

**EVALUATION OF DIURETIC AND LAXATIVE ACTIVITY OF METHANOLIC EXTRACT OF *CASSIA ALATA LINN.* (CAESALPINIACEAE) LEAVES IN ALBINO WISTAR RATS****\*Dr. P.V. Madhava Reddy,<sup>1</sup>Vamsi Krishna Murthy Ginjupalli,<sup>2</sup>C. Bhuvaneshwar Rao,<sup>3</sup>K. Yalla Reddy and<sup>4</sup>M. Santhosh Aruna**

\*Professor, Jagan's College of Pharmacy, Nellore, Andhra Pradesh, INDIA.

<sup>1</sup>Research Scientist, Department of Medicine, State University of New York, Brooklyn, U.S.A.<sup>2</sup>Assistant Professor, Jagan's College of Pharmacy, Nellore, Andhra Pradesh, INDIA.<sup>3,4</sup>Associate Professor, Jagan's College of Pharmacy, Nellore, Andhra Pradesh, INDIA.**\*Corresponding Author: Dr. P. V. Madhava Reddy**

Professor, Jagan's College of Pharmacy, Nellore, Andhra Pradesh, INDIA.

Article Received on 13/09/2021

Article Revised on 03/10/2021

Article Accepted on 23/10/2021

**ABSTRACT**

*Cassia alata Linn.* (Caesalpinaceae) is a shrub commonly known as "candle bush or ringworm shrub. *C. alata* is extensively used for treating gastrointestinal conditions, skin diseases, allergic reactions and inflammatory conditions. The present study was evaluated for diuretic and laxative activity of methanolic extract on albino wistar rats. The acute toxicity study of the extract had shown no sign of toxicity up to a dose level of 2000mg/kg body weight. The methanol extract of *Cassia alata Linn.* (200mg/kg) showed lesser diuretic activity compared to MECA 400mg/kg during the 5h of the test duration (Diuretic action (4.53 and 5.75) when compared to control the MECA 200mg/kg MECA 400mg/kg both doses showed more significant diuretic activity. The laxative activity of *Cassia alata* was after oral administration of extract showed the significant and dose-dependent increase in fecal output of rats in regards to the accumulation of water in the intestine. *Cassia alata Linn.* (200mg/kg) showed lesser laxative activity compared to MECA 100mg/kg during the 8h of the test duration (Laxative action (2.71 and 3.23) when compared to control the MECA 100mg/kg MECA 200mg/kg both doses showed more significant laxative activity.

**KEYWORDS:** *Cassia alata Linn.*, Diuretic, Laxative, ringworm shrub, Significant etc.**INTRODUCTION**

Diuretics are the drugs that increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids in variety of clinical situations. Diuresis is beneficial in many life-threatening disease conditions such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, hypertension and pregnancy toxemia<sup>[1]</sup> only few drugs produce diuresis by increasing the filtration rate at the glomeruli, and there are weakly in action most of the diuretics used therapeutically act by interfering with sodium reabsorption by the tubules. Most diuretics drugs have the adverse effects including fatigue, impotence, and weakness, natural diuretics like caffeine in coffee, tea and cola, which inhibit Na<sup>+</sup> reabsorption. Alcohol, beer, wine and mixed drinks inhibit the secretion of ADH.<sup>[2]</sup>

Diuretics are used to treat heart failure, liver cirrhosis, hypertension and certain kidney diseases. Some diuretics, such as acetazolamide, help to make the urine more alkaline and are helpful in increasing excretion of substances such as aspirin in cases of overdose or poisoning. Diuretics are often abused by sufferers of

eating disorders, especially bulimics, in attempts at weight loss.<sup>[3]</sup>

Laxatives, purgatives, or aperients are substances that loose stools and increase bowel movements. They are used to treat and prevent constipation. Laxatives vary as to how they work and the side effects they may have. Certain stimulant, lubricant and saline laxatives are used to evacuate the colon or rectal and bowel examinations, and may be supplemented by enemas under certain circumstances. Sufficiently high doses of laxatives may cause diarrhea. Some laxatives combine more than one active ingredient.<sup>[4]</sup>

