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Evaluation of Aqueous Extract of Indigofera Suffruticosa (AEIS) Leaves for Diuretic Activity.

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Abstract:

Indigofera suffruticosa, commonly known as Indigofera anil L, is a plant traditionally used in various folk medicines for its diuretic properties. This study aimed to evaluate the diuretic activity of Indigofera suffruticosa through experimental models. The plant material was extracted using aqueous solvents, and the extracts were screened for diuretic activity using spargue dawley rats. Diuretic activity was assessed by measuring urine output, electrolyte excretion, and urine pH. Results indicated that the aqueous extract of Indigofera suffruticosa exhibited significant diuretic activity compared to the control group, with a notable increase in urine volume and electrolyte excretion. The findings suggest that Indigofera suffruticosa possesses potential diuretic properties, supporting its traditional use in folk medicine. Further studies are warranted to elucidate the underlying mechanisms and identify active constituents responsible for its diuretic effects.

Keywords: Indigofera suffruticosa, diuretic activity, urine output, electrolyte excretion, urine pH. HPTLC.

INTRODUCTION

Herbal medicine has become the remedy for most of the diseases. In conjunction with a healthy diet and lifestyle

they target specific health goals providing every cell the most appropriate and optimal nourishment. These herbal supplements do not have any harmful side effects that might disturb physical health unlike synthetics. The Indigofera suffruticosa genus of the Fabaceae family is well-known for its use as feed, green fertilizer, and ground cover.(1)

The Fabaceae family is the second-largest family of medicinal plants, with more than 490 species used in traditional medicine. *Indigofera suffruticosa* is the third-largest genusin this family and consists of approximately 750 species, distributed across all tropical and subtropical regions of the world. Some 75% of these species are restricted to Africa and Madagascar, and many species are also encountered in theSino-Himalayan region, Australia, and Central and South America. (2) The leaves, flowers and tender shoots are cooling and demulcent. The leaves are used for leprosy, cancerous affections, abscesses, dandruff and also for edematous tumors.(3)

The word diuretic has a Greek stem, Diu (through) *ουρειη* (to urinate), and a diuretic is defined as any substance that increases urine flow and thereby water excretion. (4) Diuretics are among the most commonly used drugs and the majorityact by reducing sodium chloride reabsorption at different sites in the nephron thereby increasing urinary sodium, and consequently, water loss. Diuretics are an invaluable and heterogeneous class of agents commonly used in the treatment of hypertension, heart failure, and electrolyte disorders. (5)

The diuretic compounds are therapeutic tools used extensively and successfully forthe treatment of various medical disorders all over the world. All diuretic agents act primarily by impairing Na+ reabsorption in the renal tubules. (6) However, they differ considerably in their chemical derivation and mechanism of action, that is, the specific tubular ion transport systems they interfere with. The latter determinesthe site of action along the nephron where each class of diuretics acts and, becausephysiologically the amount of Na+ reabsorbed differs between the various segments of the nephron, it further determines the natriuretic efficacy, pharmacological effects and specific clinical indications of each diuretic. Thus, knowledge of the for glaucoma or osmotic diuretics for cerebral oedema. The available diuretic compounds could be also used in certain combinations to help towards effective volume control in difficult-to-treat patients with multiple underlying problems. (7)

Diuretic drugs generally exhibit an overall favourable risk/benefit balance. However, they are not devoid of side effects. Diuretics induce a loss of electrolytesand fluid, thereby stimulating several compensatory haemostatic mechanisms suchas the RAS, which result in increased renal sodium retention by all nephron segments, a phenomenon known as diuretic resistance. If dietary salt intake is sufficiently high, a daily net negative sodium balance may not be achieved even with several daily doses of loop diuretics. (8) The major side effect of loop and thiazide diuretics is the deficiency of the main electrolytes, particularly sodium andpotassium. Hypokalaemia may increase the risk of arrhythmia and cardiac arrest. Mild hypokalaemia caused by these diuretics may result in leg cramps, polyuria, and muscle weakness. Most diuretics also decrease urate excretion and increase blood uric acid, causing gout in predisposed patients. Serum cholesterol levels mayincrease after diuretic therapy, and high doses of diuretics can impair glucose tolerance and precipitate diabetes mellitus, probably through an increase in insulin resistance.(9) Diuretics use, particularly loop diuretic use, was suggested to be a risk factor of

sarcopenia.[10]

Diuretics are drugs that increase the flow of urine. They are commonly used to treatedema, hypertension, and heart failure. Typically, the pharmacological group consists of five classes: thiazide diuretics, loop diuretics, potassium-sparing diuretics, osmotic diuretics, and carbonic anhydrase inhibitors.[11]

MATERIALS AND METHODS

Experimental animals:

Rat: Healthy Sprague Dawley rats of either sex weighing between 180-300gm willtake for study. The rats were kept in standard polypropylene cages. Animals were maintained under standard laboratory conditions of temperature (25±2°), relative humidity (50±15%), 12 h light-dark cycle, standard diet, and water. The care and handling of the animals were in accordance with the internationally accepted standard guidelines for use of animals, and the protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Navsahyadri Institute of Pharmacy, Naigaon in accordance with the regulations of CPCSEA.

