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PHYTOCHEMICAL & *IN VITRO* ANTIUROLITHIATIC STUDIES ON THE LEAF EXTRACTS OF *BAUHINIA VARIEGATA* LINN.

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Keywords:

Fluorescence, Quantitative Determination, *In vitro*, Antiurolithiatic Study

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ABSTRACT: The aim of the present study is to carry out fluorescence analysis, phytochemical extraction, preliminary phytochemical screening, estimation of total flavonoids, tannins, alkaloids, steroids saponins and *in vitro* antiurolithiatic studies on the leaf aqueous and ethanolic extracts of *Bauhinia variegata.* The results of fluorescence analysis indicate that the powder on treatment with 50% H_2SO_4 shows dark brown colour under UV. The results of preliminary phytochemical screening indicated the presence of saponin glycosides, tropane alkaloids and acidic compounds. The results of quantitative determination indicated that the aqueous extract contains highest amount of flavonoids expressed as 54.6 mg/gm equivalents of quercetin, maximum amount of tannins in ethanolic exract as 56.30 mg/gm equivalents of quercetin, equal amounts of alkaloids are present in both extracts as 25mg/gm equivalents of atropine sulphate, steroids and saponins in lowest amount. The *in vitro* antiurolithiatic activity was studied as percentage inhibition of stones by nucleation, growth and aggregation assays for aqueous and ethanolic extracts at 200-1000 µg/ml taking cystone tablets as standard. The results indicated that the inhibition of growth of crystals increased with increase in concentration of the extract. Therefore, the plant claimed to possess antiurolithiatic activity and further *in vivo* studies as well as isolation of individual compounds responsible for the activity are necessary.

INTRODUCTION World Health Organization manifests that approximately 75% of the global population, of the developing world, depends on botanical medicines for their basic healthcare needs. [1] Exact identification and quality of the starting materials is an essential prerequisite to ensure reproductive quality of herbal medicine which will contribute to its safety and efficacy. [2]

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Deposition or formation of stones in any part of the urinary system i.e the kidney, the ureters or the urinary bladder is called Urolithiasis. Stone formation is the culmination of a series of physiochemical events *i.e.* super saturation and nucleation, growth of the crystal and aggregation that occurs as the glomerular filtrate traverses through the tubules of nephron.

Urine remains supersaturated with most stone forming salt components as well as chemicals that prevent or inhibit the crystals from urinary tract. These crystals remain tiny enough. [3] They will travel through the urinary tract and pass out of the body in the urine without being noticed. [4] Calcium oxalate stones represent upto 80% of analyzed stones. [5] It is considered as the third most common

affliction of the urinary tract. [6] However, the presence of certain molecules raise the level of supersaturation of salts needed to initiate crystal nucleation or reduce the rate of crystal growth or aggregation and prevents stone formation. [7]

Though technological advancements have made dramatic improvement, still some of the drawbacks of the methods exist which includes their being too costly and recurrence of stone formation along with number of other side effects [8]. The problem of urinary stones is an ancient one, and still remains a common problem worldwide.

Some medicinal plant**s** contain chemical compounds like Glycosaminoglycans (GAGs) which themselves possess an inhibitory effect in the crystallization of calcium oxalate. Antioxidant constituents of the plants also help in ameliorating the crystal/oxalate induced renal cell injury. Thus, antiurolithiatic activity of plants or herbal formulation may be due to synergism of their diuretic activity, crystallization inhibition along with antioxidant activity [9]. The activity of the extracts was evaluated by measuring the ability of the extracts to inhibit the formation of calcium and phosphate precipitates [10]. In order to find new potential antiurolithiatics the plant selected was *Bauhinea variegata* belonging to the family *Leguminosae* as there were no scientific reports published on the leaves of the plant for antiurolithiatic activity.

The plant *Bauhinea variegata* has been used traditionally, the root is carminative, used in dyspepsia, flatulence and as an antidote to snake poison. [11] The bark, flower uice are used as astringent, tonic, anthelmintic, scrofula and skin diseases [12].