*Cassia alata* is a plant originating from Argentina.<sup>[5]</sup> Commonly referred to as Candle brush, Candlestick, Senna alata and others.<sup>[6]</sup> *C. alata* root can be used to treat rheumatism and laxative<sup>[7]</sup> Seeds and leaves have high potency as fungicides<sup>[8]</sup> and medicine for eczema in India.<sup>[9]</sup> *C. alata* can be used to reduce stomach pain during pregnancy, headaches and paralysis. *C. alata* ex tracts are used in the practice of traditional herbs medicine to cure skin diseases<sup>[10]</sup> in some countries *C.*

alata leaves are used to treat constipation. anti-inflammatory agent.<sup>[11]</sup> Several studies have been reported the biological activity of *C. alata*. Crude extract of *C. alata* leaf has very strong antioxidant activity with IC50 value of 2.27 µg/mL.<sup>[12]</sup> Secondary metabolite compounds in *C. alata* include alkaloids, saponins, steroids, flavonoids and terpenoids.<sup>[13]</sup>

## MATERIALS AND METHODS

### Animals used

Albino wistar rats (150-200g) were obtained from the animal house in Sree Vidyanikethan College of Pharmacy, Tirupati, A.P. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet fed (Hindustan Level Limited, Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAE (Institutional Animal Ethics Committee) of CPCSEA (Ref. No./AEC/XIII/05/SVCP/2008-09).

### Treatments

Animals were divided into four groups, each consisting of six albino wistar rats the methanol extract of *Cassia alata* Linn. (Caesalpinaceae) was blackish oily extract divided into two doses MECA-200mg/kg, MECA 400mg/kg given orally.

Group-I received normal saline (25ml/kg) and served as normal control,

Group-II received Furosemide (20mg/kg) and served as standard.

Group-III & Group-IV are received 200mg/kg, 400mg/kg of MECA respectively.

### Acute Toxicity Study

The acute toxicity PECB was determined as per the OECD guideline no. 423 (Acute toxic class method). It was observed that the test extract was not mortal even at 2000mg/kg dose. So, 1/10<sup>th</sup> (200mg/kg) and 1/5<sup>th</sup> (400mg/kg) of the dose were selected for further study.<sup>[18]</sup>

### Diuretic activity

The method by Lipschitz *et al.*, was employed for the assessment of diuretic activity.<sup>[14,15]</sup> Healthy albino wistar rats of either sex were divided into six groups of six animals each. Methanol extract of *Cassia alata* Linn. were evaluated for diuretic activity. Furosemide (20mg/kg) was used as standard reference drug. Before the equipment the rats were fasted for 18 hours with free access to water. On the day of experiment, the animals of group-I was administered saline orally (2.5 ml of 0.9% NaCl/100g body weight) and this group served as control. Group-II rats were treated with standard drug Furosemide (20mg/kg) formulated in saline solution. Group-III and Group-IV rats received MECA 200mg/kg and MECA 400mg/kg respectively. Immediately after the treatment, the animals were individually placed in metabolic cage.<sup>[16]</sup>

The urine was collected in measuring cylinder up to 5h for all control and treated groups. During this period no food and water was made available to the animals. The volumes of urine, electrolyte (Na<sup>+</sup>, K<sup>+</sup>) content were estimated in the urine for assessment of diuretic activity. Na<sup>+</sup>, K<sup>+</sup> estimation was carried out using flame photometry (ELICO CL361 flame photometer).<sup>[17]</sup> The diuretic action of tested drug was calculated by using the following formula.

$$\text{Diuretic action} = \frac{\text{urinary excretion in test drug}}{\text{urinary excretion in control}}$$

### Statistical analysis

The statistical significance of the results of diuretic activity are analysed using ANOVA, followed by Tukey-kramer multiple comparison test, the p-values <0.05 were considered as significance.

### Evaluation of laxative activity

#### Treatments

Animals were divided into four groups, each consisting of six albino wistar rats the methanol extract of *cassia alata* was blackish oily extract divided into two doses meoc-200mg/kg, meoc 400mg/kg given orally.

Group-i received normal saline (25ml/kg) and served as normal control,

Group-ii received furosemide (20mg/kg) and served as standard.

Group-iii & group-iv are received 200mg/kg, 400mg/kg of meoc respectively.

