Screening of Phytochemical Compounds and Assessment of Antiurolithiatic Activity of *Pisonia Alba* Leaves Extracts against DNA Damage

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Abstract: ***This work investigate the Phytochemical constituents of Pisoniaalba (PA) leaves using standard protocols and to determine its DNA damage inhibition and Antiurolithiatic capability. Dried leaves of Pisoniaalba were used to screen and quantify the phyto constituents in different organic solvents extracts that are analysed by GCMS, FTIR and HPTLC. In the presence of PUC18 plasmid DNA, the DNA damage inhibition assay was performed by photolysing H_2O_2 with UV radiation and agarose gel electrophoresis with irradiated DNA. The invivo antiurolithic assay was performed by nucleation method. Pisoniaalbain's phytochemical analysis reveals the existence of secondary metabolite components including glycosides, resins, phenols, terpenoids, flavonoids, tannins, steroids, and alkaloids, etc.. ELPA express the maximum quantity of phenols, flavonoids, alkaloids and terpenoids which were 30.44 ±0.65 mg TAE/g extract, 28.51 ± 1.19 mg RE/g extract, 28.08 ± 0.08 mg of AE/g of extract, 29.94 ± 0.32 mg RE/g extract respectively. The GC-MS assessment findings confirmed the presence of nine ELPA phyto compounds accompanied by eleven ALPA phytoc ompounds. It was also observed that Pisoniaalba leaves prevents DNA damage in UV and H_2O_2 treated plasmid DNA. The invitro Antiurolithiatic research indicates that ELPA was more efficient in blocking calcium oxalate nucleation. From the obtained results it was proven that the Pisonia alba leaves has potential secondary metabolites with therapeutic role mainly against urolithiasis. Both invitro and in vivo analysis demonstrates that ELPA significantly reduces crystal formation that are causative of renal stones. These evidences suggest that Pisoniaalbacan be further investigated for the prevention and treatment of urolithiais.***

Index Terms**: *ALPA, DNA fragmentation, ELPA, urolithiasis, Pisoniaalba, Nucleation***

I. INTRODUCTION

Urolithiasis is a common disease growing in prevalence with the prospective for significant morbidity. Urolithiasis condition affects kidneys due to the formation of calculi in theurinary tract, bladder, urethra and ureters. Among urinary tract infection and prostate conditions, urolithiasis is the third most popular disease in the world. Approximately,12% of global population was afflicted by urinary stones with a higher percentage of incidence in males (70-81%) than in females (47-60 %). The common symptoms of urolithiasis were flank pain, hematuria, nausea/vomiting, dysuria, haematuria, pyuria and oliguria [1]. Calculi formation occurs due to supersaturation of urine with stone forming constituents resulting in crystallization, followed by crystal nucleation, aggregation and growth leading to their adherence to the renal tubules.Fundamental calcium carbonate, cystone stones, calcium oxalate monohydrate, uric acid, dehydrate, and potassium ammonium carbonate form various types of stones based on the chemical nature [2].

Various surgical strategies were followed to control urolithiasis namely, shock wave lithotripsy, digital endoscopy, percutaneous nephrolithotomy and robotic surgery [3,4]. However, several drawbacks occurs due to these techniques such as renal casualities in the long run leading to nosocomial infections, hypertension and recurrence of renal stones [5]. Compared to the adverse effects implicated in allopathy, herbal therapies are safer with reduced side effects and gives increased efficacy in dissolving stones and also prevents disease recurrence [6]. Also frequently discussed in both the ancient medical literature and the ayurvedic medical scheme was the implementation of therapeutic plants in renal stone regulation [7].

