ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal
www.orientjchem.org
ISSN: 0970-020 X
CODEN: OJCHEG
2026, Vol. 42, No.(2):
Pg. 653-660

Acute Oral Toxicity Evaluation of Hydroalcoholic Extracts of *Saraca indica* and *Bauhinia variegata* with the HPTLC -Fingerprinting and IR characterization of Targeted Phytoconstituents

DIKSHA JOSHI*, HEMANT KHAMBETE and SANJAY JAIN

Faculty of Pharmacy, Medicaps University
*Corresponding author E-mail: Diksha.joshi@medicaps.ac.in

http://dx.doi.org/10.13005/ojc/420226

(Received: January 05, 2026; Accepted: February 17, 2026)

ABSTRACT

The Present study investigates the acute toxicity of hydroalcoholic extract of *Saraca indica*, *Bauhinia variegata*. The hydro-alcoholic extracts of SA and BV were prepared by Soxhlet extraction method. The temperature for Soxhlet extraction was 90°C for both the extracts. The obtained extract of BV is slight sticky in appearance and the extract of SA is powder. The phytochemical Screening were performed for both the extracts. These plants are traditionally used for the various therapeutic purposes like diabetes, gynecological disorders, obesity etc. Extracts were evaluated for its safety profile in accordance with OECD guidelines for acute toxicity study. Single doses of extract of varying concentrations of 150,350,2000mg/kg body were administered to the healthy adult Wistar rats and the signs of toxicity that include, behavioral alteration, body weight and mortality were observed. The findings show that all herbal extracts of SAHE and BVHE do not cause any serious side effects or death at a dose of 2000mg/kg body weight despite the significant toxicity being present. The paper concludes that the extracts can possibly possess a positive safety profile with acute administration.

Keywords: SAHE (Hydroalcoholic extract of *Saracaasoca*), BVHE (Hydroalcoholic extract of *Bauhinia vareigata*), *Saracaasoca, Bauhinia vareigata* PCOS, Polycystic Ovarian Syndrome,herbal medicine, OECD.

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is a highly common endocrine disorder in women of menopausal age. It is an intricate association of hormonal imbalances, metabolic disorders 5- 10% of women and, therefore, plays a major role in female infertility. The disorder usually occurs with abnormal or no menstual cycles, hyperandrogenism (elevated levels of male hormones), polycystic ovarian

morphology as revealed in ultrasonography, and insulin resistance. All these physiological disturbs tend to translate into clinical changes of hirsutism, acne, obesity, alopecia and menstrual abnormalities. Along with reproductive complications, type 2 diabetes mellitus, cardiovascular disorde3rs, and endometrial hyperplasia or cancer are also the long-term health risks related to PCOS. PCOS is regarded as a hyperandrogenic disorder that is often followed by persistent oligo-ovulation or anovulation. Polycystic ovarian structure may be frequently determined with the help of ultrasonography or other imaging procedures, but not every woman with polycystic ovaries can have the clinical and biochemical conditions of PCOS. It has been reported that pregnancy prevalence of polycystic ovarian morphology has a range of between 20 and 33 percent in the general population. PCS women can exhibit a number of typical signs such as menstrual problems, obesity, hirsutism, acne, and changes in the biochemical parameters of leutinizing hormone (LH), testosterone, androstenedione, and insulin. Because of the complex nature and rising prevalence, there is need to create more awareness and research on the topic to enhance therapeutic interventions of managing PCOS.

The Precise etiology is unclear but, it is believed that a mixture of genetic predisposition, environmental factors and lifestyle factors are the cause of the etiology as hyperinsulinemia can stimulate excessive androgen secretion by the ovarian theca cells, which further worsens hormonal imbalance. The Rotterdam criteria are the most commonly used to diagnose PCOS by the presence of two or more of the following: oligo- or anovulation, clinical or biochemical hyperandrogenism, and ovarian polycystic or morbpholy on ultrasound.

The traditional treatment methods involve change in lifestyle, hormonal therapy (e.g., oral contraceptives), insulin-sensitizing drugs such as metformin and ovulation-inducing infertility medications. Nonetheless, more attention towards herbal medicines has been developing given their natural source and the possibility of fewer side effects that provides herbal medicines with a potential complementary option in the management of PCOS symptoms.

