Amount of Precipitates (mg)		% of inhibition	
	Calcium	Phosphate	Calcium	Phosphate
Control	680	560	-	-
Test	550	521	19.1	6.9

Formula:

Amount= (sample absorbance/standard absorbance) × standard concentration

% inhibition = $\frac{\text{Control} - \text{test}}{\text{Control}} \times 100$

RESULTS: The results of the present study (**Table 2 to Table 9**) indicates that the ethanol extract of both pomegranate and garlic was found to have more inhibiting property than the ethyl acetate, petroleum ether and aqueous extracts of plants. Among garlic and pomegranate, garlic has showed better result in inhibiting precipitation of calcium and phosphate.

TABLE 7: PERCENTAGE INHIBITION USING PETROLEUM ETHER EXTRACT OF *ALLIUM SATIVUM*

Petroleum ether extract (0.3mg/ml)	Amount of Precipitates (mg)		% of inhibition	
	Calcium	Phosphate	Calcium	Phosphate
Control	630	432	–	–
Test	460	373	26.9	13.6

TABLE 8: PERCENTAGE INHIBITION USING ETHANOL EXTRACT OF *ALLIUM SATIVUM*

Ethanol extract (0.3mg/ml)	Amount of Precipitates (mg)		% of inhibition	
	Calcium	Phosphate	Calcium	Phosphate
Control	545	449	–	–
Test	360	379	33.9	15.5

TABLE 9: PERCENTAGE INHIBITION USING AQUEOUS EXTRACT OF *ALLIUM SATIVUM*

Aqueous extract (0.3mg/ml)	Amount of precipitates (mg)		% of inhibition	
	Calcium	Phosphate	Calcium	Phosphate
Control	530	504	–	–
Test	460	468	13.2	9.3

DISCUSSION AND CONCLUSION: In the present study, pomegranate and garlic were used to evaluate the antiurolithiatic activity by *in vitro* methods. Garlic stimulates the production of nitric oxide and hydrogen sulphide which relax the blood vessels; this improves the circulation of the kidneys and prevents the formation of infection stones. Garlic is also believed to contain vitamin B-6 which helps to reduce the levels of urinary oxalate and the seeds of pomegranate can be considered as natural remedy for kidney stones. This may be related to their astringent property. To determine the antiurolithiatic activity we have selected titrimetry for the estimation of calcium and colorimetry for the estimation of phosphate. Based on the results obtained in **table 4 and table 8** we found that garlic has showed better results than

pomegranate. But individual isolation and further studies are to be carried out to confirm this activity.

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