### Evaluation of laxative activity

Laxative activity was evaluated according to the method of meite *et al.*<sup>[18]</sup> with slight modification. Animals fasted for 12 h before the experiment. The animals were placed individually in cages lined with filter paper. Rats were divided into five groups, the first group (negative control) received saline (5 ml/kg, p. O.). The second group (positive control) received sodium picosulfate (5 mg/kg, p.o). The third and fourth groups received 100 and 250 mg/kg p.o. Of the *cassia alata* aqueous extract. Immediately after dosing, the animals were kept in individual cages lined with clean filter paper, to collect feces. The fecal production (total number of normal as well as wet) in all groups was monitored for 16 h.<sup>[19]</sup>

### Laxative activity on loperamide-induced constipation

This study was carried out according to the mikhail o nafi. *Et al.*<sup>[20]</sup> animals were divided into five groups of four animals each, they were individually placed in cages lined with clean filter paper, allowed to fast for 18 hours and. The first two groups were treated with the aqueous extract of *cassia alata* (100 and 250 mg/kg, p.o.). Group third of received normal saline (5 ml/kg, p.o) and served as a control. Group fourth received the standard drug sodium picosulfate (5 mg/kg). After 1 h, all group received loperamide (5 mg/kg, p.o.) By gavage. The feces production (total number) in all groups was monitored for 8 h.

**Statistical analysis (p<0.05)**

The results were expressed as the mean  $\pm$  standard error of the mean (sem), and data was statistically analyzed by one-way analysis of variance followed by dunnett's multi- ple comparison tests. All the results obtained in this study were compared with the vehicle control group.

**RESULTS****Effect on urine volume**

In this method the diuretic activity assessed. ANOVA followed by Tukey-krammer multiple comparison tests, the p-values <0.05 were considered as significant activity. The methanol extract of *Cassia alata* Linn. at two doses (200, 400mg/kg body weight) show marked diuresis during the 5h of test duration. The MECA 200 mg/kg having less diuresis (9.6  $\pm$ 0.43mg) compared to MECA 400mg/kg (12 $\pm$ 2.01mg) versus control 2.12 $\pm$ 0.09 ml, P<0.001). Where as both methanol extracts of 200 &

400 mg/kg doses significantly increased urinary output compared to that of the control (MECA 200 & 400 mg/kg, 9.6 $\pm$ 0.43mg & 12.2 $\pm$ 0.26 ml versus control 2.12 $\pm$ 0.08 ml, P>0.001 but the effect less than that of standard drug Furosemide (21.65 $\pm$ 0.15ml) versus control 2.12 $\pm$ 0.08 ml; P<0.001, at 5hrs the animal were found normal and no evidence of dehydration was observed.

**Effect on urinary electrolyte excretion**

The effect of single doses of Furosemide (20mg/kg) and two doses of MECA 200mg/kg & MECA 400mg/kg on urinary electrolyte Na<sup>+</sup> and K<sup>+</sup> concentration at 5h post administration in represented in table 2 MECA 200mg/kg, MECA 400mg/kg both extracts significantly enhanced the excretion of electrolytes (P<0.001) compared to the control. The Na<sup>+</sup>/K<sup>+</sup> excretion ratio was uniform (2.0 to 2.5) in all the groups studied.

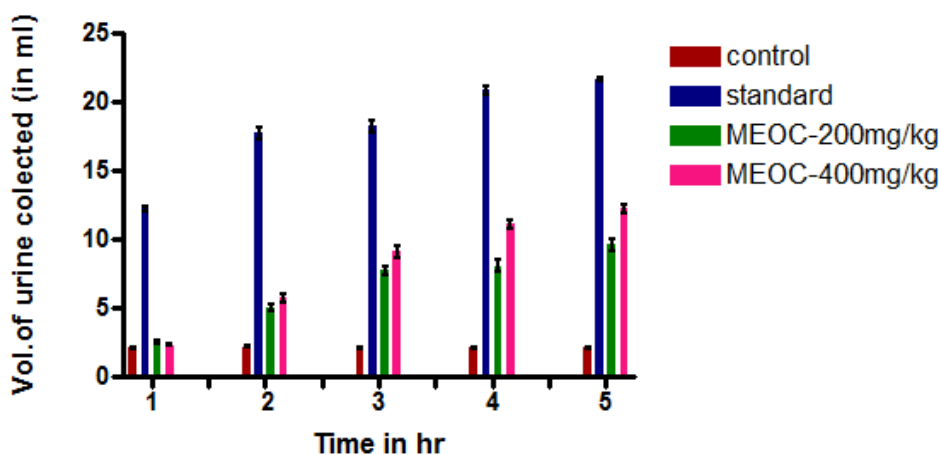
**Table 1: Effect of *Cassia alata* Linn. on excretion of urine.**