The Indian traditional medicinal plant *Pisoniaalba*, belongs to Nyctaginaceae family. It was titled after Willem Piso (1611-1678), a Dutch physician and naturalist.*Pisoniaalba* is a large evergreen shrub and the tree rarely flowers in India. The flowers are small, green, and inconspicuous. The leaves are edible that are used as important ingredient in many ayurvedic formulations [8]. From previous evidences on the phytochemical analysis of *P. alba*, It has been demonstrated to constitute alkaloids , proteins, fats, thiamin, riboflavin, vitamin C, nicotinic acid (vitamin B3), and vitamin A Ethanolic leaf extracts disclosed the existence of significant secondary metabolites such as phenolic elements, flavanoids, steroids, tannins, terpenoids, saponins, kerolyticallantoin ,insulin omimeticpinitol, coumarins betacyanins and pulmonary glycosides [9]. The occurrence of these molecules in the Pisoniaal excerpts authenticates the use of this plant in tribal and traditional healers therapy of multiple diseases. The plant leaves were shown to inhibit rheumatic arthritis, fungal infections, inflammation, filariasis and dysentery etc. There was also strong radical scavenging and antimicrobial action against Staphylococcus aureus, Bacillus subtilis, Pseudomonas aureginosa , Escherichia bacteria, and Bacillus cereus in the ethanolic

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extract of plant leaves [10]. Therefore, the current study is investigating in evaluating the phytochemical components of Pisonia alba and determining its ability to inhibit DNA damage in invitro situation.

II. MATERIALS AND METHODS

Identification and sampling of plants

The new leaves of Pisoniaalba (Synonyms: Pisoniagrandis) were gathered from Kolli hills located in Nammakal district, Tami Nadu, India. The gathered sample from the Chennai Plant Anatomy Research Center (PARC) was acknowledged by Prof. P Jayaraman, a plant taxonomist. Certificate Reg No: PARC/2015/3251. The collected leaves were washed in distilled water until the water was clear and the leaves dried in shadow and finely powdered using electrical blender.

Extraction of plant samples

Preliminary screening of the phytochemical constituents in Pisoniaalba were done by plant leaves extractions carried out in different organic solvents using ethanol, methanol, chloroform, hexane, petroleum ether, ethylacetate and aqueous, as per standard protocols. The final extracts were used for phytochemical screening and characterization analysis.

FTIR analysis

Functional groups present in *Pisoniaalba* leaves extracts (ethanol and aqueous) were analyzed using Fourier Transform Infra Red spectral (FTIR) analysis. The experiment was conducted with a combination of potassium bromide powder (KBr) in ethanol and aqueous Pisoniaalba leaves extract. Using the Perkin-Elmer FT-IR spectrum-e1 spectrophotometer, the molecular functional vibrations of chemical components intended for biological behavior were evaluated at 2 cm^{-1} precision varying from 4000 to 400 cm^{-1}.

HPTLC chromatogram analysis

To screen the possible bioactive compounds in *Pisonia alba* leaves extract (Ethanol and aqueous) with the help of CAMAG Linomat 5 "Linomat5_170147" S/N 170147 HPTLC system designed with a sample applicator with sample loading syringe 100µl. 10 µl of ethanol and aqueous sample was applied and the plate was developed in 10ml of Toluene, ethylacetate and Dimethylamine in the proportion of 7:2:1(v/v/v) at 85 mm distance. After the mobile phase had run the plate were dried at 60 °C for 5 Minutes in oven. The plate was detected under 190 – 550 wavelength under D2 &W lamp in CAMAG Visualizer : 170503TLC scanner. Slit dimensions were set as 6.00 x 0.40 mm, macro, scanning speed of the detector is 20 mm/s, the resolution of data is 100 Fm/step. The TLC plate was detected before and after under two derivation at two different wavelength 254 and 354 nm. The RF (Retardation factor) value and the color of the determined band was noted.

Invitro DNA Cleavage assay

Ethanolic and aqueous extract of Pisoniaalba leaves was screened in the existence of PUC 18 DNA by photolysing H_2O_2 with UV radiation for its DNA damage inhibition function.The 20µl reaction mixture contains 10 µl extract, 5 µl PUC 18 DNA, 50mM Tris-HCL, 50mM NaCl, 2mM H2O2 in one tube and the residual tubes have been left untreated as irradiated controls. For 10 minutes at room temperature, the microcentrifuge tubes containing the mixed solutions were placed directly on the UV transilluminator surface. Following irradiation, 4µl of tracking dye (0.25% bromophenolblue, 0.25% xylene cyanol FF and 30% glycerol) was coupled individually with 5µl of untreated plasmid DNA samples and the banding pattern was observed in 1% agarose liquid (including ethidium bromide) in the TAE solution (pH 8).