The leaves of the plant were evaluated for the pharmacognostic, powder microscopy and physiochemical studies [13]. The non woody aerial parts contain 6 flavonoids, namely kaempferol, ombuin, kaempferol 7,4'-dimethylether-3-o-β-D-glucopyranoside, kaempferol – 3 – o - β-D-gluco pyranoside, isorhamnetin-3-o-β-D-glucopyranoside & hesperidin together with one triterpene caffeate, 3β trans-(3,4 dihydroxycinnamoyloxy) olean-12-en-28-oic acid. The root contains novel flavonol glycosides [14].

The root bark possesses a new flavonone (2S) -5, 7-dimethoxy-3, methylenedioxy flavonone and new dihydrodibenzoxepin 5, 6 d i h y d r o -1, 7-dihydro-1, 7-dihydroxy-3, 4-dimethoxy-2-methyldibenzoxepin, together with three known flavonoids [15].The Stem contains an unknown compound naringenin 5, 7 dimethyl ether 4 – rhamnoglucoside, a new phenanthraquinone named Bauhinione has been isolated from *B. variegata* L. [16]. The leaves contain two new long chain compounds heptatriacontane- 12, 13-diol 7 dotetracont-15-en-9-ol. Anti cancer activity was studied on the ethanolic extract of stem [17]. Antimicrobial activity was studied on the ethanolic extract of leaf and bark. [18] Antiinflammatory avtivity for the flavonoid compounds isolated from the non woody aerial parts. [19] Hepatoprotective activity was studied on the ethanolic extract of stem.[20] Antiulcer activity was studied on the ethanolic extract of stem of *Bauhinea variegata.* [21] The aimof the present study was to carry out UV-Fluoresence analysis of the powder, preliminary phytochemical screening, to determine total amount of flavonoids, tannins, alkaloids, steroids and saponins and to evaluate *in vitro* antiurolithiatic studies by nucleation, growth and aggregation assays for the aqueous and ethanolic extracts of leaves of *Bauhinea variegata*.

MATERIALS AND METHODS:

Materials: The Plant material *Bauhinia variegata* was collected in the month of December during afternoon from the grounds of Vijaya institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada. Herbarium was prepared and the sample was authenticated by Dr. D.T. P. Satyanarayana Raju, plant taxonomist, department of Botany and Microbiology, Acharya Nagarjuna University, Guntur. The photographs of the plant and leaves were depicted in **Fig. 1** and **Fig. 2** The authentication letter was also enclosed. The Chemicals used were purchased from Finar chemicals. The dried leaves were coarsely powdered and depicted in **Fig. 3 and Fig. 4.**

Powder Analysis:

Powder Analysis using Chemical Reagents with naked Eye: The leaf powder was studied with naked eyeby using the chemicals and the results were noted and given in **Table 1** [22].

Fluorescence Analysis of the Leaf Powder:
The leaf powder was studied for fluorescence analysis under UV (Lab India) and the results were noted after treating with different chemical reagents and given in **Table 2**.[22]

Extraction: The powder was subjected to Soxhlet extraction by using water and alcohol as solvents, produced in **Fig 5**. The crude extract was dried using vaccum pump and weighed.

Phytochemical Study:
Qualitative screening: The aqueous and ethanolic extracts of the leaves of *Bauhinia variegata* were screened for the phytochemical constituents according to the standard methods. The results were given in **Table 3**. [23, 24, 25, 26]

Quantitative Determination:
Flavonoids: The extract (1.5 mL) was added to 1.5 ml of 2% methanolic $AlCl_3$ solution. The mixture was vigorously shaken on Centrifuge for 5 minutes at 200 rpm and the absorbance was read at 367 nm after 10 minutes of incubation. Quercetin was used as a standard for the calibration curve, given in fig 6. The assay was carried out in triplicate, results were calculated using the given formula, produced in **Table 4** and expressed as mg/g equivalent of quercetin [27, 28].

$$C = c.V/m$$

C – Total phenolic compounds mg/g of plant extract;
c – The concentration of standard established from the calibration curve mg/ml
V – The volume of extract in ml
m -The weight of pure plant extract

Tannins: This was determined by Folin-Denis colorimetric method with slight modification. 0.5 gmof the sample was dispersed in 50 ml of distilled water and shaken. The mixture was left undisturbed for 30 minutes at 28°C and filtered through Whatmann No. 1 filter paper. The filtrate (2 ml) was dispersed into a 50ml volumetric flask and 2.5 ml of 10% Na_2CO_3 solution was added. The content of each flask was made up to 50 ml with distilled water and incubated at 28°C for 90 minutes. Absorbance was read at 260 nm using the reagent blank. Tannic acid was used for the calibration curve, given in **Table 7**. The procedure was repeated three times results were calculated using the given formula and values were given in **Table 4**, expressed as mg/g equivalent of quercetin. [28, 29]

Alkaloids:
Preparation of Reagents: Bromocresol green solution was prepared by heating 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water.Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2M sodium phosphate (71.6 g Na_2HPO_4 in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 L distilled water).

