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**IN-VITRO AND IN-VIVO EVALUATION OF ROOT AND LEAF EXTRACTS OF  
*CROTALARIA PALLIDA AITON* FOR ANTIUROLITHIATIC PROPERTIES IN  
GENTAMYCIN INDUCED UROLITHIASIS MODEL**

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**ABSTRACT**

**Background:** Kidney Stone is a conglomeration of solid deposits of salts and minerals leading to accumulation in renal system. *C. pallida* is a significant medicinal herb with antiurolithiatic action. Nevertheless, no in-vitro and in-vivo studies were identified to bolster the proposed theory. Therefore, the purpose of this study was to investigate the phytochemistry of *C. pallida* and assess its in-vitro and in-vivo antiurolithiatic efficacy in gentamycin induced model.

**Methods:** Acute toxicity studies for Methanolic and Ethanolic extract of Roots and Leaves was conducted according to OECD guideline 420 using Wistar albino rats. The inhibitory activity of the extracts on the nucleation of Calcium oxalate (CaOx) crystals was determined by a spectrophotometric assay. Ethanolic and methanolic root and leaf extracts of *crotalaria pallida* was treated to animals who were given with gentamycin 40mg/kg sc for 1-8 days and calculi producing diet from 1-15 days. Urine parameters such as urea, uric acid, oxalate etc were measured.

**Results:** There were no signs of toxicity in the initial investigation on a single rat given a dosage of 2000 mg/kg of methanolic and ethanolic root and leaf extracts. The maximum % inhibition observed was 70.11% at 1000mg/mL of methanolic root extract concentration, followed by ethanolic leaf extract had 68.91% inhibition at 1000mg/mL concentration. All the

urine parameters such as urea, uric acid, oxalate, phosphorus, creatinine was greatly lowered by extracts of *C. pallida* whereas magnesium levels were improved.

**Conclusion:** *Crotalaria pallida* Aiton was discovered to possess therapeutic value in the management of urolithiasis.

**Keywords:** *Crotalaria pallida*, Urolithiasis, In-vitro evaluation, Phytochemical evaluation, Animal Model Study

## INTRODUCTION

Kidney Stone is a conglomeration of solid deposits of salts and minerals leading to accumulation in renal system. The illness is notable for relapsing, which causes excruciating suffering. Accordingly, the proportion of cases of urinary calculi fluctuates between 1% to 20% [1]. For all of humankind's existence, Mother Earth offers medicine, sustenance, shelter, flavours, fertiliser, and scents. Plants have been used for medicine from the inception of time owing to their number of therapeutic qualities [2]. Globally, they serve as the main source of medicine. Because of the lack of available medical resources, the high expense of care, the negative effects of many synthetic medications, and the emergence of drug resistance in infectious illnesses, an increasing population leads to an increased use of medicinal plants. According to a recent WHO estimate, around 21,000 plant species are utilised as medicinal plants, and 80% of people worldwide rely on them for their basic medical needs [3].

The Fabaceae family, which makes up the third biggest group of angiosperms, has about 700 genera and around 20,000

varieties of trees, shrubs, vines, and herbs throughout the world's geography [4]. India is home to over 81 species of *Crotalaria*, with *Crotalaria pallida* Aiton being one of the more noteworthy ones [5]. The majority of *Crotalaria* species are utilised as medicinal agents in India to treat a wide range of illnesses [6]. These applications have increased access to care, including palliative care for a number of illnesses that contemporary medicine is unable to effectively cure. For a long time, the pharmacology and phytochemistry of several *Crotalaria* species have been reported; however, only a small number of species have been thoroughly examined and explored [7, 8]. *C. pallida* is a significant medicinal herb with antiurolithaitic action. Nevertheless, no in-vitro and in-vivo studies were identified to bolster the proposed theory. Moreover, extrapolating inferences from the put forward theories casts scepticism on their significance. It's critical to evaluate the plant component and active ingredient that work best for treating urolithiasis. The need for further thorough study on *C. pallida* Aiton in the

management of kidney stones has been brought to light by this stark gap. Therefore, the purpose of this study was to investigate the phytochemistry of *C. pallida* and assess its in-vitro and in-vivo antiurolithiatic efficacy.