Antiurolithiatic study

Nucleation and aggregation assay were conducted using conventional processes to explore Pisoniaalba's in vivo antiurolithic impact. Percent inhibition of nucleation was calculated by:

Percent inhibition=[(C-S)/C×100],

where, C=turbidity of control set, S=Sample turbidity. The dosage of the leaves extracts were fixed after the characterization of invitro antiurolithiatic assays and further used for the invivo study.

Table:1 Phytochemical analysis in *Pisonia alba* leaves extracts

Tests/extract	ethanol	methanol	chloroform	ethylacetate	hexane	Petroleum ether	aqueous
Alkaloids	+	+	+	+	-	-	+
Steroids	+	+	-	-	+	+	+
Flavanoids	+	-	+	-	-	-	+
Phenols	+	+	+	-	+	-	+
Tannins	+	+	-	+	+	+	+
Glycosides	+	+	+	-	+	+	+
Terpeinoids	+	+	+	+	-	+	+
Quinines	+	-	-	-	-	+	+
Resnins	+	-	+	+	+	+	+

III. RESULTS

Phytochemical analysis

Phytochemical analysis of *Pisonia alba* were performed in different organic solvents such as, hexane, ethylacetate, aqueous chloroform, methanol, petroleum ether, and ethanol. The screened phytoconstituents were represented in table: 1, which displayed the existence of secondary metabolite components, such as, phenols, tannins, steroids, glycosides, resins, flavonoids, terpenoids and alkaloids etc. Ethanol and aqueous extracts revealed nine phytocomponents followed by methanol, chloroform and petroleum ether exhibited six components, finally ethyl acetate and hexane showed four phytocompounds. Majorly found phytocompounds are alkaloids, steroids, phenols, terpenoids, tannins and glycosides.

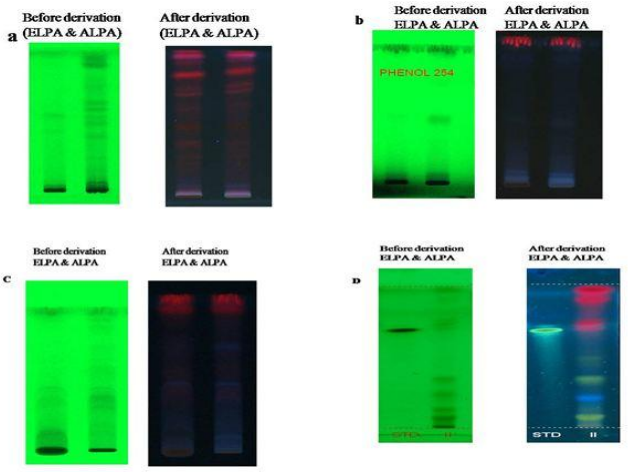

Fig:1 High performance Thin Layer Chromatography analysis HPTLC analysis in ELPA(Ethanolic leaves extract of *Pisonia alba*) and ALPA (Aqueous leaves extract of *Pisonia alba*), a) represent the screened image of alkaloids b) exhibit the screened compound of Phenol c) shows the terpenoids presence d) state the screen of flavonoids presence in both before and after derivation under the ultra voilet spectroscopy.

+: depicts the compound presence, -: depicts the compound absence in seven leaves extract of *Pisonia alba*, most of phytocompounds present in ethanol, methanol and aqueous extracts.

HPTLC analysis

The ethanol and aqueous leaves extract showing the presence of alkaloids reveals Rf value in the range of 1.22 and 1.21. ELPA reveals 5 peaks and ALPA 6 peaks, the peaks are visible at 366nm when Toluene, ethylacetate and Dimethyle amine (7:2:1) were used as mobile phase in chromotogram technique. The figure:1 shows the before and after derivation of the respective compound peak and exhibited Rf value showing area percentage corresponding to alkaloids, flavonoids, terpenoids, phenols, tannins in ELPA and ALPA.