EXPERIMENTAL DESIGN:

Grouping and Dosing of Animals:

Rats were randomly assigned into five groups each consisting of six rats for diuretictest.

Group I (control group) was treated with 2 mL/100 g of distilled water.

Group II (standard group) was treated with standard drug 10 mg/kg furosemide.

Groups III (extraxt group) were treated with doses of 200 mg/kg

Group IV (extract group) were treated with doses of 400 mg/kg

Group V (extract group) 800 mg/kg of *Indigofera suffruticosa* leaves aqueous extract,respectively.

Dose selections was made based on the acute oral toxicity test performed, taking one tenth of the limit dose as the middle dose. Route of administration used for allgroups was oral using gavage.

EXPERIMENTAL MODEL:

Diuretics Model:

The Kau method with some modification was used to screen all test substances fortheir diuretic activity. Food and water were withdrawn 24 hours prior to the experiment, and the test animals were individually placed in metabolic cages withgraduated and transparent tubes to collect their urine and determine the volume every hour. The total urine volume was measured every hour up to the end of the 5th hour for all groups. The parameters determined were cumulative urine volumeand urinary ionic concentration of Na^+ and K^+. The volume of the urine excreted until the 5th hour of the study by each group was expressed as the percent of the liquid (NS) administered giving rise to the measure of "urinary excretion" independent of group weight. The ratio of urinary excretion in the test group to urinary excretion in the control group was considered as the measure of the diuretic index for a given dose of the drug. As the diuretic index is prone to variability, a parameter known as Lipschitz value was calculated. To obtain Lipschitz value, thediuretic index of the test substance was compared to that of the standard drug in thetest group. The ratio of urinary excretion in test group and urinary excretion inthe control group was denoted as "diuretic index", which was used as the measureof degree of diuresis:

Urinary excretion = $(V_o / V_i) \times 100$

where Vo is the total urinary output and Vi is the total volume of fluid administered:

Diuretic index = V_t / V_c

where Vt is the mean urine volume of test group and Vc is the mean urine volumeof control group:

Diuretic activity = V_t / V_r

where Vt is the mean urine volume of test group and Vr is the mean urine volumeof reference group:

Evaluation parameter for Diuretic activity:

Determination of urine pH: The pH of the fresh urine samples of all groups wasmeasured with a calibrated digital pH meter (Mettler Toledo, Columbus, OH, USA). [23]

Determination of urinary Na^+, K^+, and Cl^-: Urinary Na^+, K^+, and Cl^- concentrations of the experimental, control, and standard groups were determined using Trans-sia instrument. [24]

Extraction of phytoconstituents:

1. **Preparation of plant extract of Indigofera suffuriticosa Mill leaves:** The leaves of Indigofera suffuriticosa Mill were dried in the shade and ground to a coarse powder by using a dry grinder. The 200g of coarsely ground plant material was soaked with a 25% ammonia solution and left to stand the next day. A Soxhlet extractor was then used to do a thorough extraction with distill water. Vacuum distillation was used to concentrate the

extract.[12]

2. **Extraction of Plant (Soxhlet extraction):** Solid material placed in thimble. Soxhlet extractor is placed onto a flask containing the extraction solvent.Soxhlet equipped with a condenser. The solvent is heated to reflux. The solventvapour travels up a distillation arm and floods into the chamber housing the thimble of solid. Solid material in chamber slowly fills warm solvent. Desired compound dissolves in the warm solvent. When the Soxhlet chamber is almostfull, the chamber is emptied by the siphon. The solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days. During each cycle, a portion of the compound dissolves in the solvent. After many cycles (72 hours) the desired compound is concentrated inthe distillation flask. After extraction the solvent is removed, typically by meansof a rotary evaporator, yielding the extracted compound. The non- soluble portion of the extracted solid remains in the thimble, and is usually discarded.[13]

Phytochemical screening of Plant: [14,15,16,17]

Preliminary Qualitative Tests: Characterization of all samples leaf powder extracts has been done for various chemical constituents as per following protocol:

1. Test for glycosides:

A. Test for cardiac glycosides

Table 2. Test for cardiac glycoside

Sr no.	Test	Observation
1	Baljets test	A section shows yellow to orange colour with sodium picrate
2	Legals test	To aqueous or alcoholic extract, add 1ml pyridine & 1ml sodium nitroprusside.
3	Test for deoxysugars(killer-killiani test)	To 2ml extract, add glacial acetic acid, one drop of 5% ferric chloride (FeCl3) sol. And conc. sulphuric acid. Reddish brown colour appears at junction of the two liq. Layers & upper layer appears bluish green.
4	Liebermann's test (test for bufanolides)	Mix 3ml exact with 3ml acetic anhydride.Heat & cool. Add few drops of conc. Sulphuric acid.

5	Kedds test (test for unsaturated/acetone)	Residue of chloroform exact + one drop of 90% alcohol. It gives purple colour.
6	Raymonds test	To test sol. Add hot methanolic alkali. It gives violet colour

B. Tests for Anthraquinone glycosides:

Table 3. Test for anthraquinone glycoside

Sr no	Test	Observation
1	Bontrager's test	To 30 ml exact, add dil. Sulphuric acid. Boil & filter. To cool filtrate, add equal volume of benzene & chloroform. Shake well, separate the organic solvent. Add ammonia. Ammoniacal layer turns pink to red.
2	Modified Bontrager's test for C-glycosides	To 5 ml extract, add 5ml 5% ferric chloride & 5ml dil. Hydrochloric acid. Heat for 5min in boiling water bath cool & addbenzene or any organic solvent. Shake well, separate the organic layer, add equal vol. dil. ammonia. Ammoniacal layer shows pinkish red colour.