#### MATERIALS AND METHODS

*C. pallida* plant was collected from various places in and around the areas of Mandya district of Karnataka state. Whole plants of it were collected and identified by comparing with herbarium specimens. The procured plants of *C. pallida* were sent to “Central Ayurveda Research Centre”, Bengaluru for the authentication process. Upon thorough examination and verification, the board certified that the plant specimens were authentic as per the requirements (Ref: RRCBI-18618). After authentication, leaves and roots were sorted from the whole-plant. The leaves and roots were air dried and mashed until it turned into powder. The powder was vacuum dried at 25<sup>o</sup> C and 50% humidity, thus preventing any oxidation. The powdered roots and leaves of the plants were stored in air-tight container and to prevent accidental hygroscopy, and a desiccator was added. The powder was used for the extraction process and the extraction was done using methanol and ethanol using Soxhlet apparatus. A basic phytochemical analysis of plants extracts (Roots and leaves) for phytoconstituents was performed using the

standard phytochemical screening procedures mentioned by Sofowara 1993 [9].

Acute toxicity studies for Methanolic and Ethanolic extract of Roots and Leaves was conducted according to OECD guideline 420 [10] using Wistar albino rats. The extracts were dissolved in Tween 80 before administration into the animals. Female Adult Wistar albino rats weighing around 180 – 200 grams were used for the testing acute oral toxicity. They remained in polypropylene cages at 25 ± 2°C with relative humidity 45-55% under 12hr light and dark cycles. All the animals were adjusted to the laboratory surroundings for a week earlier use. They were nourished with standard animal feed and water ad libitum. The animals were divided into 5 groups; each group containing 6 animals. The initial dose was chosen at 2000 mg/kg with the consideration that there were in vivo and in vitro toxicity data. One animal in each group is from the sighting study dosed at 2000mg/Kg. Animals (e.g. with the rat, food but not water should be withheld) were fasted overnight prior to administration. Group I was considered as control, whereas group II to V acted as test groups. After administration of the extracts, food was withheld for a further 3 to 4 hrs. The animals were then individually observed (with special attention during the first 4 h) for possible behavioural changes, allergic

reactions (skin rash, itching), eyes and mucous membrane, or any alterations unceasingly and mortality for next 24 hours and the observation was continued for 14 days.

The inhibitory activity of the extracts with concentrations of 100, 200, 400, 600, 800, and 1000 µg/mL on the nucleation of Calcium oxalate (CaOx) crystals was determined by a spectrophotometric assay as described by Bawari *et al* [11]. Solution of calcium chloride (CaCl<sub>2</sub>) and sodium oxalate (Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub>) were prepared at the final concentrations of 5 mmol/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. 1 ml of each concentration was mixed with 1 ml CaCl<sub>2</sub> solution followed by the addition of 1 ml Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub> solution. Final mixtures were incubated for 30 min at 37°C. The optical density (OD) of the mixtures was measured at 620 nm with an UV-visible spectrophotometer (UV-1900I, SHIMADZU, Japan). Percentage inhibition of nucleation was calculated using the following formula:

$$\% \text{ inhibition} = \left[ 1 - \frac{OD \text{ Test}}{OD \text{ Control}} \right] \times 100$$

where % Inhibition is percentage of inhibition of the crystals, OD Test is optical density with plant extract/standard drug, and OD Control is optical density without plant extract/standard drug. CaOx crystallization