FTIR Analysis

The FTIR spectra of both aqueous and ethanol extract of *Pisoniaalba* leaves are depicted in figure 2. The significant peaks of aqueous leaf extracts show distinct wave numbers corresponding to distinct functional groups and were noted at frequency ranges between 4000 – 400 cm^{-1}. Table: 2 explains the frequency range and their corresponding functional groups which revealed the presence of secondary metabolite compounds, alkanes, aromatic amines, carbon stretch, bending, amide groups, alkyl halides, 1°, 2° amines, and alcohols etc.The ethanolic leaves extracts of *Pisonia alba* produced peaks in twelve different frequency ranges showing the presence of amines, alcohol, and alkyl halides etc. The aqueous leaves extracts of *Pisonia alba* reveals one broad peak at 3251.98 showing the existence of hydrogen bonded alcohols, phenols /O-H stretching and also produced thirteen peaks at different frequency ranges.

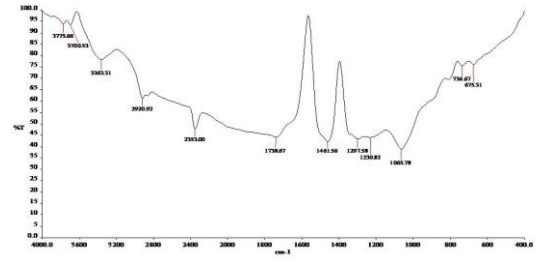

Figure: 2 (a) FTIR spectrum analysis in ELPA

Protective role of *Pisoniaalba* against DNA damage

Figure 3 shows the electrophoretic model of PUC 18 DNA after H_2O_2 UV-photolysis in the existence of Pisoniaalba aqueous, ethanolic and methanolic materials. Two bands of agarose fluid electrophoresis (Lane 2) were generated by the transformation of supercoiled linear (scDNA) to open circular form (ocDNA) extracted from PUC plasmid DNA, the faster migrating band on the agarose fluid was the indigenous type of scDNA and the slower migrating band was ocDNA. It was also noted that the intensity of ocDNA banding pattern was very light in ELPA treated lane-5(ethanolic leaves extract of *Pisoniaalba)*as compared to the banding intensities in other lane. Thus, ELPA therapy was discovered to be more efficient than Pisoniaalba's aqueous and methanol excerpts in stopping UV-induced DNA damage.

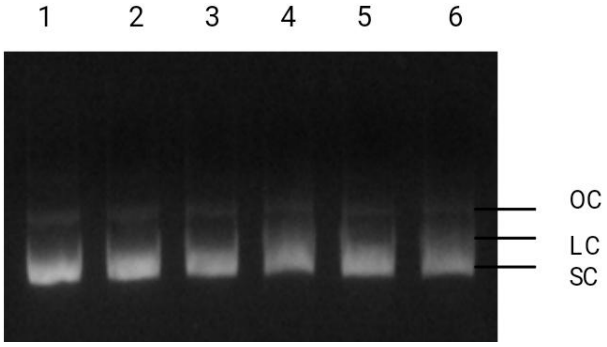

Figure: 3 DNA Damage assay in *Pisoniaalba* leaves extract

Lane 1: Positive control PUC18 DNA Lane 2: DNA + H_2O_2 Lane 3-5: MLPA, ELPA, ALPA at 70 µm Lane : H_2O_2 2mM, OC: Open Circular LC: Linear Circular MLPA methanolic leaves extract of *Pisonia alba* ELPA: ethanolic leaves extract of *Pisonia alba* ALPA: aqueous leaves extract of *Pisonia alba.*

Invitro Antiurolithiatic study

In invitro nucleation assay, Pisoniaalba leaves exhibited significant reduction in crystal formation compared to standard controls (cystone). The level of calcium oxalate crystal were determined like as nucleation and aggregation assays. The result were expressed as % variant in compare to control as mean ± SD (n=3), the % of cystone, ELPA and ALPA activities corresponds to 69.225 ± 2.389 100µg/ml of cystone, 40.509±1.538 100µg/ml of ELPA, 32.609 ± 1.598 100µg/ml of ALPA.