MATERIALS AND METHODS

Plant Material and Extraction

Bark of *Saracaindica* and *Bauhinia variegata* were collected and processed. The plant materials underwent ethanol and aqueous(1:1 ratio) extraction using Soxhlet apparatus. Further HPTLC and IR has been done for the extract and their targeted phytoconstituents.

Preliminary Phytochemical screening

Standard qualitative texts for alkaloids , flavonoids tannins and saponins has been done .

HPTLC fingerprinting

A CAMAG Llinomat-5applicator with a Hamilton microcrsyringe was used to apply bands in the chromatography analysis. The stationary phase was the use of pre-coated silica gel 60 F254 TLC plates.

FT-IR Analysis

Scanned under UV 254 nm and 366 nm. ATR spectra of extracts were captured in 4000- 400 cm-1 range of the functional groups that appeared as characteristic peaks on the instrument Shimadzu.

Experimental Animals

Animals were monitored during 14 days after the administration.Wistar rats of 190 ±10 g, 190 g were placed in a healthy condition in controlled laboratory conditions. The animals were put in a standardized environment and light and dark cycle was in a 12-hour cycle, temperature was maintained at 25 ±2 and relative humidity at 50-60. The ad libitum pellet diet and drinking water were administered during the period of study. All the experimental procedures were done in line with ethics, and they were approved by the Institutional Animal Ethics Committee (IAEC).

Acute Toxicity Study Protocol

The experiment was based on OECD guideline 423 (Acute Oral Toxicity - Acute Toxic Class Method). The animals were assigned to four groups (n = 6 rats each group) and given one dose of extract *Saracaindica* and *Bauhinia variegata* at the rates of 5 mg/kg, 50 mg/kg, 300mg/kg, and 2000mg/kg orally.

RESULTS AND DISCUSSION

Preliminary studies of Extracts

HPTLC fingerprinting of *Saracaindica* and *Bauhinia variegata*

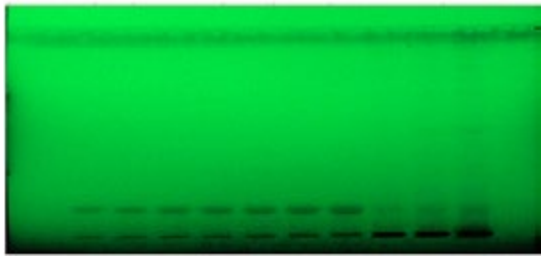
HPTLC fingerprinting of *Saracaindica*

Application- Linomat-5 Applicator (CAMAG)
Sample Volume Applied- 20 µl
Solvent material- Chloroform: Acetone: Formic Acid =[75:16.5: 8.5]
Scan Wavelength- 254nm, 366nm
TLC Plate Development- Pre-saturated Twin Trough Chamber
Reference Standards:Catechin

3.1 Preliminary studies of Extracts

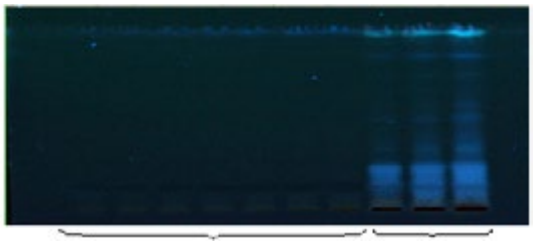
S. No.	Chemical Test	HE-SA	HE-BV
1.	Alkaloid- Test	+	+
2.	Tannin-Test	++	+
3.	Flavonoid-Test	+	+
4.	Amino acid-Test	-	-
5.	Saponin-Test	-	+

HE-SA: Hydroalcoholic extract of *Saracaasoca*, HE-BV: Hydroalcoholic extract of *Bauhinia variegata*,
+: Present, - :Absent