was observed under a light microscope in the presence and absence of extracts. Cystone, a standard herbal therapy in kidney stones was used as a standard/control. The basic calculations were performed using Microsoft Excel and the add-on facility of graph design was used to create the graphs. Gentamycin induced urolithiasis (prophylactic) was used to assess in-vivo activity of *Crotalaria pallida* in which post-one hour upon administering the ethanolic and methanolic leaves and root extracts of *Crotalaria pallida*, Gentamycin 40mg/kg/day s.c from 1-8 days was provided along with calculi producing diet from 1 to 15 day, p.o. The animals procured was divided into 7 groups: Group I serving as normal control treated only with distilled water 10ml/ kg p.o. for 15 days; Group II serving as negative control; Group III serving as standard control treated with cystone; Group IV received Methanolic Root extract of *Crotalaria pallida* Aiton; Group V received Ethanolic Root extract of *Crotalaria pallida* Aiton; Group VI received Methanolic leaf extract of *Crotalaria pallida* Aiton and Group VII received Ethanolic leaf extract of *Crotalaria pallida* Aiton. Urine parameters such as urea, uric acid, creatinine, oxalate, phosphorus, calcium and magnesium was assessed using standard tests. All the hypothesis testing statistics were performed using SPSS v.29.0.1.0. One-way ANOVA was employed with post-hoc study of

Dunnett Test to compare the means between the control and tests. All the variables are expressed in Mean  $\pm$  Standard Deviation.

## RESULTS

The yield of Methanolic Root Extract (MRE) and Methanolic leaf Extract (MLE) of *C. pallida* was found to be 3.9% and 11.9% respectively, whereas yield of Ethanolic Root Extract (ERE) and Ethanolic Leaf Extract (ELE) was 2.33% and 15.93%.

Using these extracts, a phytochemical

screening resulted in identification of secondary metabolites such as carbohydrates, resins, alkaloids, phenols, flavonoids, monoterpenes, saponins, terpenoids with varying amounts in each extract. The same has been summarised in **Table 1 and 2**. It was discovered that methanol and ethanol leaf extracts showed higher phytoconstituents presence than root extracts.

**Table 1: Phytochemical Constituents Present in Methanolic and Ethanolic Leaf Extracts of *C. pallida***

Phytochemicals	MLE	ELE
Carbohydrates	-	-
Resins	+	-
Alkaloids	-	+
Phenol	-	+
Saponin	-	+
Tannin	-	+
Flavonoids	-	+
Monoterpenes	-	+
Steroids	-	+
Terpenoids	-	+
Sesquiterpenes	-	-

- indicates absence of the constituents and + indicates the presence

**Table 2: Phytochemical Constituents Present in Methanolic and Ethanolic Root Extracts of *C. pallida***

Phytochemicals	MRE	ERE
Alkaloids	+	-
Gums	-	+
Saponin	+	+
Tannin	-	+
Flavonoids	+	+
Glycosides	-	+
Steroids	-	+

- indicates absence of the constituents and + indicates the presence

There were no significant changes observed in animals given with methanolic and ethanolic extracts of roots and leaves of *C. pallida* either in first 24 hours or at 72 hours or till the 14 days of observation. A dose up to 2000 mg/kg body weight was found to be safe in all the groups tested.

A range of in-vitro antiurolithiatic activity was seen in the nucleation assay employing

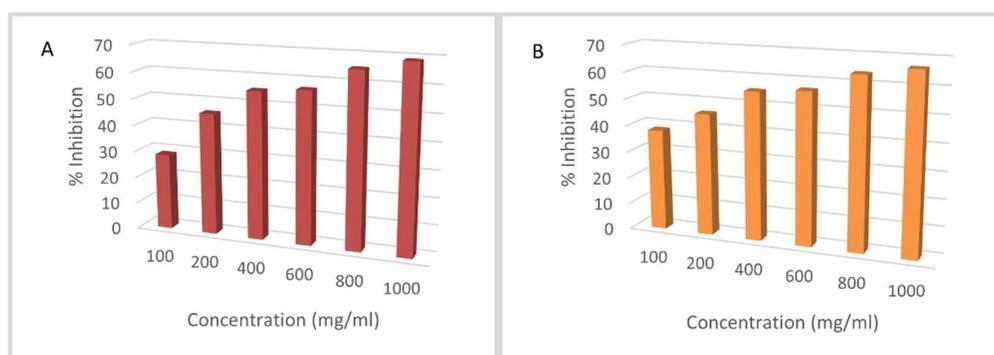
Cystone as the standard and MRE, MLE, ERE, and ELE as tests. The percentage of inhibition was computed in relation to the standard inhibition. The % inhibition of extracts of *C. pallida* has been summarized in **Table 3**. The maximum % inhibition observed was 70.11% at 1000mg/mL of methanolic root extract concentration, followed by ethanolic leaf extract had