C. Test for saponin glycosides: To 5ml aqueous extract was vigorously shaken with 10ml distilled water for 2min. the appearance of foam persists for at least15 min. confirmed the presence of saponins.

D. Test for cyanogenetic glycosides

Table 4. Test for cyanogenetic glycosides

Sr.no.	Test	Observation

1	Guignard reaction or sodium picrate test	Soak a filter paper strip in 10% picric acid, then in 10 % sodium carbonate, dry. In a conical flask. Place moistened powdered drug cork it, place the above filter paper strip in the slit in cork. The filter paper turns brick red or marom.
2	To dry drug powder or extract, add 3% aq. Mercuric nitrate solution	Metallic mercury forms
3	Dip a piece of filter paper in guaiacum resin & moist it with dilute copper sulphate solution. Expose it to freshly cut surface of drug	Blue stain is produced

E. Test for Coumarin glycosides

Table 5. Test for Coumarin glycosides

Sr. no	Tests	Observation
1	Coumarin glycosides	Aromatic odor.
2	Alcoholic extract when made alkaline	Blue or green fluorescence
3	Take moistened dry powder in test tube. Cover test tube with filter paper soaked in dilute NaOH. Keep in water bath. After sometimes expose filter paper to ultra violet light. It shows yellowish	Green fluorescence

2. Test for Flavonoids:

Table 6. Test for Flavonoids

Sr.no	Test	Observation
1	**Sulphuric acid test**	On addition of sulphuric acid (66% or 80%) flavones &flavanols dissolve into it & gives a deep yellow. Solution chalcones & aurones give red or reddish solution. Flavones give orange to red colours.

3. Test for tannins & phenolic compounds

To 2-3 ml of aqueous or alcoholic extract, add few drops of following reagents.

Table 7. Test for tannins &phenolic compound

Sr.no.	TEST	OBSERVATION
1.	5%FeCl3solution	It gives deep blue – black colour.
2.	Lead acetate solution	It gives white ppt
3.	Gelatine solution	It gives white ppt
4.	Bromine water	Decolouration of bromine water.
5.	Acetic acid solution	It gives red colour solution
6.	Potassium dichromate	It gives red ppt

4. Test for Alkaloids

Evaporate the aqueous, alcoholic and chloroform extract Separately to residue, adddilute HCl, shake well % filter with filtrate perform following tests:

Table 8. Test for alkaloids

Sr. no	Test	Observation
1.	Dragendorff's test	To 2-3 ml filtrate, add few drops of Dragendorff's' reagent. Orangebrown ppt is formed.
2.	Mayers test	To 2-3 ml filtrate add few drops of Myers reagent. It gives ppt
3.	Hager's test	2-3 ml filtrate with Hager's reagent gives yellow ppt
4.	Wagner's test	2-3 ml filtrate with Wagner's reagent it gives brown ppt
5.	Murexide test for purine alkaloids	3-4 ml test solution add 3-4 ml drops of conc. HNO3. Evaporate to dryness. Cool & add 2 drops of NH4OH. Purple colour is observed.
6.	Ammonium riebeckite test	Add 0.2 ml hydroxylamine to a saturated solution of 4% ammonium riebeckite & acidify with dil.HCl, alkaloids give ppt. the ppt is soluble in 50% acetone which is used to recrystallize it.
7.	Tannic acid test	The test solution treated with tannic acid solution gives buff colored ppt.
8.	Picrolonic acid test	The test solution on treatment with picronic acid gives yellow ppt.

TLC (18.19)

Step1: Preparation of mobile phase

Mobile phases are prepared by mixing all different sample in different solvent in the ratios of Chloroform : Methanol (8:2) degassed to remove trapped air. Pour solvent into the TLC glass chamber to a depth of just less than 0.5 cm. To aid in the saturation of the TLC chamber with solvent vapours, line part of the inside of the beaker with filter paper. Cover the beaker with a watch glass, swirlit gently, and allow it to stand while you prepare your TLC plate.

Step2: Preparation of the TLC plate

TLC plates used are purchased as 5 cm x 20 cm sheets of silica gel 60F254. Each large sheet is cut horizontally into plates that are 5 cm tall by various widths. Measure 0.5 cm from the bottom of the plate. Using a pencil, draw a line across theplate at the 0.5-cm mark. This is the origin line on which the plate is located. Underthe line, lightly mark the names of the samples you will spot on the plate. Leave enough space between the samples so that they do not run together; about 4 sampleson a 5 cm wide plate are used.

Step 3: Spotting of the TLC plate

50 mg were suspended in 50 ml of methanol & sonicated for 10 min. Thesuspensionwas centrifuged, & the supernatant used. 0.5 g of each powdered sampleA were suspended in 30 ml of water & boiled for 10 min. Each sample solution isfiltered into 50 ml vol. flask & the volume is made up with water i.e., sample – A respectively. With the help of a micro capillary dip the microcap into the solution and then gently touch the end of it onto the proper location on the TLC plate. Don'tallow the spot to become too large - if necessary, touch it to the plate, lift it off andblow on the spot.