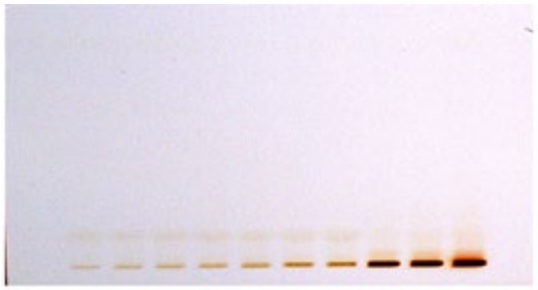
Catechin Standard *Saracaindica*

Fig. 1. Illustrate of HPTLC Plate at 254nm



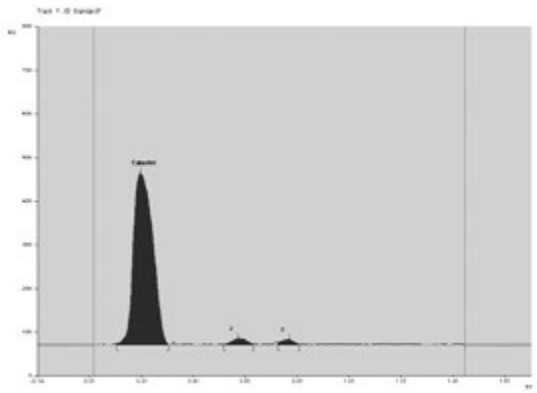
Catechin Standard *Saraca Indica*

Fig. 2. Illustrate of HPTLC Plate at 365nm



Catechin Standard *Saracaindica*

Fig. 3. Image of HPTLC Plate at White Light



Standard Peak

Catechin

HPTLC fingerprinting of Bauhinia variegata

Application- Linomat-5 Applicator (CAMAG)
Sample Volume Applied- 20 µl
Solvent System:-Toluene : Ethyl acetate : Formic acid (5:4:1)
Scan Wavelength- 254nm, 366nm
TLC Plate Development- Pre-saturated Twin Trough Chamber

Apigenin standard typically shows a sharp fluorescent yellow band under 366 nm.Rfvalue of Apigenin: ~0.45–0.60 (depending on mobile phase system). *Bauhinia variegata* extract shows multiple bands due to mixed phytoconstituents.A band

Table 1: Interpretationgraph of *Saracaasoca* and Catechin

Peak	Start position	Initial Height	Maxm Position	Max. height	Maxm %	End point	heig ht End	Area	Area %	Assigned substance
1	0.11RF	3.8 Au	0.2.1 Rf	394.6 Au	93.21	0.31 R.f	0.5 Au	141485.2 Au	94.99	Catechin
2	0.53RF	1.1Au	0.5.8 Rf	15.6Au	3.69	0.64 R.f	1.4Au	435.8 Au	2.92	Unknown
3	0.74 RF	5.8 Au	0.7.8 Rf	13.1 Au	3.10	0.82 R.f	1.5 Au	312.8 Au	2.09	Unknown

Table 2: IR interpretation of *Saracaasoca* and Catechin

S. No.	Functional Group	Observations cm^{-1} Saracaasoca	Observations cm^{-1} Catechin	Reference range
1	-OH	Stretching at at 3273	Stretching at 3398	Stretching at 3550- 3200
2	-CH	Stretching at 2924	-	Stretching at 3000- 2840
3	-CH2	Bending at 1352	Bending at 1352	Bending at1350- 1150
4	-CO	Stretching at 1284	Stretching at 1284	Stretching at 1260-1000
5	-C=C	Stretching at 1609	Stretching at 1612	Stretching at 1600-1500

Table 3: IR interpretation of *Bauhinia variegata* and *Apigenin*

S. No.	Functional Group	Observations cm^{-1} Saracaasoca	Observations cm^{-1} Catechin	Reference range
1	-OH	Stretching at at 3242	Stretching at 3257	Stretching at 3550- 3200
2	-C= C	Stretching at group at 1609	Stretching at 1649	Stretching at 1600-1500
3	-CH2	Bending at 1346	Bending at 1346	Bending at1350- 1150
4	-CO	Stretching at 1282	Stretching at 1282	Stretching at 1260-1000
5	-CH	Stretching at at 3242	Bending at 754.17	Stretching at 3550- 3200

