International Journal of Pharmaceutics and Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *ARTEMISIA NILAGIRICA* WITH REFERENCE TO ITS ANTI-UROLITHICAL ACTIVITY

Ashutosh Mishra*, Sachin Soni
Daksh Institute of Pharmaceutical Sciences, Chatarpur (M.P.)

***Correspondence Info:**
Ashutosh Mishra
Daksh Institute of Pharmaceutical Sciences, Chatarpur (M.P.)
Email:
researcharticle78@gmail.com

***Article History:**

Received: 21/07/2024
Revised: 10/08/2024
Accepted: 25/08/2024

ABSTRACT

In the present study, successive extracts of *Artemisia nilagirica* were prepared and evaluated for pharmacognostic and pharmacological activities. The preliminary phytochemical investigation of *Artemisia nilagirica* extracts showed presence of glycosides, saponins and triterpenoids. Three extracts MeE of *Artemisia nilagirica* possessing optimum bioactivity were subjected to fractionation using non-polar to polar solvents and all fractions were further subjected to TLC/HPTLC finger printing. Although, the exact phytochemistry of isolated compound (lupeol) remains unidentified; further systematic phytochemical studies would elucidate the probable structural entities in these plants and their structure activity relationship with similar biomolecules from other lithontriptic plants. Neverthe less, there are reports which correlate the antiurolithiatic activity ofthelupeol with its diuretic potentials. Accordingly, the diuretic potentials exhibited by extracts of Artemisia *nilagirica*) suggest that it could also be the contributing factor for lithontriptic properties shown by these plant extracts. Diuresis reduces the risk of stone formation by forbidding the saturation product of CaOx. Further, the study of these plant extracts at molecular level would elaborate the exact modus operandi of these plant extracts as a diuretic and antiurolithiatic agent.

Keywords: Phytochemical, Pharmacological evaluation, *Artemisia nilagirica*, Anti-urolithical activity.

INTRODUCTION

India has an ancient heritage of traditional medicine. Materia medica of India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural product. Indian traditional medicine is based on phytochemical, pharmacological & allied approaches including instrumental techniques like chromatography, microscopy and others. There is accumulating evidence suggesting medicinal plants are unlimited reservoirs of drugs. The amazing structural diversity among their active components makes them a useful source of novel therapeutic compounds. Researchers with interest in natural products have intensified their effort to towards scientific evaluation of traditional medicines (Mukherjee, 2001).

Kidney stone

Urolithiasis is also called nephrolithiasis. "Nephrolithiasis" is derived from the Greek nephros- (kidney) lithos (stone) ± kidney stone. It is a process of forming stones in the kidney, bladder, and/or urethra (urinary tract). Kidney stones occur in 1 in 20 people at some time in their life. The development of the stones is related to decreased urine volume or increased excretion of stone-forming components such as calcium, oxalate, urate,

cystine, xanthine, and phosphate. Kidney stones are most likely to form in the renal pelvis, which can range in size from microscopic to 10 to 20 mm in diameter. The entry of a kidney stone into a ureter may cause intense pain (renal colic) and bleeding. Obstruction of a ureter by a stone may cause backup of urine and possible kidney damage (Vyas *et al*., 2010; Agnas *et al*., 2024).

Epidemiology

A large number of people are suffering from urinary stone problem all over the globe. Not only the humans but animals and birds also suffer from the urinary stone problem. The occurrence in some areas is so alarming that they are known as 'Stone Belts' .Urinary stone disease is a common disorder estimated to occur in approximately 12% of the population, with a recurrence rate of 70–81% in males, and 47–60% in females . Approximately 50% of patients with previous urinary calculi have a recurrence within 10 years. Stone disease is 2-3 times more common in males than in females. Most urinary calculi occur in patients aged 20–49 years (CoeFL, 2005).

Classification

Based on the chemical composition of the stone and the severity of the disease different categories of stone formers can be identified (Alelign and Petros, 2018).

Calcium stones: Calcium is the most common constituent of urinary tract calculi. Such stones are radio-opaque.