Separation of Alkaloids: A part of extract residue was dissolved in 2N HCl and then filtered. 1 ml of this solution was transferred to separating funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and complex extracted with 1, 2, 3 and 4 ml chloroform by vigorous shaking, the extract was then collected in a 10 ml volumetric flask and diluted with chloroform.

Method: The sample (0.5 g) was dispersed in 10% acetic acid solution in ethanol in a ratio of 1:10 (10%). The mixture was left undisturbed for 4 hours at 28°C and filtered with Whatmann No. 1 filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with 3 drops of conc. NH_4OH. The alkaloid precipitate was received in a weighed filter paper, washed with 1% ammonia solution, and oven dried at 80°C. Alkaloid content, expressed as a percentage of the weight of sample analyzed and converted to mg/ml. Atropine was used as standard for the calibration curve, produced in fig 8.This was done in triplicate results were calculated using the given formula, expressed as mg/g equivalents of atropine and the values were produced in **Table 4**. [28; 30]

$$C = c.V/m$$

Steroids: Preparation of Reagents: 20% $MgSO_4$: 20 g of magnesium sulphate was dissolved in 100 ml of distilled water. [31]

Method: This was done by the alkaline precipitation gravimetric method described by Harborne. The sample (0.5g) was taken and dissolved in 50 ml of butanol and it was stirred for about 5 hours for homogenous mixing and the above solutions was filtered by using Whatmann filter paper and add 20 ml of 20% Magnesium sulphate and was filtered. The above solution was analysed by using UV spectrophotometer at 380nm and this procedure was repeated for triplicate. Diosgenin was used as standard for the calibration curve, depicted in fig 9, results were calculated using the given formula and expressed as mg/g equivalents of diosgenin, produced in table 4.[28;32]

$$C = c.V/m$$

Saponins:

Preparation of Reagents: 40% Magnesium carbonate: 40 g of $MgCO_3$ was dissolved in 100 ml of water.5% $FeCl_3$ solution: 5 g of ferric chloride was dissolved in 100 ml of water.

Method: A known mass (1 g) of finely ground sample was weighed into a 250 mL beaker and 100 ml of isobutyl alcohol was added. The mixture was shaken for 5 hours to ensure uniform mixing and filtered through Whatmann No. 1 filter paper into a 100mL beaker, after which 20 ml of 40% magnesium carbonate solution was added. The resulting mixture was again filtered through Whatmann No. 1 filter paper to obtain a clear colorless solution. A known volume (1 ml) of the colorless solution was pipetted into a 50-mL volumetric flask and 2 ml of 5% $FeCl_3$ solution was added and made up to the marked level with distilled water. It was left undisturbed for 30 minutes for blood red color to develop. The absorbance was read after color development at a wavelength of 380 nm. Standard Saponin diosgenin was used for calibration curve, depicted in **Fig 10,** results were calculated in same way as above, given **Table 4.**[28, 33]

$$C = c.V/m$$

***In vitro* antiurolithiatic activity:**

Nucleation assay Method:

Preparation of Reagents: 10% Trichloro acetic acid was prepared by dissolving 10g of Trichloroacetic acid in 1000 ml of water. [34]

Method: It is the classical model for the study of oxalate crystallization because of its simplicity and satisfactory reproducibility. This model includes the study of crystallization without inhibitor and with it, in order to assess the inhibiting capacity of any chemical species used. Solution of calcium chloride and sodium oxalate were prepared at the final concentrations of 5mmol/L and 7.5 mmol/L respectively in a buffer containing Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. 950 µL of calcium chloride solution mixed with 100 µL of herb extracts at the different concentrations (100 µg/ml–1000 µg/ml). Crystallization was started by adding 950 µL of sodium oxalate solution.