Table 4: Body weight changes and Mortality

Animal group	(mg/kg.)	No. of Rat	Sex	Day of Death	Body Wt. (gm) 0-Day	7-Day	14-Day	No. dead/ Dosing
I	5 mg/kg.	R1	F	--	213.45	217.33	225.00	0/3
	"	R2	F	--	209.23	214.37	221.57	
	"	R3	F	--	216.54	219.21	228.44	
II	50 mg/kg	R1	F	--	207.25	211.39	218.51	0/3
	"	R2	F	--	196.37	201.55	208.71	
	"	R3	F	--	195.48	201.73	211.82	
III	300 mg/kg	R1	F	--	203.67	207.33	214.38	0/3
	"	R2	F	--	215.68	220.72	226.16	
	"	R3	F	--	212.52	216.92	224.68	
IV	2000 mg/kg	R1	F	--	190.78	196.44	201.34	0/3
	"	R2	F	--	203.55	207.67	210.76	
	"	R3	F	--	192.73	195.63	202.59	

Table 5: Acute toxicity behavior changes of *Saracaindica*

Parameters	Observations of isolated compoundof SA			
	(5.mg/kg)	(50 .mg/kg)	(300 mg/kg)	(2000 mg/kg)
Changes in skin and fur	No Change	No Change	No Change	No Change
Eyes	No Change	No Change	No Change	No Change
Mucous membranes	No Change	No Change	No Change	No Change
Salivation	No Change	No Change	No Change	No Change
Stool	No Change	No Change	No Change	No Change
Diarrhea	None	None	None	None
Sleeping pattern	No Change	No Change	No Change	No Change
Behavior pattern	No Change	No Change	No Change	No Change
Somatomotor activity	Not-visible	Not-visible	Not-visible	Not-visible
Mortality (14 days)	No	No	No	No

Table 6: Body weight changes and Mortality

Animal groups	(mg/kg.)	No. of Rat	Sex	Day of Death	Body Wt. (gm) 0-Day	7-Day	14-Day	No. dead/ Dosing
I	5.mg/kg	R1	F	--	211.45	214.09	220.33	0/3
	"	R2	F	--	205.67	209.54	215.64	
	"	R3	F	--	210.37	213.54	221.22	
II	50 mg/kg	R1	F	--	204.56	206.42	213.51	0/3
	"	R2	F	--	199.88	203.00	209.66	
	"	R3	F	--	191.88	195.55	202.00	
III	300 mg/kg	R1	F	--	203.55	207.13	213.58	0/3
	"	R2	F	--	204.66	207.89	214.11	
	"	R3	F	--	206.75	209.22	216.16	
IV	2000 mg/kg	R1	F	--	202.00	206.13	211.98	0/3
	"	R2	F	--	201.25	204.93	211.31	
	"	R3	F	--	190.56	194.12	203.88	

Table 7: Acute toxicity behavior changes of *Bauhinia Vareighata*

Parameters	Observations of isolated compoundof SA			
	(5.mg/kg)	(50 .mg/kg)	(300 mg/kg)	(2000 mg/kg)
Changes in skin and fur	No Change	No Change	No Change	No Change
Eyes	No Change	No Change	No Change	No Change
Mucous membranes	No Change	No Change	No Change	No Change
Salivation	No Change	No Change	No Change	No Change
Stool	No Change	No Change	No Change	No Change
Diarrhea	None	None	None	None
Sleeping pattern	No Change	No Change	No Change	No Change
Behavior pattern	No Change	No Change	No Change	No Change
Somatomotor activity	Not Visible	Not Visible	Not Visible	Not Visible
Mortality (14 days)	No	No	No	No