Step4: Development of the plate

Place the prepared TLC plate in the developing beaker which is filled withdeveloping solvent i.e., mobile phase, cover the TLC glass chamber with the glasslid, and leave it undisturbed on your bench top. The solvent will rise up the TLC plate by capillary action. Make sure the solvent does not cover the spot. Allow theplate to develop until the solvent is about half a centimeter below the top of the plate. Remove the plate from the TLC glass chamber and immediately mark the solvent front with a pencil. Allow the plate to dry.

Step 5: Visualization of the spots:

A 10 ml solution of anisaldehyde sulphuric acid reagent is sprayed onto a TLC plate to visualize the spots. 0.5 ml of anisaldehyde and 10 ml of glacial acetic acidare combined to create the anisaldehyde sulphuric acid reagent. Next, 85 ml of methanol and 5 ml of concentrated sulfuric acid are added in that sequence. After the

TLC plate was sprayed, it was heated for five to ten minutes at 100°C before

being assessed at visible or UV-365 nm. Reagent loses its usability as the color turns reddish-violet and has only a limited stability.

Step 6: Calculation of the Rf value

$$R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent front}}$$

The retention factor, or Rf, is defined as the distance travelled by the compound divided by the distance travelled by the solvent.

HPTLC (20.21)

1. **Preparation of mobile phase**

Mobile phase is prepared by mixing all the solvent in the different ratios ofand degassed to remove trapped air, Chloroform : Methanol (8:2)

Stock and working standard solution

The standard stock solution prepared by sample-A (Indigofera suffuriticosa), was weighed 50 mg and suspended in 50 ml of methanol and transferred separately to 50 ml of volumetric flask and sonicated for 10 min. The weighed powder was dissolved in methanol & make up the volume to obtain stock sol. of 100 µg/ml appropriate dilutions of the above-mentioned standard stock sol. Were prepared 10ml vol. flask with methanol.

3. Preparation of sample solutions

0.5 g of each powdered leaf sample A were suspended in 30 ml of water & boiledfor 10 min. Each sample solution is filtered into 50 ml vol. flask & the volume is made up with water i.e., sample – A respectively.

Results and Discussion

Preliminary Qualitative tests

Table 9: Summary of Preliminary Qualitative tests

Sr.no.	Test	Methanol	Ethanol	Aqueous

1	Glycosides	Baglet's test	-	+	+
		Legal's test	-	+	+
		Keller-killiani test	+	+	+
		Liebermann's test	-	+	-
		Kedd's test	+	-	+
		Raymond's test	-	-	-
A.	Anthraquinone glycosides	Bontrager's test	-	+	+
B.		Modified Bontrager's test	-	-	+
C.	Saponin glycosides	Foam test	+	+	-
D.	Cyanogenetic glycosides	Grignard reaction test	-	+	+
E.	Coumarin glycosides	Coumarin glycosides test	-	+	+
2	Flavonoids	Shinoda test	+	+	+
		Sulphuric acid test	+	+	+
3	Tannins & Phenols	FeCl3test	+	+	-
		Lead acetate test	+	+	-
		Gelatine test	+	+	-
		Bromine water test	+	+	-
		Acetic acid test	-	+	+
		Potassium dichromate test	+	+	-
		Dil.NH4OH & Potassium ferricyanide test	+	-	-
		Dil. potassium permanganate test	+	-	+
4	Alkaloids	Dragendorff's test	-	+	+
		Mayer's test	-	+	+
		Hager's test	-	+	+
		Wagner's test	-	+	+
		Murexide test	-	+	+
		Ammonium riebeckite test	-	+	+
		Tannic acid test	+	+	-
		Picronic acid test	-	+	+

The result analysis of physicochemical properties tested for modified & unmodified starches are reported in table

TLC METHOD

Optimization of mobile phase

The plant extract solutions sample-A (*Indigofera suffruticosa)* were spotted on the TLC plates and run in different solvent systems. Initially, Chloroform : Methanol (8:2) in varying ratios were tried.

Analysis of plant extract

The extract, sample – A, were analysed using optimized mobile phase or developedmethod. The Rf value of samples show different Rf value of plant extract. The bands of these compounds of samples A well separated on HPTLC plate, for sample- A, at Rf= 0.04, 0.49 and 0.82 respectively.

(A)TLC of samples a, by using Chloroform: Methanol (8:2) at UV-254 nm

Fig no 7: TLC of samples a, by using Chloroform: Methanol(8:2)

HPTLC

Optimization of mobile phase

The plant extract solutions sample-A (*Indigofera suffruticosa*) were spotted on theTLC plates and run in different solvent systems. Initially, Chloroform: Methanol (8:2) in varying ratios were tried.

Analysis of plant extract

The extract, sample – A, sample were analyzed using optimized mobile phase or developed method. The Rf value of samples show different Rf value of plant extract. The bands of these compounds of samples A were well separated on HPTLC plate, for sample- A, at Rf=0.04, 0.49 and 0.82 respectively.