The temperature was maintained at 37 °C. The OD of the solution was monitored at 620 nm. The rate of nucleation was estimated by comparing the induction time in the presence of the extract with that of control. The Cystone tablets are used as standard solution.[35] The results were given in **Fig 11** and **12** and **Table 5**.

The growth of crystals was expected due to the following reaction:

$CaCl_2+NaC_2O_4 \rightarrow 2CaCO_4+2NaCl.CaCl_2+Na_2C_2O_4 \rightarrow CaC_2\ O_4+2NaCl$

Growth Assay:

Preparation of Reagents: 4mM $CaCl_2$ was prepared by 4 g of calcium chloride was dissolved in 1 litre of water.4mM of Sodium oxalate was prepared by dissolving 4 g of sodium oxalate was dissolved in 1 litre of water.10mM NaCl was prepared by dissolving 10 g of NaCl was dissolved in in 1 litre of water.[14]

Method: Newly formed crystals may combine to form a small hard mass, called calculus. The percentage inhibition of calcium oxalate crystal growth was evaluated in presence and absence of drug extracts. 4mM calcium chloride and 4mMsodium oxalate of 1ml each were added to a 1.5ml of solution containing sodium chloride (10mM) buffered with Tris (10mM) at pH 7.2. To this 30µl of calcium oxalate monohydrate crystal slurry (1.5mg/ml acetate buffer) was added. Consumption of oxalate begins immediately after calcium oxalate monohydrate crystal slurry addition and was monitored for 600 seconds for the disappearance of absorbance at 214 nm, with and without extract. The results were produced in **Fig. 13** and **14** and **Table 6.**[36, 37, 38]

The Cystone tablets are used as Standard drug solution.[10]

The relative inhibitory activity was calculated as follows:

% relative inhibitory activity = ((C-S)/C) × 100

Where 'C' is the rate of reduction of free oxalate without any extract and 'S' is the rate of reduction of free oxalate with drug extract.[39]

Aggregation Assay:

Preparation of Reagents: 50 mM $CaCl_2$ was prepared by 50 g of calcium chloride was dissolved in 1 liter of water. 50 mM of Sodium oxalate was prepared by dissolving 50 g of sodium oxalate was dissolved in 1 liter of water. 0.05 mM NaCl was prepared by dissolving 0.05 g of NaCl was dissolved in in 1 liter of water.[40]

Method: CaOx monohydrate (COM) crystals were prepared by mixing calcium chloride and sodium oxalate at 50 mmol/L. Both solutions were equilibrated to 60 °C in a water bath for 1 h and then cooled to 37 °C overnight. The crystals were harvested by centrifugation and then evaporated at 37 °C. CaOX crystals were used at a final concentration of 0.8 mg/ml, buffered with Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. Experiments were conducted at 37 °C in the absence or presence of the plant extract after stopping the stirring. The Cystone tablets are used as Standard drug solution .The results were given in **Fig. 15** and **Table 7**.[41, 42] The percentage aggregation inhibition rate (Ir) was then calculated by comparing the turbidity in the presence of the extract with that obtained in the control using following formula:

Ir= (1−Turbiditysample/Turbidity control) × 100

RESULTS AND DISCUSSION:

FIG: 1 *BAUHINEA VARIEGATA* PLANT

FIG.2: *BAUHINEA VARIEGATA* LEAVES UPPER AND LOWER SIDE

FIG.3: *BAUHINEA VARIEGATA DRIED* LEAVES UPPER AND LOWER SIDE

FIG. 4: DRIED LEAF POWDER OF *BAUHINEA VARIEGATA*

Powder Analysis:

Powder analysis using chemical reagents with nacked eye:

TABLE 1: POWDER ANALYSIS WITH CHEMICAL REAGENTS FOR THE LEAVES OF *BAUHINIA VARIEGATA*

S.no.	Reagents	Colour observed
1.	Powder as such	Light green
2.	Powder + concentrated HCl	green
3.	Powder + concentrated HNO_3	Light brown
4.	Powder + concentrated H_2SO_4	Dark brown
5.	Powder + 5% NaOH Solution	Brownish yellow
6.	Powder + 5% KOH solution	Brownish yellow
7.	Powder + 5% $FeCl_3$	Black
8.	Powder + picric acid	Light green
9.	Powder + ammonia	Dark brown