Calcium oxalate

Calcium phosphate

Calcium oxalate and phosphate

Non-calcium stones

Magnesium ammonium phosphate (Struvite)

Uric acid

Cystine

Calcium oxalate: Also called whewellite or 'mulberry' stones, these stones are characteristically dark brown/black in color, with a dense, smooth appearance shows the crystals under electron microscopy.

Calcium phosphate: Calcium hydroxyphosphate stones commonly comprise a significant proportion of carbonate to form apatite stones.

Magnesium ammonium phosphate (Struvite): These are also called triple phosphate or struvite stones, named after Heinrich von Struve who first described them.
Uric acid: Uric acid is the end product of purine metabolism.

Cystine: Cystinuria occurs due to an inherited defect in the transport of amino acids cystine, lysine, arginine and ornithine.

There are many herbal plants which have been used in the treatment of urinary stones. A number of plant drugs used in India and elsewhere which claim efficient cure of urinary stones. Urinary tract infections (UTI), acute and chronic, can be effectively treated with herbal medicine. Two strategies are essential in utilizing herbal medicine. The choice of herbs, through their herbal actions, along with appropriate therapeutic dosing strategies, will determine the effectiveness of herbal treatment and prevent the need to intervene with antibiotics. In relation to various medicinal properties of plants we have selected *Artemisia nilagirica* from

different geographic region for their biological activity related to urolithiasis. *Artemisia nilagirica* (Indian Wormwood) is an aromatic shrub, 1-2 m high, yellow or dark red small flowers, grows throughout India in hills up to 2400 m elevation. This medicinal herb is erect, hairy, often half-woody. The stems are leafy and branched. The leaves are pinnately lobed, 5-14 cm long, gray beneath. Mugwort blossoms with reddish brown or yellow flowers. The flowers are freely small and stand in long narrow clusters at the top of the stem. The fruit (achene) is minute. It is believed that Indian Wormwood drives away insects. So the leaves and flowers are put in boxes and cupboards (Suresh*et al*., 2011). *Artemisia nilagirica* (Indian Wormwood) have been claimed in traditional literature to be valuable against wide varieties of diseases. India materica medica describes the use of plant in the treatment of a number of ailments, including burning of sensation, diuretic, urinary disorders, nephrolithiasis, dermatopathy (Abad *et al*., 2012).

The herbs are preferred because they do not produce any adverse effect having their popularity and therapeutic excellence. Few studies have been performed but there are no evidence of isolated constituents of plant, that can prove beneficial for urolithiasis. So, here the main target is to isolate the unknown phytoconstituents and to perform their bioactivity followed by characterization of active constituents. The overall aim of this project is to perform phytochemical investigation and pharmacological evaluation of *Artemisia nilagirica* (Seed) for Antiurolithiatic activity.

MATERIALS AND METHODS

Plant material

Seeds of *Artemisia nilagirica* were collected in the month of June 2023, the locality of pune district of Maharashtra, India.

Identification and authentication of plant material

Artemisia nilagirica was recognized and validated by Department of Botany, Saifia College, and Bhopal.

Phytochemical studies

The phytochemical assessment of *A. nilagirica* was done by standard methods (Bisht *et al*., 2021).

Percent Yield

It was calculated by using this formula:

$$W2\text{-}W1/W0\times 100.$$

Where W2 is the weight of the extract and the container, W1 is the weight of the container alone and W0 is the weight of the initial dried sample (Anokwuru *et al*., 2011).

In-vivo evaluation of antiurolithiatic activity

Ethylene glycol induced urolithiasis

Rats were isolated in 9 groups containing 6 in each and placed in metabolic enclosures. All rats had free access to rodent chow and drinking water ad libitium for 28 days. Renal calculi were initiated in II to IX group by enhancing with 0.75%v/v ethylene glycol in drinking water. III to IX Group were treated with plant concentrates beginning from fifteenth day to 28th day (Curative routine). VI to VII group were treated with plant

concentrates beginning from first day to 28th day (Preventive routine).

Routine analysis

Routine analysis was performed using Urocolor 10 test strips including quantitative pH and specific gravity determination along with occurrence of bilirubin, occult blood, proteins, urobilinogen, ketone bodies, nitrite, glucose and leucocytes in urine.