68.91% inhibition at 1000mg/mL concentration. The least %inhibition observed was 19.05% in ethanolic root extract at 100mg/mL concentration. The % inhibition increased as the concentration of *C. pallida* extracts increased. It was

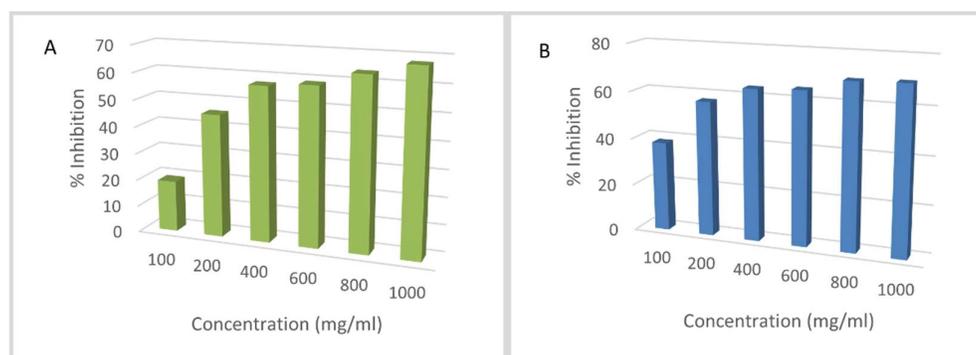
observed that in in-vitro nucleation assay, *C. pallida* exhibited antiurolithiatic activity. Preferably, at a concentration of 1000mg/mL, it was found to be more effective than any other concentrations.

**Table 3: In-vitro Percentage Inhibition of Methanolic and Ethanolic Roots and Leaf Extracts of *C. pallida***

Concentration mg/mL	% Inhibition of MRE	% Inhibition of MLE	% Inhibition of ERE	% Inhibition of ELE
100	38.10	38.10	19.05	28.57
200	57.14	45.71	45.71	45.71
400	63.83	55.32	57.45	55.32
600	64.71	56.86	58.82	56.86
800	69.57	63.77	63.77	65.22
1000	70.11	66.67	67.82	68.97



**Figure 1: The in-vitro Percentage Inhibition of A: ELE and B: MLE**



**Figure 2: The in-vitro Percentage Inhibition of A: ERE and B: MRE**

The urine pH was greatly reduced by the test groups but was not equivalent to standard control group ( $8.26 \pm 0.67$  pH vs Negative control  $10.38 \pm 0.27$  pH vs Vehicle control  $7.27 \pm 0.32$ ). Ethanolic and methanolic root extract of *C. pallida* exhibited  $8.32 \pm 0.61$  and  $8.46 \pm 0.31$  pH respectively. Similarly,

ethanolic and methanolic leaves extract of *C. pallida*  $9.44 \pm 1.58$  and  $9.06 \pm 0.71$  pH respectively. There was a statistical significance between the methanolic and ethanolic root extracts vs negative control group.

Table 4: Effect of Methanolic and Ethanolic Root and Leaf Extracts of *C. pallida* on Urinary Urea, Uric Acid and Creatinine in Gentamycin Induced Urolithiasis Model – Prophylactic Model