Fluorescence Analysis of the Leaf Powder:

TABLE 2: FLUORESCENCE ANALYSIS OF *BAUHINIA VARIEGATA* LEAF POWDER:

S.no	Treatment with Chemical Reagents	Fluorescence Observed
1.	Powder as such	Green
2.	Powder + 1N NaOH in methanol	Brown yellow
3.	Powder + 1N NaoH in water	yellow
4.	Powder + 50% HCL	Green
5.	Powder + 50% H_2SO_4	Dark brown
6.	Powder + 50% HNO_3	Light brown
7.	Powder + petroleum ether	Colour less
8.	Powder + NH_3	Light green
9.	Powder + picric acid	Light green
10.	Powder + $FeCl_3$ solution	Black
11.	Powder + 5% KOH	Green

The results of powder analysis indicate that when the powder was observed with naked eye on treatment with 5% KOH solution shows brownish yellow which on UV shows green florescence. When the powder is observed by adding ammonia shows dark brown colour which under UV observation shows light green florescence. This analysis was helped in powder material of *Bauhinia variegata.*

Phytochemical Study:

FIG. 5: PHYTOCHEMICAL EXTRACTION *BAUHINIA VARIEGATA* LEAF POWDER USING WATER AND ETHANOL AS SOLVENTS

Qualitative Screening: The results of preliminary phytochemical screening show the presence of Acidic compounds, saponins glycosides, alkaloids, Tropane alkaloids.

TABLE 3: QUALITATIVE PHYTOCHEMICAL SCREENING OF AQUEOUS AND ETHANOLIC EXTRACTS OF *BAUHINIA VARIEGATA* LEAVES

S.no.	Phyto chemical	Test	AqEBVL	EEBVL
1.	Saponin glycosides	Emulsification test	+	+
		Froth test	+	+
2.	Alkaloids	Dragodorffs reagent	+	-
		Mayers test	+	+
		Wagners test	+	+
		Tannic acid reagent test	+	+
3.	Tropane alkaloids	Vitalimorin test	+	+
		Tannic acid test	+	+
		Iodine test	+	+

AqELBV- aqueous extract of leaves of *Bauhinia variegata,* **ELBVTA-** ethanolic extract of leaves of *Bauhinia variegata.*

Quantitative Determination:

TABLE 4: QUANTITATIVE PHYTOCHEMICAL SCREENING OF AQUEOUS AND ETHANOLIC EXTRACTS OF BAUHINIA *VARIEGATA* LEAVES

S.no	Phytochemical	AqELBV	EELBV
1.	Flavanoids (mg/g)	54.6±1.616	48.4±1.568
2.	Tannins (mg/g)	21.46±1.54	56.30±0.558
3.	Alkaloids (mg/g)	25.26±1.75	25.32±1.677
4.	Steroids (mg/g)	15.58 ±0.585	13.585 ±0.577
5.	Saponins(mg/g)	13.48 ±0.458	12.585 ± 0.414

Values represented mean ± **S.D.** of three parallel measurements. **AqELBV, EELBV-** aqueous extract of roots of *Bauhinia variegata,* ethanolic extract of leaves of *Bauhinia variegate*

