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Comparative Study of the Antiurolithiatic Activity of *Leea macrophylla, Alangium lamarkii, Saccharum officinarum, Marsdenia tenacissima, Chonemorpha macrophylla, Maerua oblongifolia*

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ABSTRACT

Introduction: Incidence of urinary tract disorders and kidney disorder including nephropathies is on the rise. In spite of tremendous development of modern medicine, still there is no appropriate medical treatment for these problems.

Aim: In Ayurved, the plant *Morat* mentioned in *Veeratarvadi gana*' [type of classification], which has prime indication in *Ashmari* (Urolithiasis). Under the name of *Morat*, botanical identity such as *Leea macrophylla, Saccharum officinarum, Alangium lamarkii, Marsdenia tenacissima, Maerua arenaria, Chonemorpha macrophylla* are considered as they exhibit same medicinal properties

Methodology: The purpose of this work was to compare anti-urolithiatic activity of these plants by Ethylene glycol induced model method. Also, comparison of antiurolithiatic property of these plants and Cystone is studied.

Result: In this article effect on urine output, pH, and concentrations of urinary calcium, phosphate, oxalate & total protein, serum concentrations of Creatinine, Uric Acid, Calcium Phosphate and BUN is studied. *Saccharum officinarum* L, *Chonemorpha macrophylla* (Roxb) G. Don, *Maerua arenaria* Hook, *Marsdenia tenacissima* exhibited significant antiurolithiactic activity.

Conclusion: Comparison suggests that *Chonemorpha macrophylla* is the most suitable source of *Morat* for Antiurolithiatic Activity is.

Key Words: *Morat, Veeratarvadi gana, Ashmari*, Cystone, Antiurolithiactic activity, Ayurveda

INTRODUCTION

Incidence of urinary tract disorders and kidney disorder including nephropathies is on the rise. The incidence is higher in developing countries including India. UTI, Urolithiasis, Diabetic Nephropathy & Nephrotoxic drugs are the most common conditions which causes injury to kidney. Prevalent environment, lifestyle, socioeconomic conditions have contributed towards rise of incidence of the same. In spite of tremendous development of modern medicine, still there is no appropriate medical treatment for these problems. There is, therefore continuous rising demand for safe & effective herbal drugs. Hence in recent years, plants documented in Ayurvedic literature like Charaka Samhita, Sushruta Samhita, and Ashtanga Hridaya has been received much attention. In Ayurved, many categories of drugs (*Gana*) are mentioned which could be useful in kidney disorders. '*Veeratarvadi gana*' is one of them, which has prime indication in *Ashmari* (Urolithiasis).[1] *Morat* is one of the controversial plants, which is mentioned in this group. Plants can be considered as Morat are *of L. macrophylla, A. lamarkii, S. officinarum, M. tenacissima, C. macrophylla. M. oblongifolia.*[2] This study was conducted to evaluate & anti-urolithiatic activity of species which are mentioned under the name of Morat in animal.

MATERIAL AND METHOD

Plant collection and authentication: All plants self-collected from its natural habitats & identified on the basis of its morphological characters with the help of by Dr. S. D. Jagtap (Senior Taxonomist & Head, Herbal Biotechnology, IRSHA, Bharati Vidyapeeth, Pune, India.) & flora. Voucher specimens of all six plants have been deposited in Region-

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al Ayurveda Institute for Fundamental Research(RAIFR), Pune, India.

Table 1: Collection and authentication of plants

Species	Collection date	Natural habitat	Specimen Voucher
Leea macrophylla Roxb. ex Hornen of Vitaceae	Roots September 2017	Vile village, located at the foothills of Tamhini hills in north Konkan, Dist. Raigad, Maharashtra, India.[3]	No.13944
Alangium lamarkii Thw. of Alangiaceae	Flowers March 2017	Jeuor village in Tal. Purandar, Dist. Pune, Maharashtra, India.[4]	No. 13946
Chonemorpha macrophylla (Roxb) G. Don of Apocynaceae	Roots May 2017	Near sacred groves Nagachi Devarai in Amboli Ghat in south Maharashtra, India.[5]	No. 13945
Marsdenia tenacissima (Roxb.) Moon. of Asclepiadaceae	Roots April 2017	Hilly region of Chitrakoot, a town in the Dist. Satna, Madhya Pradesh, India. [6]	No. 13943
Saccharum officinarum L. of Poaceae	Root September 2017	Mahadevwadi village, Tal. Bhor, Dist. Pune, Maharashtra, India. [7]	No. 1312
Maerua arenaria Hook of Capparaceae	Roots April 2017	Khambatki Ghat, Dist. Satara (Western Ghats) Maharashtra, India.[8]	No. 1313