Groups	Urea mg/dL	Uric Acid mg/dL	Creatinine mg/dL
Vehicle Control	12.62 ± 2.65	0.43 ± 0.75	1.47 ± 1.02
Negative Control	27.17 ± 1.13 <sup>s</sup>	3.05 ± 0.45 <sup>s</sup>	4.98 ± 7.52 <sup>s</sup>
Cystone	12.63 ± 1.12 <sup>***</sup>	0.65 ± 0.21 <sup>***</sup>	1.91 ± 7.11 <sup>***</sup>
ELE of <i>C. pallida</i> (400mg/kg)	25.85 ± 2.33	2.29 ± 0.48*	2.89 ± 0.04**
MLE of <i>C. pallida</i> (400mg/kg)	16.73 ± 3.23 <sup>**</sup>	1.06 ± 0.44 <sup>**</sup>	2.03 ± 0.14 <sup>***</sup>
ERE of <i>C. pallida</i> (200mg/kg)	21.32 ± 3.00*	0.85 ± 0.37 <sup>***</sup>	2.24 ± 0.13 <sup>***</sup>
MRE of <i>C. pallida</i> (200mg/kg)	18.92 ± 0.99 <sup>**</sup>	1.33 ± 0.17 <sup>**</sup>	1.99 ± 0.10 <sup>***</sup>
Sp<0.001 vs vehicle control; *p < 0.05 vs negative control group; **p<0.001 vs negative control; ***p<0.001 vs negative control group			

Ethanolic Leaf Extract and Methanolic Leaf Extract of *C. pallida* – Urine urea for ethanolic and methanolic leaf extract of *C. pallida* was 25.78±2.33 and 16.73±3.23 respectively. Urine uric acid for ethanolic and methanolic leaf extract was 2.29±0.48 and 1.06±0.44 respectively. Urine creatinine was in the range of 2.89±0.04 and 2.03±1.14 for ethanolic and methanolic leaf extract respectively. Ethanolic and Methanolic Root Extract of *C. pallida* – Urine urea range for

ethanolic and methanolic root extract of *C. pallida* was in the range of 21.32±3.00 and 18.92±0.99 respectively. Urine uric acid was 0.85±0.37 and 1.33±0.17 for ethanolic and methanolic root extract respectively. Urine creatinine ranged from 2.24±0.13 and 1.99±0.10 respectively (Table 4). Similarly, urine oxalate, phosphorus, calcium and magnesium levels are summarised in Table 5.

Table 5: Effect of Methanolic and Ethanolic Root and Leaf Extracts of *C. pallida* on Urinary Oxalate, Phosphorus, Calcium and Magnesium in Gentamycin Induced Urolithiasis Model – Prophylactic Model

Groups	Oxalate mg/dL	Phosphorus mg/dL	Calcium mg/dL	Magnesium mg/dL
Vehicle Control	1.11 ± 2.95	2.41 ± 1.20	47.65 ± 7.41	3.65 ± 1.18
Negative Control	6.89 ± 0.79 <sup>s</sup>	3.95 ± 0.32 <sup>s</sup>	62.03 ± 8.14 <sup>s</sup>	1.079 ± 0.58 <sup>s</sup>
Cystone	1.56 ± 3.15 <sup>***</sup>	2.05 ± 0.41 <sup>***</sup>	47.28 ± 5.18 <sup>***</sup>	3.21 ± 0.69 <sup>***</sup>
ELE of <i>C. pallida</i> (400mg/kg)	1.76 ± 0.06 <sup>***</sup>	2.47 ± 0.40 <sup>***</sup>	57.50 ± 0.36	1.11 ± 0.33
MLE of <i>C. pallida</i> (400mg/kg)	2.48 ± 0.04 <sup>**</sup>	2.27 ± 0.44 <sup>***</sup>	53.36 ± 0.38*	1.50 ± 0.28
ERE of <i>C. pallida</i> (200mg/kg)	2.22 ± 0.04 <sup>**</sup>	2.71 ± 0.31 <sup>**</sup>	52.06 ± 0.49*	1.82 ± 0.36*
MRE of <i>C. pallida</i> (200mg/kg)	2.96 ± 0.06 <sup>**</sup>	3.31 ± 0.30	53.89 ± 0.45*	2.18 ± 0.20*
Sp<0.001 vs vehicle control; *p < 0.05 vs negative control group; **p<0.001 vs negative control; ***p<0.001 vs negative control group				

## DISCUSSION

Biological resources are essential to the process of finding and creating new therapeutic compounds. The secondary metabolites obtained from plant sources have several pharmacological effects and may have therapeutic relevance [12]. The extensive research and investigation of the literature revealed the Indian *Acalypha*'s potent antiurolithiatic properties [13-15]. The purpose of this study was to identify the phytochemicals found in the plant and assess the antiurolithiatic efficacy of ethanolic and methanolic extracts of *C. pallida* leaves and roots in vitro.