Microscopic evaluation

Fresh urine samples collected (day 14 and day 28), were examined at ×50 magnification using compound microscope to ascertain the presence of characteristic crystals of CaOx and CaPh. Their photomicrographs were taken using a digital Microtel CCD camera supported with Aver cap software (Aver media Technologies, version 1.0.0.10)

Urine biochemistry

Before being used at 4°C Conc. HCl was added to the urine. Using biochemical estimation kits, calcium and phosphate content was analyzed in urine; while, urinary oxalate content was estimated using modified method of Hodgkinson and Williams (1972).

Assessment of serum

After experimental period, anesthesia was given to rat and blood was collected from retro-orbital vein. Serum was placed to centrifuge at 10,000 rpm for 10 minute and examined for uric acid, urea nitrogen and creatinine.

Assessment of kidney

Finally all rats were sacrificed using appropriate method of euthanasia. Kidneys are removed from rats abdomen by giving cut. Kidneys are then washed and leaned and placed in neutral formalin 10%. One kidney (randomly selected) from each rat was processed for preparing kidney homogenate. From remaining kidneys as representative of the whole group was selected randomly and processed for histopathology examination.

Kidney homogenate analysis

Each selected kidney was dried using hot air oven at 80°C.A dried kidney sample of 100mg placed for boiling in 10 ml of 1 N HCL acid for 30 min and homogenized. Then homogenized sample was placed for centrifugation at 2000 rpm for 10 minute and supernatant was removed. The kidney homogenate were determined for calcium, phosphate and oxalate content as mg/g kidney weight using the methods as described earlier.

RESULTS AND DISCUSSION

Yield of successive solvent extracts

Extraction of *Artemisia nilagirica* showed maximum percent yield with methanol; The summary of colour, percent yield and consistency of each successive solvent is given in table 1.

Preliminary phytochemical screening of various extracts

The preliminary qualitative phytochemical investigation of various extracts of *Artemisia nilagirica* indicated steroids, carbohydrates (primary metabolite), tri-terpenoids, volatile oils, alkaloids, tannins and phenolic compounds (secondary metabolites). Summary of results given in table 2.

Fluorescence analysis

The extracts plant materials in various solvents showed characteristic fluorescence at

long and short wavelength of UV light. Summary of results given in table 3.

Evaluation of antiurolithiatic activity

Ethylene glycol induced urolithiasis

Continuous oral administration of 0.75%v/v ethylene glycol in drinking water for 14 days rendered a condition of experimentally induced hyperoxaluria in rats. The microscopic examination of urine collected on 14th day from randomly selected groups showed characteristic crystals of calciumoxalate(CaOx) and calciumphosphate (CaPh) under 50x and 100x magnifications. Further, curative treatment with crude extracts showed characteristic alterations in serum and renal biochemical, and renal histological parameters.

Routine urinalysis

In CaOx urolithiasis, the pH of urine remarkably increases beyond 7.2, which initiates the nucleation of phosphate and oxalate with calcium. In calculi induced animals the mean pH of 7.58 was observed. However, the AqE of *Artemisia nilagirica* significantly decreased the elevated urinary pH to nearly normal values (6.67 to 6.92).

Microscopic examination of urine

The microscopic examination under 50x magnification showed that the urine of normal group animals was devoid of any crystal or similar structure.In calculi induced rats, the urine sample showed abundant, large crystals of CaOx with characteristic rectangular shape. The cystone treated animal showed very lessor almost dissolved smallc rystals. The AqE and MeE of *Artemisia nilagirica showed* less abundant crystals and on visual comparison, the size of crystals were found to be reduced with respect to that of calculi induced rats. *Artemisia nilagirica* was found to be equally effective in dissolving the preformed crystals; however, smaller and discrete fragments of crystals were seen among these groups.

Urine biochemistry

Increased urinary calcium is a factor favouring the nucleation and precipitation of CaOx or apatite (calcium phosphate) from urine and subsequent crystal growth. Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces CaOx deposition. However, supplementation with AqE of *Artemisia nilagirica* significantly (P<0.01) lowered the elevated levels of oxalate, calcium and phosphate in urine and kidney as compared to cystone treated animals.