Initial HPTLC fingerprinting study was performed on the pure marker compounds*Indigofera suffruticosa*. The

bands of marker compounds were scanned and their spectra were recordedat400nm.Thus, for better quantitative analysis all the plates were scanned at 400nm. Fig shows the chromatograms obtained at 400nm from thestandard marker compounds separately and as standard mix.

Table 10: Analysis of plant extract sample A

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.04	4.4	0.07	22.3	39.20	0.09	0.9	389.1	43.91
2	0.49	2.1	0.51	12.3	21.70	0.53	3.9	203.1	22.92
3	0.82	9.7	0.83	22.2	39.10	0.87	1.8	294.0	33.18

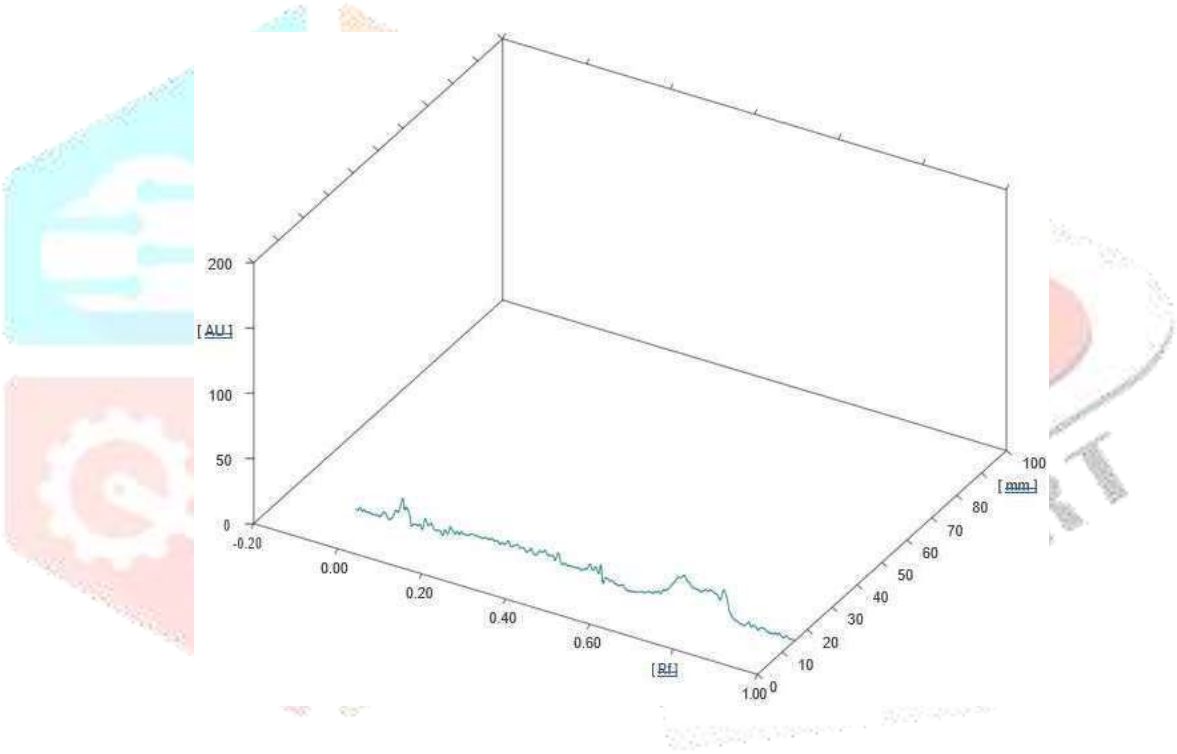

Fig no 8: 3-D chromatogram of Samples-A

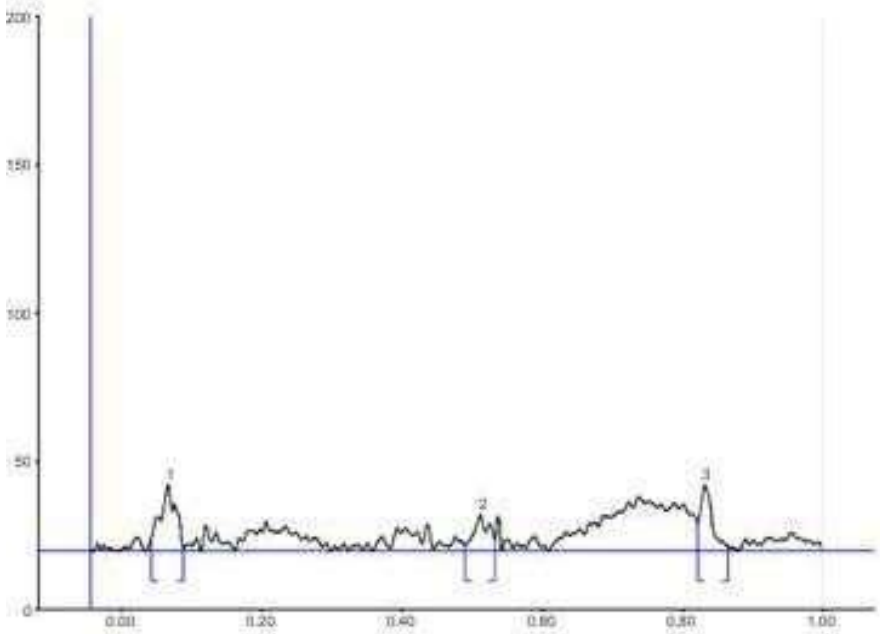

Figure no 9: Chromatogram of Indigofera suffruticosa Leaf extract usingmobile phase, Chloroform: Methanol (8:2)

EVALUATION OF DIURETICS

Urine output

Table no 11 demonstrates changes in urine output during 5hrs, following administration of the interventions. The treatment with I. suffruticosa increased urine volume at each time interval that was obvious from the first hour after the administration. This effect was at 1hrs (0.408 ml) and also significant at 5hrs (1.48 ml) as compared to distal water. Furosemide caused significant increase in the urine volume as compared to distal water: however, its effect was insignificant as compared to I. suffruticosa also increases with compared to furosemide.