The results of preliminary phytochemical screening of plant revealed the presence of alkaloids, saponins, flavonoids in MRE; only resins MLE; gums, saponins, tannins, flavonoids, glycosides, steroids in ERE; and alkaloids, phenol, saponin, tannin, flavonoids, monoterpenes, steroids in ELE. This was seen in studies conducted by Alalade JA *et al* [15] and Hassan Mohammad M *et al*. [16]. The acute oral toxicity of the MRE, MLE, ERE and ELE did not result in any significant toxicities and hence was found to be safe up to 2000mg/kg body weight of rats.

By employing in vitro techniques—which were ascertained by nucleation assay—different phases of the development of calcium oxalate crystals were investigated in conjunction with the plant preparation. The

development of the stone first occurs with the nuclei coalesce. The inhibitory action on different stages of CaOx crystallisation was identified by the duration course of the turbidity observed in counterfeit urine at varied doses. The most important stage in the production of stones is called nucleation, which starts when the stone salts in the solution combine to create loose clusters that can become larger by incorporating other elements [17]. CaOx crystals are generated by immersing artificial urine containing sodium oxalate and calcium chloride [18]. The turbidity of the reaction solutions was measured and compared to the control to assess the impact of plant extracts on CaOx crystallisation. It was discovered that *C. pallida* extracts showed inhibitory action during the nucleation process, hence impeding the development of new stones. These data suggest that the plant acts more as a preventative measure than a cure, which can greatly lower the likelihood of recurrence of stone formation. Further in-vivo research is being performed to acquire a better understanding of the plant's putative mode of action and the dose at which it demonstrates antiurolithiatic efficacy.

All the urine parameters were greatly reduced in test groups which symbolises that the *crotalaria pallida* has antiurolithiatic activity. Magnesium is vital in preventing urinary stones by inhibiting the crystallization of minerals like calcium

oxalate, competing with calcium for binding sites, and maintaining acid-base balance. It promotes the dissolution of existing stones, hydrates crystals, and contributes to overall kidney health, reducing the risk of stone formation and supporting urinary tract well-being [19, 20]. However, the *Crotalaria pallida* could be able to improve the magnesium levels as depicted in **Table 5** compared to negative control group.

## CONCLUSION

*Crotalaria pallida* Aiton was discovered to possess therapeutic value in the management of urolithiasis. There were no adverse events detected in the acute toxicity investigations even after 14 days of monitoring. The antiurolithiatic activity of *C. pallida* was demonstrated by the considerable nucleation inhibitory properties observed in the methanolic and ethanolic roots and leaf extracts during the in-vitro nucleation experiment. *Crotalaria pallida*'s potential antiurolithiatic mechanisms may involve inhibiting crystallization through compounds that interfere with mineral nucleation, chelating minerals like calcium to prevent aggregation, and exerting anti-inflammatory effects, reducing inflammation in the urinary tract. Its diuretic properties may increase urine flow, flushing out stone-forming substances, while antioxidant activity could neutralize free radicals, lowering oxidative stress

associated with stone formation. Additionally, *Crotalaria pallida* may offer renal tubular protection, preserving cell integrity and promoting repair.

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## CONFLICT OF INTEREST

None

## AUTHORS' CONTRIBUTIONS

**Mr. Anil Kumar:** Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Writing Original draft along with editing. **Dr. Ashish Kumar Sharma:** Supervision, Validation, Resources and Project Administration.

## REFERENCES

- [1] Tzelves L, Türk C, Skolarikos A, European association of urology urolithiasis guidelines: where are we going? *European Urology Focus*, 7(1), 2021, 34-38.
- [2] Kumari R, Kumar S, Pharmacological, phytochemical and their application of *Crotalaria* L. Genus. *Genus* (April 15, 2022). 2022 Apr 15.
- [3] WHO Global Centre for Traditional Medicine.