Serum biochemistry

In urolithiasis, the glomerular filtration rate (GFR) diminishes, because of the impediment to the surge of urine by stones in urinary framework and the waste items, especially nitrogenous substances, for example, urea, creatinine and uric corrosive get gathered in blood. Oxalate has been accounted for to ins tigate lipid peroxidation and to cause renal tissue harm by responding with polyunsaturated unsaturated fat sincell film. The serum uric corrosive and creatinine were astoundingly expanded in calculi-prompted creatures; while, serum was just marginally raised in gathering II showing checked renal harm. In any case, *Artemisia nilagirica* (fruits) treatment essentially

(P<0.01) brought down theraised serum levels of creatinine, uric corrosive. The critical diuretic impact might be contributory and rushes the way toward dissolving the preformed stones and further counteractive action of new stone development in urinary framework on corrective treatment for 14 days.

Table 1: Yield of successive solvent extracts

Extracts	Colour	Consistency	Yield (%w/w)
***Artemisia nilagirica* (Seeds)**			
Petroleum ether (40- 60^0C)	Yellow	Greasy	11.6
Chloroform	Green	Sticky	3.8
Methanol	Brown	Sticky	22.2
Aqueous	Brown	Sticky	15.8

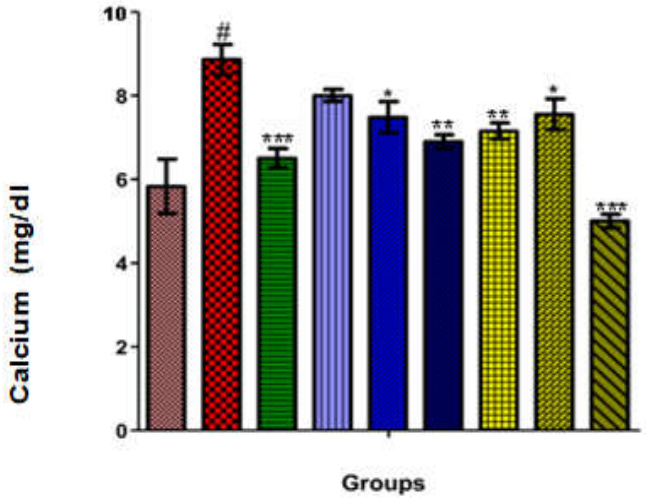
Table 2: Preliminary phytochemical screening of extracts of *Artemisia nilagirica* (Seeds)

Sr. No	Phytoconstituents	Pet ether (60^o-80^oC)	Chloroform	Methanol	Aqueous
1.	Alkaloids	-	-	-	-
2.	Carbohydrates	-	-	+	+
3.	Glycosides	-	-	-	-
4.	Flavonoids	-	-	+	+
5.	Phenol& tannins	-	+	+	+
6.	Steriods	-	-	-	-
7.	Triterpenoids	-	-	+	-
8.	Saponins	-	+	-	-
9.	Proteins	-	-	-	+
10.	Amino acids	-	-	-	-
[-] =Absent; [+] = Present					

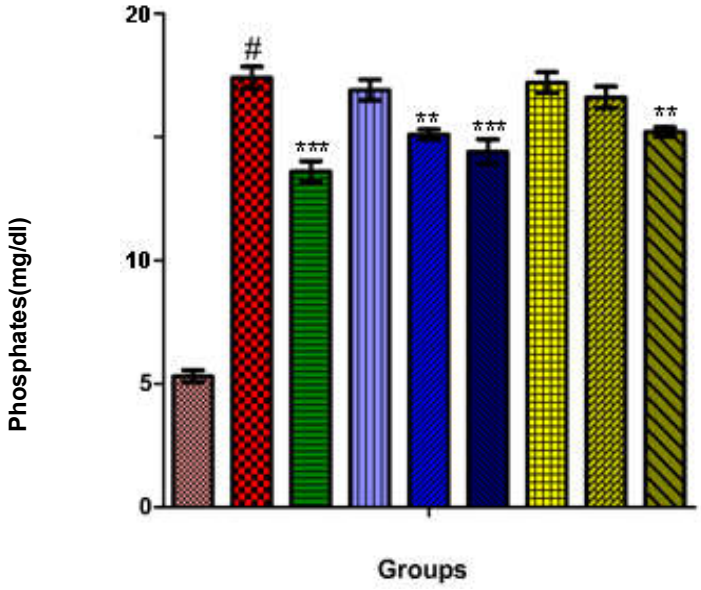
Table 3: Effect of *Artemisia nilagirica* (seed) on urine calcium, phosphate and oxalate levels in ethylene glycol induced urolithiasis in rats