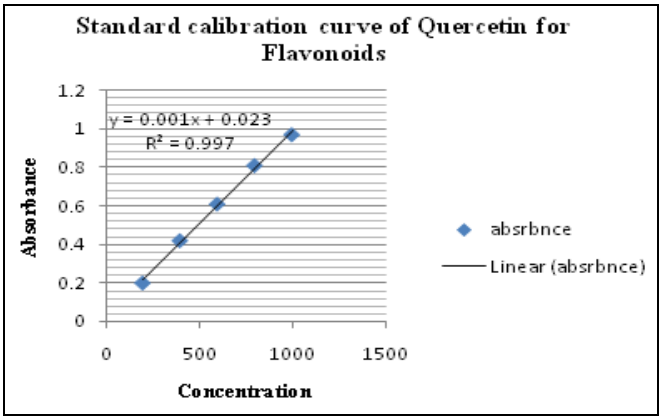

FIG. 6: STANDARD CALIBRATION OF FLAVONOIDS FOR QUERCETIN

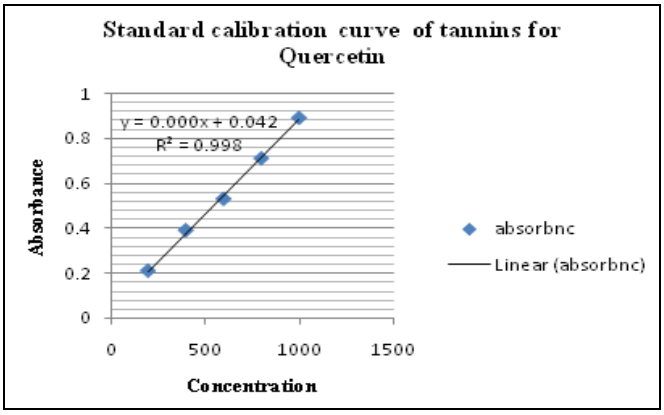

FIG. 7: STANDARD CALIBRATION OF TANNINS FOR QUERCETIN

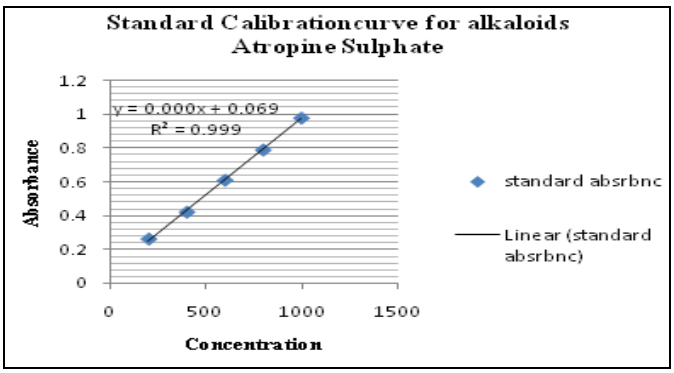

FIG. 8: STANDARD CALIBERATION CURVE OF ATROPINE FOR ALKALOIDS

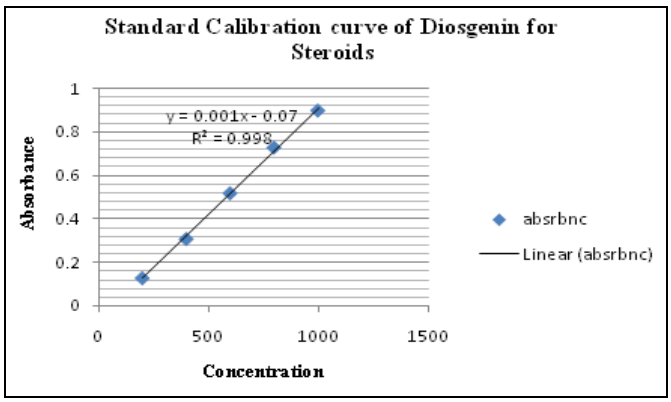

FIG. 9: STANDARD CALIBRATION OF STEROIDS FOR DIOSGENIN

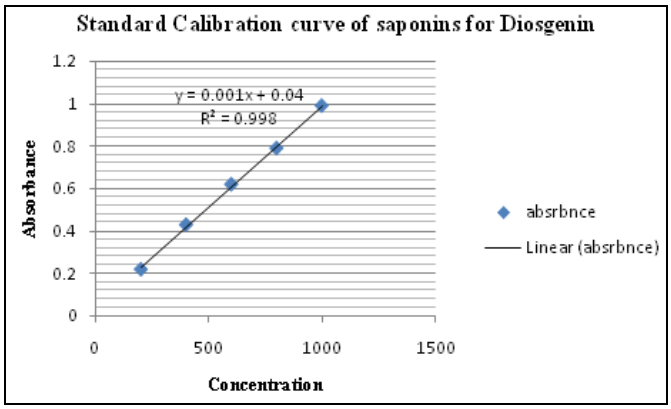

FIG.10: STANDARD CALIBRATION CURVE OF SAPONINS FOR DIOSGENIN

The results of quantitative determination indicated that the leaf powder of *Bauhinia variegata* contains highest amount of Saponins in Aqueous extract. It contains lower amounts of steroids in Aqueous and ethanolic extracts of *Bauhinia variegata*. The amounts of Alkaloids are equal in both aqueous and ethanolic extracts.