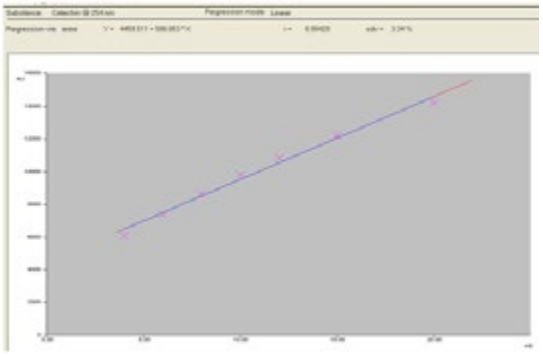


Fig. 4. Calibration curve of catechin

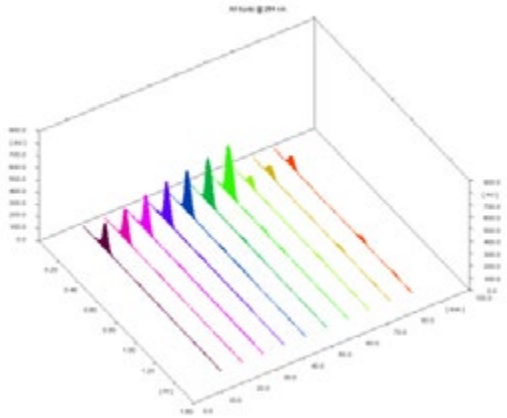


Fig. 5. 3D-Plot at 254nm

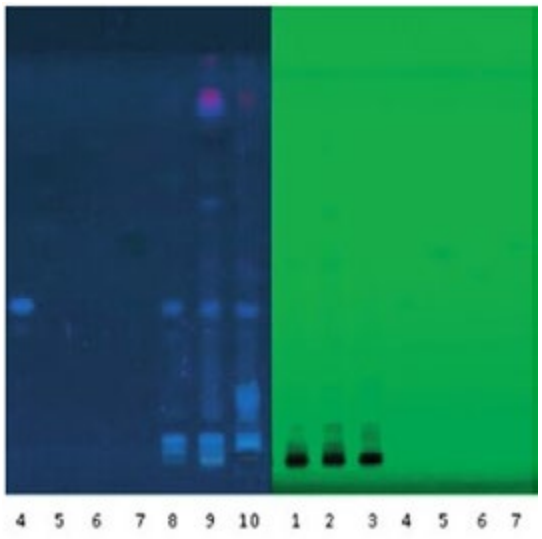


Fig. 6. Image of HPTLC Plate of Apigenin at366nm and 254nm

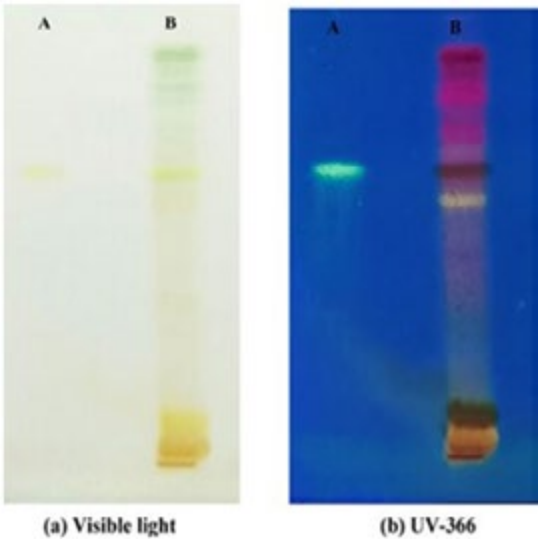


Fig. 7. Image of HPTLC Plate of extract and apigenin (fluorescent col.) at254nm and 366nm