Sr. No.	Groups	Calcium(mg/dl)	Phosphates (mg/dl)	Oxalate(mg/dl)
1.	Normal	5.83±0.65	5.31 ±0.24	6.03±0.14
2.	Control	8.86 ±0.36	17.4 ±0.44	9.00 ±0.09
3.	Standard (Cystone)	6.5 ±0.23***	13.6 ± 0.42***	6.02 ±0.12***
4.	AN100mg/kg	8.00±0.14	16.9 ±0.42	8.38 ±0.16
5.	AN200mg/kg	7.48 ±0.37*	15.1 ±0.20**	7.97 ±0.50*
6.	AN400mg/kg	6.90±0.16**	14.4 ± 0.50***	6.94 ±0.34***
7.	AN100 mg/kg	7.15 ±0.19**	17.2 ±0.42	8.40 ±0.10
8.	AN200 mg/kg	7.55±0.37*	16.6 ±0.44	7.76 ±0.10**
9.	AN400 mg/kg	5.00±0.16***	15.2 ± 0.185**	6.17±0.20***

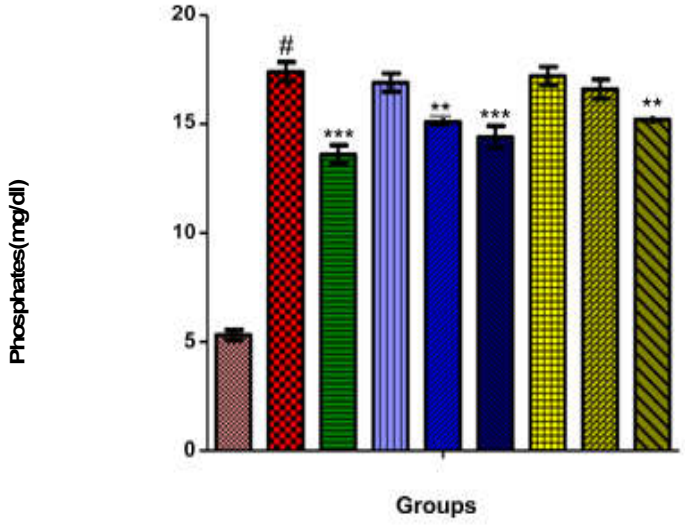
Artemisia nilagirica methanolic extract, *Artemisia nilagirica* aqueous extract Data are expressed as mean ± S.E.M.; n=6 rats per group. Two way ANOVA followed by Bonferonni post hoc test when compared with vehical control $^{*}P<0.05, ^{**}P<0.01, ^{***}P<0.001$.



Graph 1: Histogram showing the effect of AqE and MeE of Artemisia nilagirica Seeds on urinary excretion of calcium



Graph 2: Histogram showing the effect of AqE and MeEof *Artemisia nilagirica.* Seeds on urinary excretion of phosphate



Graph 3: Histogram showing the effect of AqE and MeE of Artemisia nilagirica, Seeds on urinary excretion of oxalate

Table 4: Effect of *Artemisia nilagirica* (seeds) on serum creatinine,urea and uric acid levels in ethylene glycol induced urolithiasis in rats

Sr. No.	Groups	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
1.	Normal	0.43±0.08	8.07±0.17	1.15 ±0.12
2.	Control	1.28±0.12	21.9±0.29	2.2 ±0.26
3.	Standard (Cystone)	0.44 ±0.04 ***	13.2 ±0.376***	1.52 ±0.06***
4.	AN100mg/kg	0.71±0.09*	20.4±0.201	2.01 ±0.19*
5.	AN200mg/kg	0.62± 0.12**	19.9±0.237**	1.93 ± 0.10**
6.	AN400mg/kg	0.44±0.06***	14.7±0.733***	1.64 ±0.15***
7.	AN00mg/kg	0.84±0.21	20.1±0.218*	2.10 ±0.12
8.	AN200 mg/kg	0.86 ±0.24	20±0.440**	2.14 ±0.17
9.	AN400 mg/kg	0.62± 0.05**	15.8±0.324***	1.79±0.11***