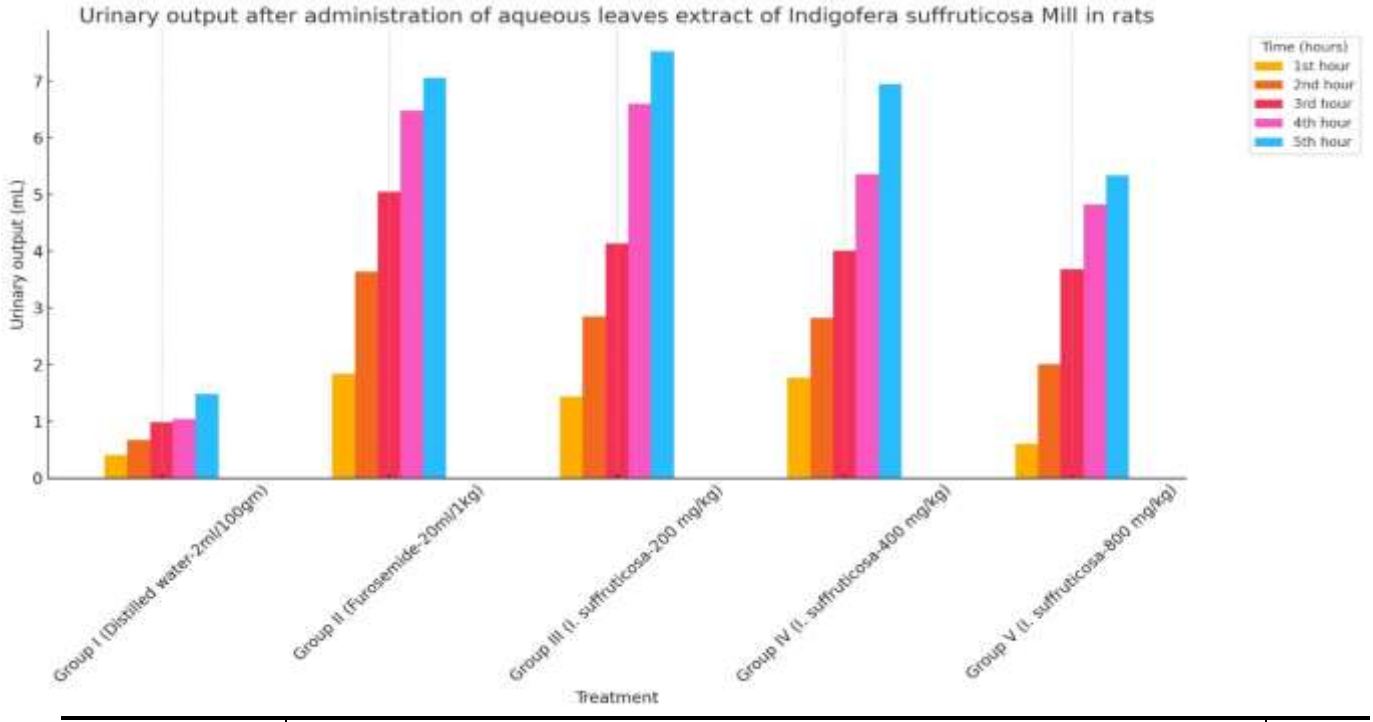

Fig no 10: The urine output after the administration of the aqueous crudeextract of *I. suffruticosa* leaves in rats

Table 11: Urinary output after the administration of aqueous leaves extract ofIndigofera suffuriticosa Mill in rats

Treatment	Urinary output (mL) with SD (±)				
	1st hour	2nd hour	3rd hour	4th hour	5th hour
Group I (Distilled water- 2ml/ 100gm)	0.408±0.016	0.671 ± 0.070	0.982 ± 0.093	1.042 ± 0.001	1.481 ± 0.077
Group II (Furosemide-20ml/1kg)	1.836±0.011	3.642 ± 0.038	5.051 ± 0.013	6.484 ± 0.019	7.058 ± 0.021
Group III (*I. suffruticosa- 200* mg/kg)	1.44±0.016	2.85 ± 0.045	4.134 ± 0.019	6.601 ± 0.050	7.525 ± 0.092
Group IV (*I. suffruticosa- 400* mg/kg)	1.77±0.016	2.82 ± 0.047	4.009 ± 0.095	5.36 ± 0.091	6.95 ± 0.012
Group V (*I. suffruticosa- 800* mg/kg)	0.606±0.024	2.011 ± 0.040	3.684 ± 0.026	4.821 ± 0.060	5.341 ± 0.045

Urine pH

Urinary pH measurement revealed that the different treatment groups that receivedaqueous leaf extract including the Normal and Standard control groups produced slightly acidic urine. The average urine pH of the normal and standard controls wasdetermined to be 6.02 and 6.70, respectively. The pH of urine from rats treated withthe aqueous extract displayed increase for the two doses 200 and 400 mg/kg (6.58and 6.91, respectively), and a slight decrease in pH (6.19) was seen for the highestdose (800 mg/kg) of the aqueous crude extract. There was no statistically significant change in urinary pH in the rats.