S. No.	Group	Dose (mg/kg)	Vol. of urine collected (in ml)					Diuretic action
			After 1hr	After 2hr	After 3hr	After 4hr	After 5hr	
1.	Control	---	2.12 $\pm$ 0.08	2.17 $\pm$ 0.08	2.09 $\pm$ 0.07	2.1 $\pm$ 0.09	2.12 $\pm$ 0.08	1.00
2.	Standard	20	12.25 $\pm$ 0.19***	17.7 $\pm$ 0.39***	18.2 $\pm$ 0.41***	20.83 $\pm$ 0.31***	21.65 $\pm$ 0.15***	10.21
3.	MECA	200	2.52 $\pm$ 0.13***	5 $\pm$ 0.21***	7.7 $\pm$ 0.34***	8.06 $\pm$ 0.43***	9.6 $\pm$ 0.43***	4.53
4.	MECA	400	2.3 $\pm$ 0.06***	5.7 $\pm$ 0.35***	9.1 $\pm$ 0.42***	11.1 $\pm$ 0.29***	12.2 $\pm$ 0.26***	5.75

Values are expressed as Mean  $\pm$  SEM, ANOVA followed by Tukey-Krammer multiple comparison test, n=6 in each group, \*\*\*P<0.0001 vs control group.

MECA: Methanol extract of *Cassia alata* Linn.

Standard : Frusemide

**Fig 1: Effect of *Cassia alata* Linn. on excretion of urine.****Table 2: Urinary electrolyte concentration of *Cassia alata* Linn. (Caesalpiniaceae)**

S. No.	Group	Dose (mg/kg)	Vol. of urine (ml)	Na <sup>+</sup> (mmol/L)	K <sup>+</sup> (mmol/L)	Na <sup>+</sup> /K <sup>+</sup> ratio
1.	Control	---	2.1 $\pm$ 0.08	138.9 $\pm$ 0.50	54.73 $\pm$ 0.60	2.54
2.	Standard	20	21.65 $\pm$ 0.15	150.1 $\pm$ 0.55***	71.06 $\pm$ 0.75***	2.11
3.	MECA	200	9.6 $\pm$ 0.43	148.3 $\pm$ 1.46***	72.01 $\pm$ 0.34***	2.05
4.	MECA	400	12.2 $\pm$ 0.26	150.16 $\pm$ 1.47***	72.21 $\pm$ 0.37***	2.07

Values are expressed as Mean  $\pm$  SEM, ANOVA followed by Tukey-Krammer multiple comparison test, n=6 in each group, \*\*\*P<0.0001 vs control group.

MECA: Methanol extract of *Cassia alata* Linn.

Standard : Frusemide

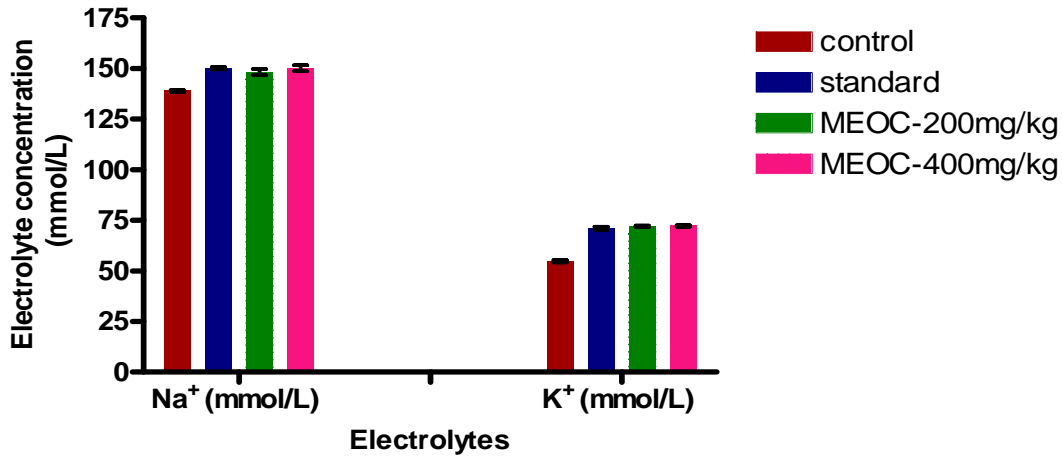


Fig 02: Urinary electrolyte concentration of *Cassia alata* Linn.