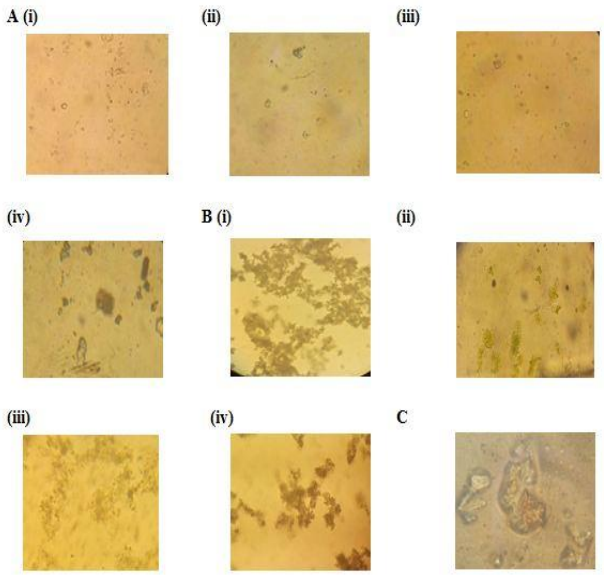

Figure: 4 Effect of Pisonialba leaves in antiurolithiatic activity. A)Nucleation assay (i) CaOx in control tube, ii) Cystone, iii) ELPA, iv) ALPA, B Aggregation assay (i) control, ii) Cystone, iii) ELPA, iv) ALPA, C) Crystal structure in microscope.

IV. DISCUSSION

Traditional medicines (TMs), such as herbal medicines, are used in the avoidance and therapy of natural illnesses for thousands of years. Researchers around the globe regard herbal species as a cause of fresh chemical compounds and are now used to isolate, atrophin, vinblastine, morphine, taxol and digoxin in allopathic medicine. However, the adverse effects of the chemical drugs led to the rapid growth and development of herbal medicinal products for treating various ailments. Large numbers of people have suffered from kidney stone, gallstone and urinary calculus in latest years. Stone disease is regarded to be the most common and old and can grow quickly owing to modifications in working circumstances, i.e. industrialization and malnutrition.

Herbal medicines, including Pashanbhedamen, are primarily used for diuretic and lithotriptic problems in the Ayurvedic medicine system. Stones and other crops such as Alternantherasessalis and Aervaspp in South India are also renowned for their diuretic property have been discovered to crack and disintegrate. Likewise, the aqueous and tobacco parts of Jasminu mauriculatum Vahl (Oleaceae) are recorded for kidney stone and Herniaria hirsute L aqueous nephrolithiasis extracts are reported. The aqueous extracts of many herbal plants such as Retamaraetam, Spergularia purpurea, Ammannia baccifera, Cratevanurvala, Sesbania grandiflora and Raphanussativus were used for kidney ailments, kidney stone, reducing urinary stone formation as well as antiurolithiatic, anti-hypercalciuric and anti-hyperoxaluric activity, respectively.

In supporting to these previous works the effect of Phyllanthusniruri on crystal deposition was experimented in rats and the results reveals that this herb has a therapeutic potential in the form and texture of the calculus are changed to a smooth, which eventually eliminate and dissolute the calculi. Based on these previous observations, the effect of Pisoniaalba against DNA damage inhibition and urolithiasis was tested in model rats in this study. It was noticeable from the earlier study that oxalate performs an significant part in stone formation and has an enhanced impact of about 15 times that of urinary calcium. The oxalate salts are small when coupled with magnesium, whereas when complexed with calcium they are insoluble creating crystalline precipitation of calcium oxalate calculus in the kidney. It is also valid to study the translational effect of Pisoniaalba and also to study the toxicological parameters which will eventually helps in using Pisoniaalba as a herbal drug prescribed for urolithiasis condition.

V. CONCLUSION

Drug of plants are less toxic, side effect of scanty and also caused effected with this optimistic and traditional folklore medicinal use the *Pisoniaalba* leaves has been identified and characterized for its bioactive potentials which has been uncovered and authentication by systematic quantification and characterization protocols. A profound quantum of phyto compounds has been seemed by this study. The vast and systematic study clearly validates that *Pisoniaalba* herbal sources which has been chosen to experiment and determined the antiurolithiatic property which has been scientifically documented by this study. The object focused in order to evaluate *Pisoniaalba* leaves as an natural nontoxic. Antiurolithiatic safe potent source to counter inflammatory response and protect the renal tissue damage and also provided a valid note that it possess membrane stabilization and regeneration capacity which ill aid developing new therapeutic option for kidney stone diseases in future.

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