Processing of plant material: Collected plant samples were shade dried, powdered with mechanical grinder, sieved through 80# mesh and stored in air tight glass vessel. These powders were utilized for various experimental studies. *Kwatha* (decoction) of test drugs were freshly prepared prior to administration to the animals. It was prepared according to the procedure mentioned in the "*Sharngadhar samhita*" *Madhyam khand* 2/77 i.e. 16 parts water and one part drug which was boiled on low flame till 1/8th part remained. This was filtered and allowed to cool before administering to the animals.[9]

Pharmacological screening for antiurolithiatic activity

Animals: Wistar strain albino rats of either sex; weighing 180 to 250 g were obtained from National Institute of Biosciences, 1091/ABC/07/CPCSEA & maintained in the animal house of Bharati Vidyapeeth University, Poona College of Pharmacy, Pune. Animals were housed on straw bedding 6 per cage & exposed to natural day and night cycles with ideal laboratory conditions in term of ambient temperature and humidity. The Institutional Animal Ethics Committee approved the protocol of this study **(CPCSEA Number: CPCSEA/PCP/PCL05/2018)**

Grouping and posology: Animals were divided into nine groups containing 6 animals in each group. The dose for experimental animals was calculated on the basis of body surface area ratio by referring to the standard table of Paget and Barnes (1969). [10]

Ethylene glycol induced urolithiasis model: The method of Ethylene glycol induced model [11] was employed for the assessment of antilithiatic activity. Rats were divided in nine groups containing six in each. Ethylene glycol (0.75% v/v) in drinking water was feed to all groups except control for induction of renal calculi till the 28th day. In the study all the groups except control were receive test drugs from 15th day till 28th day.[11] Cystone was used as standard drug. During the study animals were allowed free access to food.[12, 13]

Treatment protocol

Table 2: The treatment received by grouped animals

Group	Treatment	
Group – I	Control	
Group – II	0.75% ethylene glycol in drinking water for 14 days	Untreated
Group – III		Cystone(750 mg/kg)
Group – IV		Leea macrophylla (Root)
Group – V		Sachharum officinarum (Root)
Group – VI		Marsdenia tenacissima (Root)
Group – VII		Chonemorpha macrophylla (Root)
Group – VIII		Alangium lamarckii (flower)
Group – IX		Maerua arenaria (Root)

From day 1 to day 14, ethylene glycol (0.75%) in drinking water was fed orally (q.s.) to rats from group II to IX for rendering them hyperoxaluric. The state of hyperoxaluria was checked and confirmed by collection and examination of urine after day 14. Then onwards till day 28, rats from group III to IX received curative treatments with individual drugs and group I and II received saline water (5 ml/kg). All doses were administered once daily by oral route.

Collection and assessment of urine: Rats were kept in individual metabolic cages for collection of 24 h urine. They

had free access to drinking water during the urine collection period. During the entire study period, rats were subjected to 24 h urine collection twice. After day 14, urine was collected and analyzed to check and confirm the extent of Urolithiasis. After completion of study period (i.e. day 28), urine was collected and analyzed for biochemical parameters.

Routine urinalysis: Routine urinalysis was carried out using diagnostic reagent & kits (Coral Clinical Systems, Gitanjali, Tulip Block, Dr Antonio DO Rego Bagh, Bambolim Complex P.O. Goa) including quantitative determination of pH and specific gravity along with presence of occult blood, bilirubin, urobilinogen, ketone bodies, proteins, nitrite, glucose and leucocytes in urine.

Urine biochemistry: A drop of concentrated hydrochloric acid was added to the urine before being stored at 4ºC. Using biochemical estimation kits, urine was analyzed for calcium and phosphate content; while, urinary oxalate content was estimated using modified method of Hodgkinson and Williams (1972).

Assessment of serum: After the experimental period, blood was collected from the retro-orbital vein under anesthetic conditions. Serum was separated by centrifugation at 10,000 rpm for 10 min and analyzed for creatinine, uric acid and urea nitrogen.

Assessment of kidney: Finally all rats were sacrificed using appropriate method of euthanasia. The abdomen was cut open to remove either kidney from each rat. Isolated kidneys were cleaned off extraneous tissue and preserved in 10% neutral formalin. One kidney (randomly selected) from each rat was processed for preparing kidney homogenate. From remaining kidneys, one kidney as representative of the whole group was selected randomly and processed for histopathology examination.