**Laxative activity**

The extract showed dose dependant increase in fecal output of rats when compared to the control group (Table 4). The effects of *Cassia alata* increased significantly fe- cal output at doses of 100 and 250 mg/kg (p.o.) of rats compared to control group (p < 0.05 and p<0.01 respectively). Extract effect at the higher dose of 250 mg/kg (p.o.) was similar to that of the standard drug sodium picosulfate (5 mg/kg, p.o.).

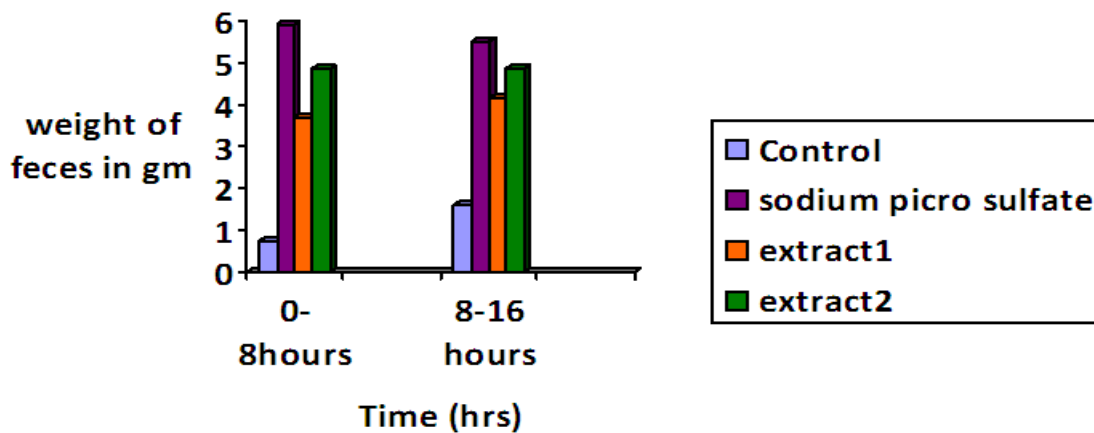
**Effect of the aqueous extract of *Argemone mexicana* on loperamide-induced constipation in rats**

In loperamide-induced constipation, the aqueous extract of *Cassia alata* increased the total number of feces in a dose-dependent manner, and the results were statistically significant (p < 0.05) (Table 5). The reduction of lop- eramide-induced constipation at 250 mg/kg (p.o.) of plant extract treatment was found to be almost comparable with that of treatment by 5 mg/kg of sodium picosulfate.

Table 3: Laxative activity of aqueous extract of *Cassia alata* in rats.

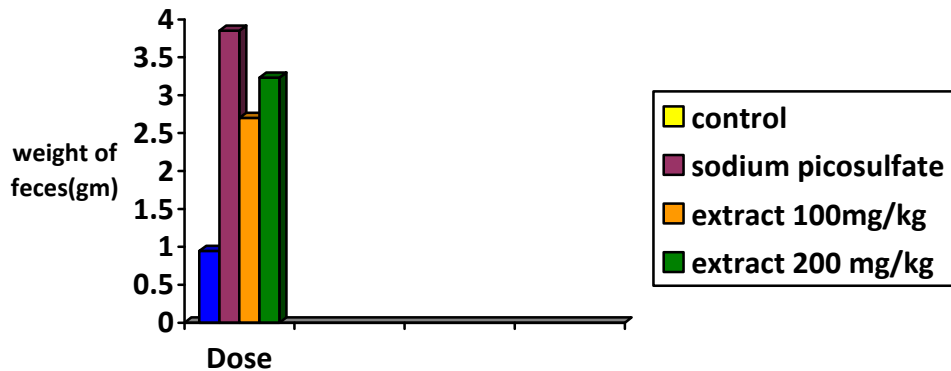
Treatment	Dose	Feces out Put(g)	
		0-8 hours	8-16 hours
Control	5 ml/kg	0.751 +0.48	1.612+ 0.62
Sodium Picosulfate	5mg/kg	5.912 + 0.14**	5.511 + 0.71 **
Extract	100 mg/kg	3.711 + 0.74*	4.131 + 0.31 *
Extract	250 mg/kg	4.871 + 0.97**	4.86 + 0.68 **

Figure 3: Laxative activity of aqueous extract of *cassia alata* in rats.