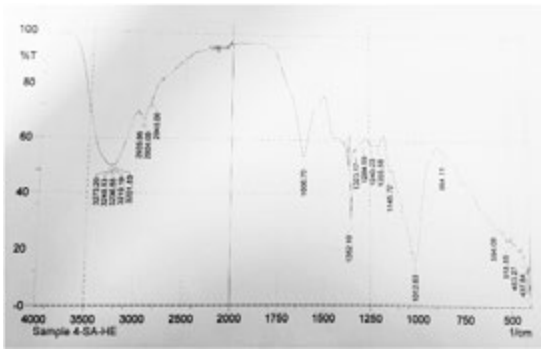


Fig. 8. IR spectra of *Saracaasoca*

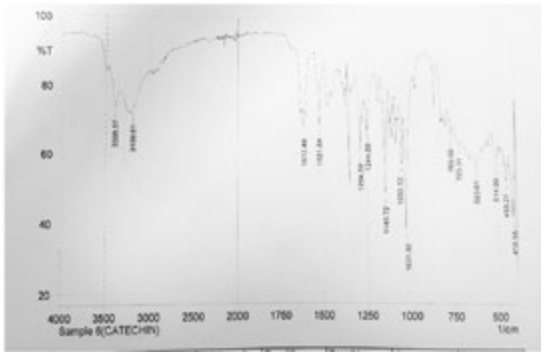


Fig. 9. IR spectra of Catechin

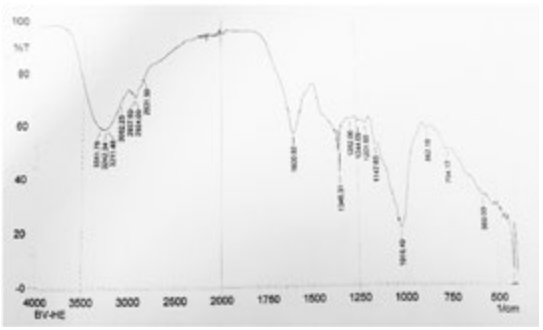


Fig. 10. IR spectra of *Bauhinia variegata*

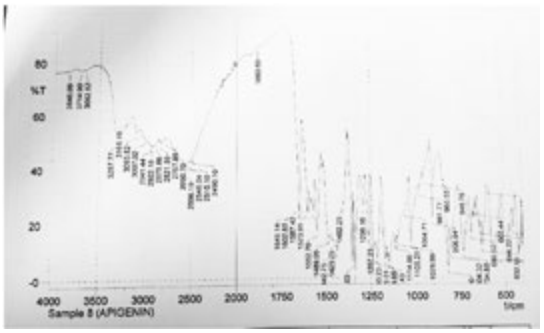


Fig. 11. IR spectra of Apigenin

matching the Rf of apigenin standard confirms its presence in the extract.

FTIR Analysis

FTIR Analysis of *Saracaindica* extract and catechin FT-IR Analysis of *Bauhinia variegata* extract and Apigenin

AcuteOral.Toxicity

Acute oral toxicity of Test sample *Saracaindica*

The acute toxicity analysis of *Saracaindica* and *Bauhinia variegata* plant extract in female Wistar rat species showed that there were no changes in its toxicity at the maximum dose of 2000mg/kg body weight. During the 14 days of observation, it was found that all the animals showed a slow and steady increase of their body weight, and there were no deaths in any group of doses. Additionally, close examination of behavioral and physiological parameters, including state of skin and fur, eye and mucous membranes looks, salivation, stool, sleep, somatomotor activity, and overall behavior revealed no deviations or any negative response. These results suggest that extraction of *Saracaindica* and *Bauhinia variegata* does not produce any acute toxicity and has a high level of tolerance in animals when used in experimental studies.

The lack of mortality along with a considerable clinical effect even at the highest dose indicate that LD50 (lethal dose of 50 percent of the population) of extract of *Saracaindica* is higher than 2000 mg/kg, which makes this extract practically non-toxic in terms of OECD guidelines. This captive safety margin is especially necessary in the light of increased interest in herbal medicines as complementary or alternative therapeutic tools in the management of chronic endocrine disorders like Polycystic Ovary Syndrome (PCOS). PCOS may be a long-term condition, so it is important to determine the safety of herbal extracts in advance to avoid the possible adverse effects.

Saracaindica and *Bauhinia variegata* extract has a high potential in the development as a therapeutic agent against PCOS based on its promising safety profile as evidenced in this study. Nevertheless, more research is suggested to be undertaken such as chronic toxicity research, reproductive toxicity research and in-depth pharmacodynamics and pharmacokinetics research. Such studies in the future will aid in assuring long term safety and efficacy which will open the way to clinical trials and a possible application of these therapeutic modalities in future in the management of PCOS.

CONCLUSION

This analysis showed that no acute toxic effects were observed with Saracaindica and bauhinia variegata extract at the highest dose of 2000mg/kg in female Wistar rats. The weight gain and lack of mortality and normal physiological and behavioral parameters indicate that the extract is practically non-toxic in acute exposure situations. This means that Saracaindica can be deemed safe to continue with further investigations on its chronic toxicity and pharmacological implications on the treatment of PCOS.

These results coincide with the other studies that suggested low toxic effect of related

plant extract. This research contributes to the non-toxicity of bauhinia variegata and *Saracaindica* as therapeutic agents due to the current interest in the natural treatment of PCOS.