Kidney homogenate analysis: Each selected kidney was dried at 80ºC in a hot air oven. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1 N hydrochloric acid for 30 min and homogenized. The homogenate was centrifuged at 2000 rpm for 10 min and supernatant was separated. The calcium, phosphate and oxalate content in kidney homogenate were determined as mg/g of kidney weight using the methods as described earlier.

Histopathological examination of kidney: Each selected kidney was embedded in paraffin using conventional methods and cut into 5 µm thick sections and stained using hematoxylin-eosin dye and finally mounted in diphenyl xylene. Then each section was observed under microscope for histopathological changes in kidney architecture and their photomicrographs were taken at x50 magnification. By visualizing different fields, a general method of scoring was adopted to observe the extent of nephritic damage and the recovery. A minimum of 10 fields for each kidney slide were examined and assigned for severity of renal damage and progression of recovery using scores on a scale of none (ND), mild (+), moderate (++) and severe (+++) damage.

Statistical analysis Results were expressed as mean ± SEM. Differences among data were determined using one way ANOVA followed by the Dunnett's posttest using Graph pad Prism software (Graph pad Prism software Inc., Version 4.00.255). Differences between the data were considered significant at $p<0.05$.

RESULTS

Effect on urine output and pH in Ethylene glycol induced urolithiasis: Details of the effect of the Cystone and other test drugs in urolithiatic animals are summarized here in table 3. It shows that, in untreated animals urine output was reduced. There was increased urine output observed in the groups treated with cystone, *S. officinarum, M. tenacissima, C. macrophylla & M. arenaria*. It was observed that there was increase in urinary pH of 7.117 in calculi-induced Group II, but it was brought down gradually toward normal value in the groups treated with *L. macrophylla, A. lamarkii*, *S. officinarum, M. tenacissima, C. macrophylla*. *M. oblongifolia* and Cystone

Table 3: Effect of six plant drugs on urine output and pH in Ethylene glycol induced urolithiasis

Group	Urine output	pH
Group – I: Vehicle Control group	7.583±1.129	6.317±0.06009
Group – II : Untreated(Ethylene Glycol control)	5.233±0.2275 ns	7.117±0.1249 ***
Group – III Cystone(750 mg/kg)	16.33±0.9545*	6.433±0.03333 ***
Group – IV: L macrophylla (Root) 7.2 ml/kg	8±0.8944 ns	6.5±0.03651 ***
Group – V : *M. tenacissima* (Root) 7.2 ml/kg	27.33±5.077 ***	6.333±0.03333***
Group – VI: S. officinarum (Root) 7.2 ml/kg	24±5.164 ***	6.267±0.03333***
Group – VII: C. macrophylla (Root) 7.2 ml/kg	19.5±2.604 **	6.533±0.04944***
Group – VIII: A. lamarckii (flower) 7.2 ml/kg	6.333±0.9545 ns	6.5±0.03651***
Group – IX: M. arenaria (Root) 7.2 ml/kg	17.83±1.641 *	6.583±0.07923***

Values are expressed as mean ± SEM.*p<0.05,**p<0.01,***p<0.001 when compared to vehicle control. ns – Nonsignificant

Effect on Calcium, Phosphate, Oxalate & total protein: Details of the concentrations of urinary calcium, phosphate, oxalate & total protein are presented in Table 4. In the present study, group treated with S. *officinarum M. tenacissima, C. macrophylla. & M. oblongifolia* and Cystone shows significant decrease in concentrations of urinary calcium, phosphate, oxalate & total protein.

Table 4: Effect of six drugs (at dose 7.2 ml/kg) on various urinary parameters at the end of 28 d experimental periods

Group	Oxalate	Calcium	Phosphate	Total Protein
Vehicle control	64.54 ±0.9034	6.495±0.0590	17.71±0.336	2.32±0.07262
Ethylene glycol	114.7 ±1.255 ***	13.25±0.175***	124.3±3.619***	10.35±0.1045***
Cystone	96.03 ±2.397***	8.502±0.0647***	45.77±0.870***	4.745±0.04787***
L macrophylla	116.3 ±1.5 ns	12.63±0.05402***	122.9±3.579 ns	10.24±0.1045 ns
S. officinarum	97.34 ±2.54***	9.478±0.118***	54.36±1.035***	5.453±0.05534***
M. tenacissima	104.6 ±1.139***	9.44±0.04472***	57.22±1.089***	5.345±0.05457***
C. macrophylla	106.6 ±1.162**	11.39±0.07265***	103.3±3.007***	7.782±0.7867***
A. lamarckii	110.7 ±1.616 ns	12.18±0.06908***	120±3.495 ns	10.04±0.1014 ns
M. arenaria	102.5 ±0.2987***	11.99±0.0677***	113.3±3.298*	9.032±0.0915***