**Table 4: Effect of *Cassia alata* aqueous extract on loperamide induced constipation in rats.**

Treatment	Dose	Weight of Feces(g)
Control	5 ml/kg	0.947 + 0.45
Sodium Picosulfate	5mg/kg	3.851 + 0.63**
Extract	100 mg/kg	2.701 + 0.33*
Extract	250 mg/kg	3.231 + 0.47**

**Figure 4: Effect of *cassia alata* aqueous extract on loperamide induced constipation in rats.**

## DISCUSSION

The aim of this study was to investigate the diuretic activity of methanol extract of *Cassia alata* Linn. According to previous ethnopharmacological survey carried out in the south Indian region. The plant materials are used in various diseases like dyspepsia, cancer, piles, convulsions, and dementia and traditionally used as diuretic, but no previous pharmacological clinical study has been carried out to test the diuretic activity of this plant.<sup>[21]</sup> In this study the methanol extracts were tested at 200mg/kg, 400mg/kg respective doses. The diuretic response was compared with that produced by furosemide, a widely used loop diuretic in clinical practice. The effect on electrolyte balance was also determined along with diuretic response.

The methanol extract of *Cassia alata* Linn. (200mg/kg) showed lesser diuretic activity compared to MECA 400mg/kg during the 5h of the test duration (Diuretic action (4.53 and 5.75) when compared to control the MECA 200mg/kg MECA 400mg/kg both doses showed more significant diuretic activity but less than that of standard furosemide (Diuretic action 10.21). Urine output continued to be enhanced throughout the study period and the cumulative urinary excretion was significantly higher compared to that of the control. Furosemide is reported to increase urinary output and urinary excretion of sodium by inhibiting Na<sup>+</sup>/K<sup>+</sup>/Cl<sup>-</sup> transports system in this thick ascending of henley. The MECA 200mg and MECA 400mg/kg both doses significantly increase the urinary excretion of Na<sup>+</sup> and K<sup>+</sup> ions was observed when compared to control.

The laxative activity of *Cassia alata* was studied in rats. Oral administration of extract showed the significant and dose-dependent increase in fecal output of rats in regards to the accumulation of water in the intestine. Our results showed that *Cassia alata* extract and sodium picosulfate (standard) exert respectively opposite effects with loperamide on the gastrointestinal function. It is well documented that loperamide abolishes experimental osmotic diarrhea by acting on intestinal motility, and consequently reducing the flow entering the colon.<sup>[22]</sup>

Sodium picosulfate related to the polyphenolic category of stimulant laxatives. After oral administration, it is converted in the intestine to an active form through the action of bacterial enzymes. Sodium picosulfate increases peristaltic movements and reduces water reabsorption, increases secretion which leads to softening stool. These results suggest that the active principle of extract acts through the same way. *Cassia alata* Linn. (200mg/kg) showed lesser laxative activity compared to MECA 100mg/kg during the 8h of the test duration (Laxative action (2.71 and 3.23) when compared to control the MECA 100mg/kg MECA 200mg/kg both doses showed more significant laxative activity.

## CONCLUSION

Finally, the results of the present study confirm that *Cassia alata* Linn. has diuretic and laxative activity. Therefore, the native practitioners using this plant material are used in various diseases like dyspepsia, cancer, piles, convulsions, and dementia and traditionally used as diuretic. There is a need for further studies in

order to isolate the active ingredients in the plant that is responsible for its biological activities and to elucidate the mechanism of action of these active ingredients.