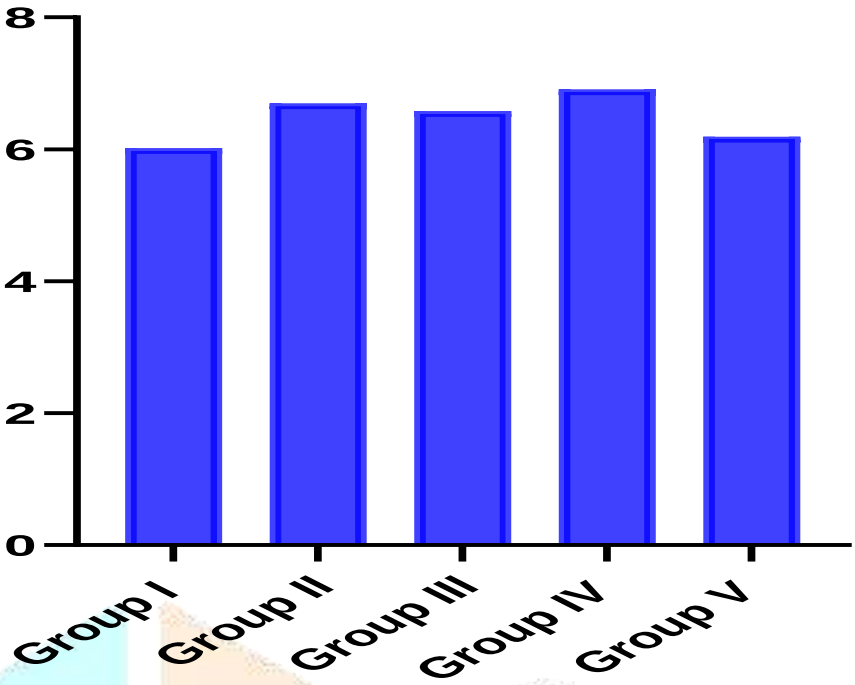

Fig no 11: Urine pH

Diuretic activity

The rats that received the lower doses 2ml/100gm and higher dose 800 mg/kg showed a significant diuretic activity from the administration of aqueous crude extract of *I. suffruticosa*. As seen in Table 11, the animals that received doses 2ml/100 gm to 800 mg/kg.

Table no 12: Diuretic activity of aqueous crude extract of Indigofera suffuriticosa Mill leaves in rats.

Treatment	Diuretic Activity				
	Dose (mg/kg)	Cumulative urine volume (mL)	Urinary excretion	Diuretic index	Diureticactivity
Group I	Distilled water-2ml/ 100gm)	0.67 ± 0.070	0.98 ± 0.93	1.04 ± 0.01	1.48 ± 0.77
Group II	Furosemide-20ml/1kg	3.64 ± 0.038	5.05 ± 1.13	6.48 ± 0.09	7.05 ± 0.020
Group III	*I. suffruticosa-200*	3.85 ± 0.045	4.13 ± 1.19	6.60 ± 0.050	7.52 ± 0.9
Group IV	*I. suffruticosa-400*	2.82 ± 0.047	4.00 ± 0.095	5.36 ± 0.01	6.95 ± 0.09
Group V	*I. suffruticosa-800*	2.01 ± 0.04	3.68 ± 0.026	4.8 ± 0.06	5.35+0.05