Values are expressed as mean ± SEM.*p<0.05,**p<0.01,***p<0.001 when compared to vehicle control. ns - Nonsignificant

Effect on Serum Levels of Creatinine, Uric Acid, Calcium Phosphate and BUN: Details of the serum concentrations of Creatinine, Uric Acid, Calcium Phosphate and BUN are presented in table 5. In the present study, in calculi-induced rats (Group II), marked renal damage was seen by the elevated serum levels of creatinine, uric acid and Blood Urea Nitrogen. However group treated with S. *officinarum, M. tenacissima, C. macrophylla. & M. oblongifolia* and Cystone restored the elevated serum levels of creatinine, uric acid and BUN

Table 5: Effects of six plant drugs on Creatinine, Uric Acid, Calcium Phosphate and BUN in blood

Group	BUN	Calcium	Phosphate	Total Protein	Uric Acid	Creatinine
V. C.	27.76±0.7543	10.45±0.3115	3.463 ±0.0518	2.32±0.07262	1.272±0.032	0.615±0.0635
E. G.	45.67±1.1 ***	6.32±0.1314 ***	8.608 ±0.1785 ***	10.35±0.1045 ***	2.253±0.069 ***	1.545±0.0596 ***
Cys.	37.04±1.129 ***	9.497±0.0896 ***	5.498 ±0.0779 ***	4.745±0.0477 ***	1.543±0.0561 ***	0.785±0.02668 ***
L.M	43.83±1.055 ns	6.56±0.07099 ns	7.78 ±0.108 ***	1024±0.1045 ns	2.185±0.0661 ns	1.507±0.0585 ns
S. O	40.85±1.275 *	9.373±0.02765 ***	5.938 ±0.084 ***	5.452±0.055 ***	1.868±0.06848 ***	0.8478±0.028 ***
M. T	40.69±1,301 *	8.557±0.07667 ***	6.413 ±0.091 ***	5.345±0.0545 ***	1.697±0.0616 ***	0.8983±0.02982***
C. M	41.5±1.327 ns	8.657±0.0736 ***	6.928 ±0.0991 ***	7.782±0.07867 ***	1.82±0.04858 ***	0.9433±0.0313 ***
A. L	44.5±1.388 ns	6.472±0.0750 ns	7.54 ±0.0735 ***	10.04±0.101 ns	2.218±0.0672 ns	1545±0.05976 ns
M. A	42.49±1.357 ns	8.382±0.0674 ***	6.785 ±0.066 ***	9.032±0.0915 ***	1.742±0.074 ***	0.991±0.034 ***

Values are expressed as mean ± SEM.*p<0.05, **p<0.01,***p<0.001 when compared to vehicle control. ns – Nonsignificant

The Calcium, Phosphate, Total Protein and Oxalate content in the kidney homogenate: Details of the Calcium, Phosphate and Oxalate content in the kidney homogenate is presented in table 6. Kidney homogenate analysis revealed increased amount ($P< 0.001$) of stone forming components Calcium, Phosphate and Oxalate content in diseased animals(Group II vs. Group I).

Kidney homogenate analysis revealed increased amount of stone-forming components calcium, phosphate, and oxalate in diseased animals (Group II vs. Group I). The animals treated with *S. officinarum, M. tenacissima, C. macrophylla & M. oblongifolia* & cystone showed decreased levels of stone-forming components. Whereas animals treated with *L macrophylla* & *A. lamarckii* didn't show reduced content of calcium, phosphate, and oxalate.

Table 6: Effects of six plant drugs on the Calcium, Phosphate and Oxalate content in the kidney homogenate.