## REFERENCES

1. Golla U, Gajam PK, Bhimathati SS. Evaluation of diuretic and laxative activity of hydro-alcoholic extract of *Desmostachya bipinnata* (L.) Stapf in rats. *Journal of integrative medicine*, 2014; 12(4): 372-8. 25.
2. Biswas S, Murugesan T, Maiti K, Ghosh L, Pal M, Saha B. Study on the diuretic activity of *Strychnos potatorum* Linn. Seed extract in albino rats. *Phytomedicine*, 2001; 8(6): 469-71.
3. Mohua Sarker, Towkir Ahmed, Md. Nazmul Islam, Bithi Biswas, Shrabanti Dev, Bishwajit Bokshi, Asish Kumar Das and Nripendra Nath Biswas, Diuretic and laxative activities of *Kandelia candel* and *Brownlowia tersa* in experimental mice, *Journal of Medicinal Plants Studies*, 2021; 9(2): 59-65.
4. Patel BR, Ashok B, Ravishankar B. Study on the diuretic activity of *Veerataru Kwatha* in albino rats. *Ayu*, 2011; 32(3): 395.
5. E.F. Gilman, D.G. Watson, *Cassia Alata* Candlebrush, Southern Group of State Foresters, 1993; 1-3.
6. T.K. Lim, "Senna alata" edible medicinal and non-medicinal plants, 2014; 7: 841-859.
7. Reezal, M.N. Somchit, M.A. Rahim, In vitro antifungal properties of *Cassia alata* (Gelenggang Besar), in: *The Regional Symposium on Environment and Natural Resources*, 2002; 654-659.
8. N. Shiddamallayya, A. Yasmeen, K. Gopakumar, Medicobotanical survey of *Kumar pavatha* Kukke Subramanya, Manglore, Karnataka, *Indian J. Tradit. Knowl*, 2010; 9: 96-99.
9. P. Monkheang, R. Sudmoon, T. Tanee, et al., Species diversity, usages, molecular markers and barcode of medicinal *Senna* species (Fabaceae, Caesalpinioideae) in Thailand, *J. Med. Plants Res*, 2011; 5: 6073-6181.
10. S. Saito, G. Silva, R.X. Santos, G. Gosmann, C. Pungartnik, M. Bredel, Astragalins from *Cassia alata* induces DNA adducts in vitro and repairable DNA Damage in the yeast *Saccharomyces cerevisiae*, *Int. J. Mol. Sci.*, 2012; 13: 2846-2862.
11. A.C. Akinmoladun, M. O. Efere, E.O. Farombi, Evaluation of antioxidant and free radical scavenging capacities of some nigerian indigenous medicinal plants, *J. Med. Food*, 2010; 13: 444-451.
12. M.T. Alam, M.M. Karim, A.N. K A. Lewis, A. Levy, "Antiinflammatory activities of *Cassia alata* leaf extract in complete Freund's adjuvant arthritis in rats, *W. Indian Med. J.*, 2011; 60: 615-621.
13. W.F. Sule, I.O. Okondo, T.A. Joseph, et al., In vitro antifungal activity of *Senna alata* Linn. Crude Leaf Extract, *Res. J. Biol. Sci.*, 2010; 5(3): 275-284.
14. Mukherjee PK, Pharmacological screening of herbal drugs in quality control of herbal drugs, 1<sup>st</sup> edition, 2002; 537.
15. Lipschitz WL, Haddian Z, Kepsar A. Bioassay of diuretics, *J Pharmacol Exp*, 1942; 79: 110.
16. Kuppast IJ and Nayak PV, Diuretic activity of *Cordida dichotoma forester* fruits, *Ind J. Pharm, Edu*, 2005; 39(4): 186-187.
17. Jeffery GH, Bassets, Mendham J and Denny, *Vogels text book of Quantitative chemical analysis*, 5<sup>th</sup> edition, addition westly longman Ltd, England, 1989; 801.
18. Hiroki Mizokami, Kaori Tomita-Yokotani, Kunijiro Yoshitama Flavonoids in the leaves of *Oxalis corniculata* and sequestration of the flavonoids in the wing scales of the pale grass blue butterfly, *Pseudozizeeria maha*, *J Plant Res*, 2008; 121: 133-136.
19. Bairagi Shripad M, Aher Abhijeet A, Nema Nitin and Pathan Inayat B. Evaluation of anti-diarrhoeal activity of the leaves extract of *Ficus Microcarpa* L. (Moraceae). *Marmara Pharm J*. 2014; 18: 135-138.
20. Muhammad Asif, Qaiser Jabeen, Muhammad Atif, Amin Ma lik Shah, Abdul Majid and Muhammad Qamar Uz Zaman. Diuretic Activity of *Achyranthes aspera* Linn Crude Aqueous Extract in Albino Rats. *Trop. J Pharm Res.*, 2014; 13(12): 2039- 2045.
21. Souleymane Meite, Calixte Bahi, Dodehe Yeo, Jacques Y Datte, Joseph A Djaman and David J Nguessan. Laxative ac tivity of *Mareya micrantha* (Benth.) mull. arg. (Euphorbiaceae) leaf aqueous extract in rats. *BMC Complementary and Alt Med.*, 2010; 10: 7.
22. Upendarrao Golla, Gajam PK and Bhimathati SS. Evaluation of diuretic and laxative activity of hydro-alcoholic extract of *Desmostachya bipinnata* (L.) Stapf in rats. *J Integr Med.*, 2014; 12(4): 372-378.