- <https://www.who.int/initiatives/who-global-centre-for-traditional-medicine>, Accessed 5 December, 2023.
- [4] Asfaw MM, Abebe FB, Traditional medicinal plant species belonging to Fabaceae family in Ethiopia: A systematic review. *International Journal of Plant Biology*, 12(1), 2021, 8473.
- [5] Rather SA, Subramaniam S, Danda S, *et al*, Discovery of two new species of crotalaria (Leguminosae, Crotalariaeae) from Western Ghats, India, *PloS one*, 13(2), 2018, e0192226.
- [6] Wal P, Shukla V, Uzzaman Khan MM, *et al*, Medicinal Properties of *Crotalaria burhia*: A Review, *Current Traditional Medicine*, 10(3), 2024, 90-100..
- [7] Anwar S, Faisal Nadeem M, Pervaiz I, *et al*, A comprehensive phytochemical, biological, and toxicological studies of roots and aerial parts of *Crotalaria burhia* Buch.-Ham: An important medicinal plant. *Frontiers in plant science*, 13, 2022, 988352.
- [8] Pandharmise P, Tambe AN, Kamble S, *et al*, Preliminary Phytochemical Screening And Hptlc Analysis Of Leaf Extract Of *Crotalaria Juncea* From Vidarbha Region, Ms, India, *Journal of the Maharaja Sayajirao University of Baroda*, 25, 0422.
- [9] Sofowora A, Screening plants for bioactive agents. *Medicinal Plants and Traditional Medicinal in Africa*, 2nd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria. 1993, 134-156.
- [10] Ridgway P, Revised fixed dose procedure: OECD Test Guideline 420, *RAPPORTI ISTISAN*, 41, 2002, 27-31.
- [11] Bawari S, Sah AN, Tewari D, Antirolithiatic activity of *Daucus carota*: an in vitro study, *Pharmacognosy journal*, 10(5), 2018.
- [12] Bi LA, Toto NK, Zahoui OS, *et al*, Acute and subacute toxicity assessment of an aqueous extract of *Crotalaria retusa* (Fabaceae) in Swiss mice and Wistar rats, *Journal of Drug Delivery and Therapeutics*, 11(6), 2021, 94-100.
- [13] Bolleddu R, Narasimhaji CV, Venkatesh S, *et al*, Establishment of quality parameters for fruits of *Crotalaria pallida* Aiton. through microscopy and phytochemical studies. *Vegetos*, 35(3), 2022, 622-632.
- [14] Nisa U, Astana PR, Triyono A, *et al*, Ethnobotanical study of medicinal plants used for treating

- urinary tract problems in eastern Indonesia. In IOP Conference Series: Earth and Environmental Science, 905 (1), 2021, 012119.
- [15] Ashitha Ganesh B, AG DP, Traditional plant-based remedies in the Western Ghats region of Kodagu district: A recent survey of ethnomedicinal knowledge, communities, 2013, 7.
- [16] Alalade JA, Proximate composition and phytochemical screenings of *Crotalaria retusa* leaves and seeds, Open Access Library Journal, 6(1), 2019, 1.
- [17] Hassan Mohammad M, Kanagasabai V, Nandini MS, *et al*, The GC-MS Analysis Of Ethyl Acetate Extract Of One Herbal Plant, 'Crotalaria Pallida. NVEO-NATURAL VOLATILES & ESSENTIAL OILS Journal NVEO, 2021, 6791-6801.
- [18] Zaki S, Jahan N, Kalim M, *et al*, In vitro antilithiatic activity of the hydro-alcoholic extract of *Cinnamomum zeylanicum* Blume bark on calcium oxalate crystallization, Journal of integrative medicine, 17(4), 2019, 273-281.
- [19] Wang P, Zhang H, Zhou J, *et al*, Study of risk factor of urinary calculi according to the association between stone composition with urine component, Scientific reports, 11(1), 8723.
- [20] Shringi S, Jie Tang MD, Dietary Magnesium Intake and Kidney Stone: The National Health and Nutrition Examination Survey 2011–2018, Rhode Island Medical Journal, 106(11), 2023, 20-25.