AN- *Artemisia nilagiric* methanolic extract, AN- *Artemisia nilagirica* aqueous extract, Data are expressed as mean±S.E.M.; n=6 rats per group. Two way ANOVA followed by Bonferonni post hoc test when compared with vehical control $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

CONCLUSION

In the present study, successive extracts of *Artemisia nilagirica* were prepared and evaluated for pharmacognostic and pharmacological activities. The preliminary phytochemical investigation of *Artemisia nilagirica* extracts showed presence of glycosides, saponins and triterpenoids. Although, the exact phytochemistry of isolated compound (lupeol) remains unidentified; further systematic phytochemical studies would elucidate the probable structural entities in these plants and their structure activity relationship with similar biomolecules from other lithontriptic plants. Nevertheless, there are reports which correlate the anti urolithiatic activity of the lupeol with its diuretic potentials. Accordingly, the diuretic potentials exhibited by extracts of Artemisia *nilagirica*) suggest that it could also be the contributing factor for lithontriptic properties shown by these plant extracts. Diuresis reduces the risk of stone formation by forbidding the saturation product of CaOx. Further, the study of these plant extracts at molecular level would elaborate the exact modus operandi of these plant extracts as a diuretic and antiurolithiatic agent.

The pharmacological screening including diuretic activity and evaluation of antiurolithiatic activity was carried out. The findings from present study support the *Artemisia nilagirica* for their diuretic actions. Aqueous and ethanol extracts of these plants do not seem to have renal toxicity in rats at doses selected in the present study. Based on the pattern of excretion of water and electrolytes, it appears that that there are at least two types of active principals present in these extracts, one having a frusemide-like activity and the other a spironolactone-like activity.

It is evident from the above data that the MeE of *Artemisia nilagirica* are endowed with potential diuretic and lithontriptic principles, supporting folklore uses of these plants as traditional medicine. Although, the mechanism underlying this effect is still unknown, but it is apparently related to significant diuretic effects and lowering of urinary concentrations of stone forming constituents. The protective effect against oxalate induced lipid peroxidation may be contributory to the inhibition and protection from further.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Abad, M.J., Bedoya, L.M., Apaza, L. & Bermejo, P. (2012) The Artemisia L. Genus, a review of bioactive essential oils. *Molecules*, 17, 2542–2566.
- Agnas, V.M., Viju, E., Manoj, A.P., Ashifa, A., Sreejitha, K.J. & Kumar, S. (2024) Plants Having Antiurolithiatic Activity; A Review, wjpmr, 10, 282–290.
- Alelign, T. & Petros, B. (2018) Kidney stone disease: An update on current concepts. *Advances in Urology*, 2018, 3068365.

- Review on Artemisia nilagirica (Clarke) Pamp. J. Suresh, N.M. Mahesh, J. Ahuja, K.S. Santilna, JBAPN 1 (2) 2011 pp 97 – 104.
- Anokwuru, C.P., Anyasor, G.N., Ajibaye, O., Fakoya, O. & Okebugwu, P. (2011) Effect of Extraction Solvents on phenolic, Flavonoid and Antioxidant activities of Three Nigerian Medicinal Plants. *Nature and Science*, 9.
- Bisht, D., Kumar, D., Kumar, D., Dua, K. & Chellappan, D.K. (2021) Phytochemistry and pharmacological activity of the genus artemisia. *Archives of Pharmacal Research*, 44, 439–474.
- Coe, F.L. Kidneystonedisease. *J. ClinInvest*, 115:2598–608, 2005Oct 1.
- Mukherjee, P.K. (2001) Evaluation of Indian traditional medicine. *Drug Information Journal*, 35, 623–632.
- Vyas, N., Keservani, R.K., Jain, S., Raghuvanshi, R., Gavatia, N.P., Agrawal, A.K. & Kesharwani, R.K. (2010) Urolithiasis and its management. *Pharmacia* [Letter], 2, 457–466.