REFERENCES

1. Sharma, A., Chandel, V. & Roy, S. Exploring the bioactive properties and diverse uses of *Bauhinia variegata*: a comprehensive review. *Discov. Plants* **2025**; *2*, 8. https://doi.org/10.1007/s44372-025-00167-7
2. Kathole, N. S., Karande, V. V., Ghumare, B. C., Mote, C. S., Lonkar, V. D., Jadhav, S. N., &Bachapalle, P. P., Evaluation of Antiangiogenic Potential of Ethanolic Extract of *Bauhinia variegata* Leaves in Mice. *Indian Journal of Veterinary Sciences and Biotechnology*, **2023**; *19(5),* 70-73. https://doi.org/10.48165/ijvsbt.19.5.14
3. Ziyaurrahman A.R, Mohammed Saad, Reehan Khan, Tufail Dana, BeldarShmashad, &Dilnawaz Pathan., Acute AndSubacute Oral Toxicity Of SaracaAsoca In Rats. *Journal for ReAttach Therapy and Developmental Diversities,* **2023**; *6(1),* 870–888. https://doi.org/10.53555/jrtdd.v6i1.2331
4. Fathima, Faiza& Parveen, Rabea& Khan, Mohammad & Malik, Zoya& Mustafa, Saad & Massey, Sheersh& Husain, Syed., Saracaasoca: From Traditional Herb to Modern Drug as a Cure for Various Diseases, 2023.
5. Khare, Pragati& Kishore, Kamal & Sharma, Dinesh., Acute Oral Toxicity of Bauhinia Variegata and MadhucaLongifoliaIn Mice. *International Journal of Current Pharmaceutical Research*. **2022**; *69-71.* 10.22159/ijcpr.2022v14i2.1966.
6. Choudhary, Y., Deshpande, S. V., Patil, S. B., & Chaudhari, A. L. , Acute toxicity study of the nanoparticles of Bauhinia variegata Linn bark hydro-alcoholic extract in Wistar Albino rats. *International Journal of Ayurvedic Medicine,* **2022**; *13(3),* 777–782. https://doi.org/10.47552/ijam.v13i3.2895
7. Jangra, Ashwani & Saharan, Vikas Anand & Dubey, R.C. &Goel, Kapil., Toxicological evaluation of Bauhinia variegata Linn. for estimating the neurological, hematological and physical alterations in the albino mice. *Environment Conservation Journal.* **2016**; *17.* 97-102. 10.36953/ECJ.2016.17318.
8. Kumar, A. ., Anand, V., Dubey, R. ., & K. Goel, K., Toxicological evaluation of Bauhinia variegata Linn. for estimating the neurological, hematological and physical alterations in the albino mice. *Environment Conservation Journal,* **2016**; *17(3),* 97–102. https://doi.org/10.36953/ECJ.2016.17318
9. Chaudhari NB, Suralkar A, ZiyaurrahmanAR. Preclinical Safety Evaluation of Aqueous Bark Extract of SaracaAsocaRoxb : Acute And Subacute Oral Toxicity., *International Journal of Pharmacological research*. **2015**; *5(12);* 332-337; http://doi.org/10.7439/ijpr.v5i12.2791
10. Mali RG, Dhake AS. Evaluation of effects of Bauhinia variegata stem bark extracts against milk-induced eosinophilia in mice. *Journal of Advanced Pharmaceutical Technology & Research.* **2011**; *2(2):*132-4. doi: 10.4103/2231-4040.82949. PMID: 22171306; PMCID: PMC3217693.
11. Makadiya, V. S., Kulkarni, Y. A., &Apte, K. G. Acute and 28-Day Repeated Dose Oral Toxicity of Bauhinia variegata (Caesalpiniaceae) Stem Bark Extract., *Journal of Herbs, Spices & Medicinal Plants,* **2014**; *21(2*), 161-172
12. Yadav NK, Saini KS, Hossain Z, Omer A, Sharma C, Gayen JR, Singh P, Arya KR, Singh RK. Saracaindica bark extract shows in vitro antioxidant, antibreast cancer activity and does not exhibit toxicological effects. Oxid Med Cell Longev. 2015:205360. doi: 10.1155/2015/205360.
13. Gupta M, Sasmal S, Mukherjee A. Therapeutic Effects of Acetone Extract of Saracaasoca Seeds on Rats with Adjuvant-Induced Arthritis via Attenuating Inflammatory Responses. 2014, ISRN Rheumatol. doi: 10.1155/2014/959687.
14. Kolhe, Rasika&Acharya, Rabinarayan & Ravishankar, Basavaiah., Chronic toxicity study of stem bark powder of Kanchanara (*Bauhinia variegata* L.) in albino rats. *Indian journal of Natural product and resourses.* **2014** ; 5. 244-248.