Antiurolithiatic Aactivity:

Nucleation Assay Method:

FIG.11: STUDY OF CALCIUM OXALATE CRYSTALS IN THE AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF *BAUHINIA VARIEGATA*

TABLE 5: ANTIUROLITHIATIC EFFECT OF DIFFERENT CONCENTRATIONS OF AQUEOUS AND ETHANOLIC EXTRACTS OF *BAUHINIA VARIEGATA* LEAVES BY NUCLEATION ASSAY METHOD

S.no	Concentration (µg/ml)	AqELBV	EELBV	STD
1.	200µg/ml	65.1%±0.0029**	68.4%±0.0012**	80.85%±0.0016*
2.	400µg/ml	76.5%±0.001**	70.6%±0.0013*	79.23%±0.0012*
3.	600µg/ml	83.2%±0.078**	80.2%±0.044*	79.56%±0.0042*
4.	800µg/ml	89.2%±0.0018**	85.2%±0.0028**	83.2%±0.004*
5.	1000µg/ml	94.2%±0.008**	93.2%±0.0025**	90.2%±0.0011**

The values are expressed as Mean ±SEM, n= 6. The values are significant,* p< 0.05;**p < 0.01 when compared with standard. **AqELBV, EELBV,** STD– aqueous extract of leaves of *Bauhinia variegata*, ethanolic extract of leaves of *Bauhinia variegata*, Standard.

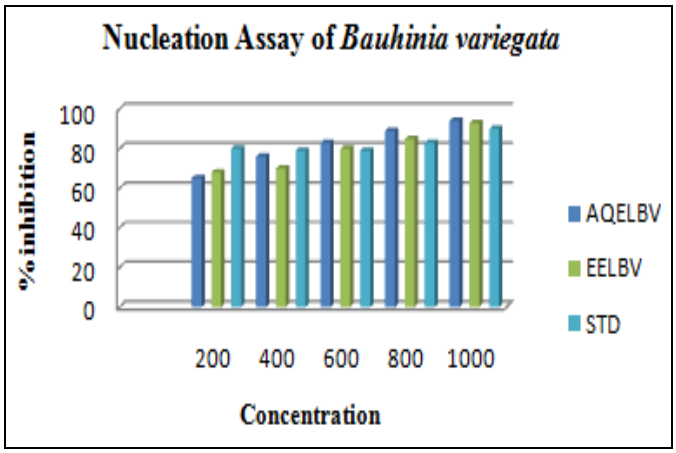

FIG. 12: NUCLEATION ASSAY ON THE LEAF EXTRACTS OF *BAUHINIA VARIEGATA*

Growth Assay Method:

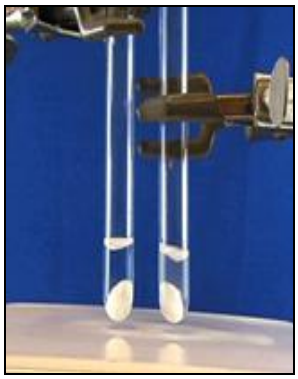

FIG.13: FORMATION OF CALCIUM OXALATE CRYSTALS

TABLE 6: ANTIUROLITHIATIC EFFECT OF DIFFERENT CONCENTRATIONS OF AQUEOUS AND ETHANOLIC EXTRACTS OF *BAUHINIA VARIEGATA* LEAVES BY GROWTH ASSAY METHOD

S.no	Concentration (µg/ml)	AqELBV	EELBV	STD
1.	200	67.1%±0.0329**	69.4%±0.082**	72.85%±0.0366*
2.	400	86.5%±0.082**	90.6%±0.063*	82.23%±0.023*
3.	600	83.2%±0.078**	85.2%±0.044*	84.56%±0.0242*
4.	800	91.2%±0.078**	92.2%±0.078**	90.2%±0.078**
5.	1000	93.2%±0.078**	95.2%±0.078**	92.2%±0.078**

The values are expressed as Mean ±**SEM**, **n= 6**. The values are significant,* **p< 0.05**;** **p < 0.01** when compared with standard. **AqLBV, EELBV**, **STD** – Aqueous extract of leaves of *Bauhinia variegata*, Ethanolic extract of leaves of *Bauhinia variegata*, Standard.