Group	Oxalate	Calcium	Phosphate	Total Protein
Vehicle Control	1.165±0.0725	1.227±0.03783	2.395±0.04209	244.8±6.979
Ethylene Glycol	7.325±0.05464***	5.657±0.2468***	6.755±0.2559***	347.7±9.997***
Cystone	5.547±0.0936***	3.66±0.01461***	4.81±0.02129***	215.6±6.198***
L. macrophylla	6.9542±0.0804 ns	5.333±0.06546 ns	6.483±0.07297 ns	319.9±9.918 ns
S. officinarum	5.995±0.1351***	3.368±0.01327***	4.518±0.02136***	257.3±7.397***
M. tenacissima	6.015±0.179***	3.5730±0.444***	4.723±0.05414***	239.9±6.89***
C. macrophylla	6.47±0.09845***	4.708±0.1407***	5.858±0.1619**	281.6±8.098***
A. lamarckii	7.36±0.1334ns	5.048±0.2199*	6.198±0.2249 ns	323.3±9.298 ns
M. arenaria	6.075±0.0734***	4.492±0.235***	5.642±0.2364***	295.5±8.497***

DISCUSSION

Many medicines have been employed during the ages to treat urinary stones. In the Ayurveda, most of the medicines were plant origin and they were shown to be beneficial, though the rationale behind their practice is not well proven through systematic pharmacological studies. *Morat* is one of effective drug, which was mentioned in conditions such as *Mutrakruchra, Mutraghata* & *Ashmari.*

Urolithiasis is one of the common kidney disorders. The majority of stones found very often in humans are composed mainly of calcium oxalate or calcium oxalate stones mixed with calcium phosphate. So in the present study, Ethylene glycol induced model was employed for the assessment of the activity.

Antiurolithiatic plants show multidimensional action & effectiveness at different stages of urolithiasis. In the present study there was increased urine output observed in the groups treated with Cystone, *S. officinarum, M. tenacissima, C. macrophylla* and *M. arenaria*. So it can be stated that above plants shows effectiveness in urolithiasis as their diuretic action increases the quantity of fluid going pass through the kidney & flush out the deposits.

Oxalate, calcium and phosphate elimination were increased significantly in the Ethylene glycol induced animals (Group II) which caused hyperoxaluria. It indicates stone formation in ethylene glycol-fed animals.

Group treated with *S. officinarum M. tenacissima, C. macrophylla*, *M. arenaria* and Cystone shows significant decrease in concentrations of urinary calcium, phosphate, oxalate & total protein.

Accumulation of the CaOx crystals in the kidney increased the urinary pH, which is one of the indications of urolithiasis. It was observed that there was increase in urinary pH of 7.117 in calculi-induced Group II, but it was brought down gradually toward normal value in the groups treated with *L. macrophylla, A. lamarkii, S. officinarum, M. tenacissima, C. macrophylla. M. oblongifolia* and Cystone.

The glomerular filtration rate (GFR) is significant parameter for confirming renal function and it gets reduced in urolithiasis due to the obstruction to the urine output by stones in urinary system, which leads to increase in urea, creatinine, and uric acid in blood. In Ethylene glycol induced animals (Group II), noticeable renal impairment was seen by the raised serum levels of Creatinine, Uric acid and Blood Urea Nitrogen (BUN). However, the groups treated *with L. macrophylla, A. lamarkii, S. officinarum, M. tenacissima, C. macrophylla. M. arenaria* and Cystone restored the elevated serum levels of creatinine, uric acid and BUN.

Kidney homogenate analysis revealed increased amount ($P< 0.001$) of stone forming components Calcium, Phosphate and Oxalate content in diseased animals (Group II vs. Group I), which indicating Ethylene glycol induced urolithiasis.

The animals treated with *S. officinarum, M. tenacissima, C. macrophylla.* & *M. arenaria* & cystone showed decreased levels of stone-forming components. Whereas animals treated with *L macrophylla* & *A. lamarckii* didn't show reduced content of calcium, phosphate, and oxalate.

CONCLUSION

Saccharum officinarum L, *Chonemorpha macrophylla* (Roxb) G. Don, *Maerua arenaria* Hook, *Marsdenia tenacissima* exhibited significant antiurolithiactic activity in animal model. Plants mentioned in name of Morat, except *Marsdenia tenacissima* exhibited significant diuretic activity in animal model. The inter-group comparison suggests that the most suitable source of Morat is *Chonemorpha macrophylla*

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Author's Contribution:

Nangare N performed the experiments, drafted the manuscript, analysed and interpreted the data.

Deshpande M, Arulmozhi S., designed the experiments, supervised, analysed, critically revised and finally approved the manuscript.

Kurulkar M interpreted the data & technically supported the study.

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