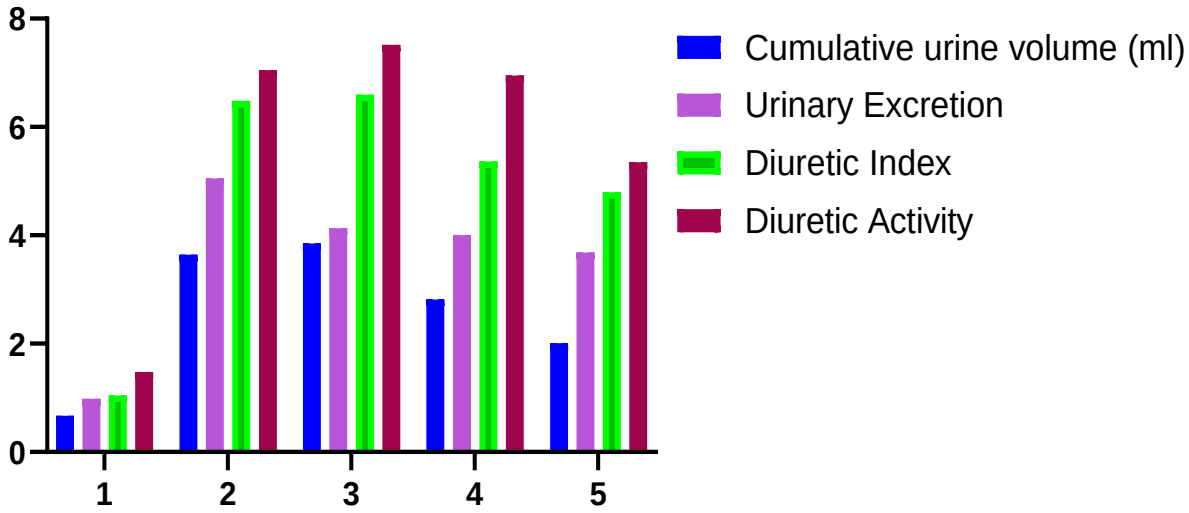

Fig no 12: Diuretic Activity

The result show in cumulative urine volume is (0.67 to 3.85). The urinary excretion is (0.98 to 5.05). The diuretic index is (1.04 to 6.60) and the diuretic activity is (1.48 to 7.52). The aqueous crude leaf extract of I. suffruticosa induced a significant diuretic activity at all tested doses (Table 11). The highest diuretic activity of was observed at 200 mg/kg comparable to the standard drug, Furosemide (20 ml/1kg).

7.4.4 Urinary electrolyte excretion

The hot tea infusion of *I. suffruticosa* leaves induced a significant urinary electrolyte at all tested doses the highest diuretic activity was observed at the dose2ml/100gm to 800mg/kg. The aqueous extract of *I. suffruticosa* leaves induced a significant urine Na^+ excretion was observed in rats that is between 79.22 to 195.64. A significant urine Cl^- excretion was observed in rats that is between 122.24 to 245.55. urine K+ excretion was observed only in rats that received the highest dose, 800 mg/kg. On the other hand, the extract showed a significantnatriuretic effect at all tested doses in table no 13.

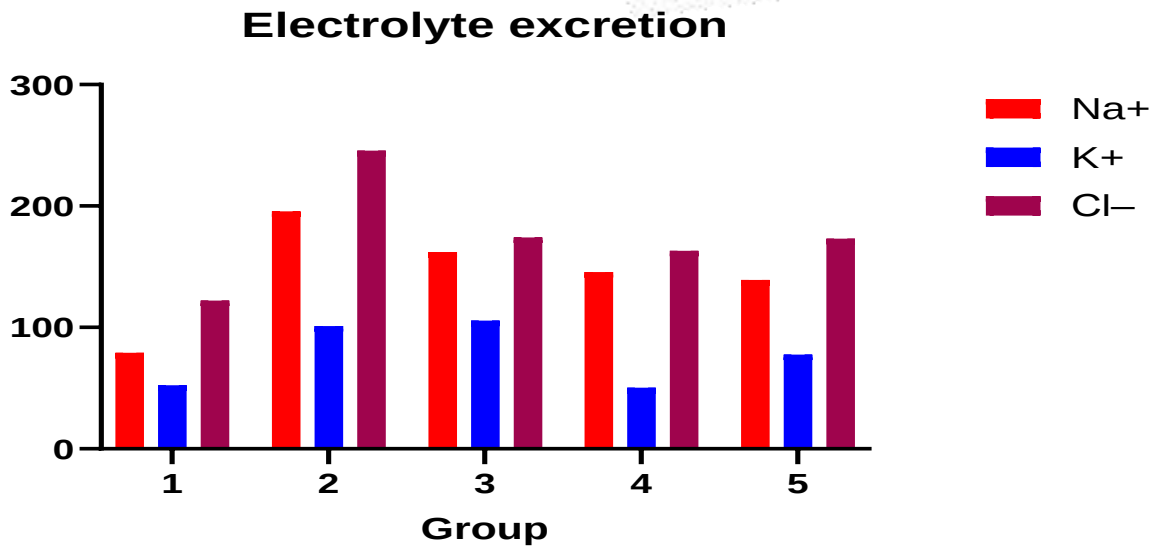

Fig no 13: Electrolyte Excretion

Table no 13: Electrolyte Excretion

Treatment	Electrolyte excretion with SD (±)			
	Dose(mg/kg)	Na+	K+	Cl–
Group I	)istilled water-2ml/ 100gm)	79.22+0.053	52.26 ± .024	122.2 ± 0.063
Group II	urosemide-20ml/1kg	195.6 ± 0.025	101.01 ± 0.041	245.5 ± 0.110
Group III	*ruticosa-200*	162.2 ± 0.011	105.6 ± 0.089	174 ± 0.57
Group IV	*I. suffruticosa-400*	145.5 ± 0.047	50.4 ± 0.080	163.2 ± 0.63
Group V	*ruticosa-800*	139.1 ± 0.056	77.6 ± 0.065	173 ± 0.054

Conclusion:

The outcome of this study implies that the aqueous crude extract of the leaves of *Indigo suffruticosa* are possess a palpable diuretic activity, comparable to that of the standard loop diuretic Furosemide. These findings therefore substantiate the plant's traditional use in the management of hypertension. Never the less, in-depth studies are still obligatory to elucidate the mechanismof action and the active components responsible for the perceived diuretic action for the extract. Therefore, it can be concluded that in future also use full extract for an important role in the development of new pharmaceuticals employing more advanced techniques and materials. The result of extract shows the better result in group III as compared to standard drug Furosemide.

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