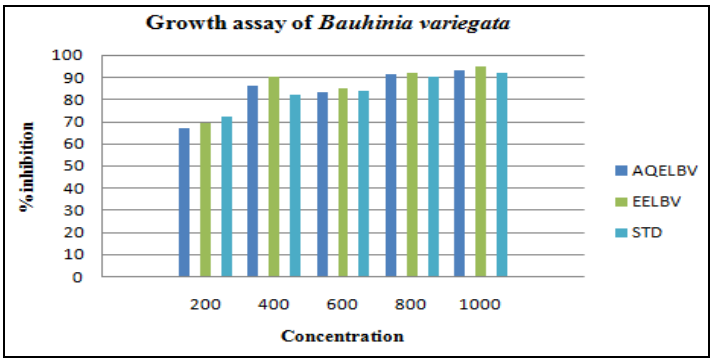

FIG. 14: GROWTH ASSAY ON THE LEAF EXTRACTS OF *BAUHINIA VARIEGATA*

Aggregation Assay:

TABLE 7: ANTI UROLITHIATIC EFFECT OF DIFFERENT CONCENTRATIONS OF AQUEOUS AND ETHANOLIC EXTRACTS OF *BAUHINIA VARIEGATA* LEAVESBY AGGREGATION ACTIVITY

S.no.	Concentration (µg/ml)	AqEELBV	EELBV	STD
1.	200	63.1%±0.0012**	66.4%±0.0002**	62.85%±0.066*
2.	400	76.5%±0.082**	78.6%±0.063*	72.23%±0.023*
3.	600	81.2%±0.078**	83.2%±0.044*	80.56%±0.0242*
4.	800	91.2%±0.078**	94.2%±0.078**	90.2%±0.078**
5.	1000	93.2%±0.078**	95.2%±0.078**	94.2%±0.078**

The values are expressed as Mean ± **SEM, n= 6**. The values are significant,* **p< 0.05;** ** **p < 0.01** when compared with standard. **AqELBV, EELBV, STD** – Aqueous extract of leaves of *Bauhinia variegata*, ethanolic extract of leaves of *Bauhinia variegata*, Standard.

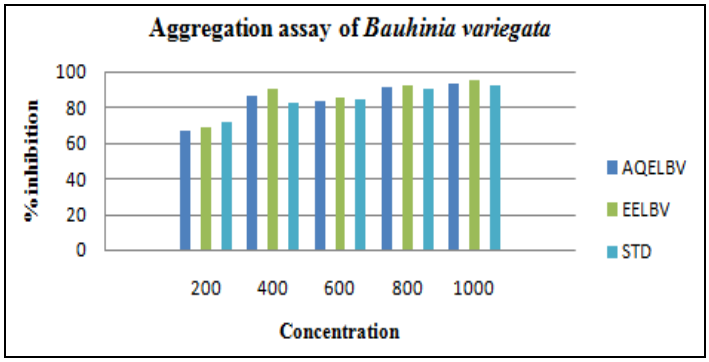

FIG. 15: AGGREGATION ACTIVITY OF *BAUHINIA VARIEGATA*

The results of Anti urolithiatic activity by nucleation assay indicated that % inhibition was more in aqueous extract at 1000µg/ml when compared to standard drug Cystone tablets. The results of Anti urolithiatic activity by growth assay indicated that % inhibition was more in ethanolic extract at 1000µg/mlwhen compared to standard drug Cystone tablets. The results of Anti urolithiatic activity by aggragation assay indicated that % inhibition is equivalent to standard drug Cystone tablets. The leaves of the plant exhibited significant antilithiatic activity due to the presence of flavonoids and tannns in rich amount (Surendra *et al.*, 2011). The statistical analysis was performed using one way Annova, Dunnetts test through Insat graph pad software.

CONCLUSION: The aqueous and ethanolic extracts of *Bauhinia variegata* leaf have inhibitory effect on CaOx crystallization thus may be beneficial in the treatment of urolithiasis. But there is a need of detailed investigation in elaborated preclinical experimentations and clinical trials to establish the use of plant as antiurolithiatic agent.

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