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Diuretic activity on different extracts and formulation on aerial parts of *Rumex vesicarius*. Linn

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ABSTRACT

The present study was undertaken to investigate the diuretic activity of Rumex vesicariys.Linn and its formulation in experimental rats. The preliminary phytochemical investigation was carried out to identify the various chemical constituents present in the alcoholic extract. It was found that the Rumex vesicarius contain carbohydrate, glycosides, saponin, steroids, and flavonoids and anthraquinones. The diuretic properties of Rumex vesicarius were evaluated by determination of urine volume, electrolyte concentration,diuretic activity,diuretic action and saluteric index in male albino rats. Different concentrations of Rumex vesicarius benzene and ethanol extract (750mg/kg, 1000mg/kg) were orally administered to hydrated rats & their urine output was immediately measured for 6 hours of treatment. Frusemide (0.10mg/10g) was used as reference drug while Tween 80 solution was used as control. Rumex vesicarius exhibited dose dependent diuretic property. The onset of diuretic action was extremely prompt (with in 1 hour) are lasted through out the study period (up to 6 hours). The result suggests that the ethanol extract (1000mg/ml) of Rumex vesicarius.Linn possess significant diuretic activity.But the formulation(syrup) shows less activity than the Ethanol extract (1000mg/ml),it may be due to the sugar base which is interfering in the urine excretion.

Key Words: - *Rumex vesicarius*, Flavonoids, Tween 80, Furosemide, Diuretic activity and rats.

INTRODUCTION

Diuretics are drugs that increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations. Drug-induced diuresis is beneficial in many life threatening disease conditions such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, hypertension, and pregnancy toxaemi[1,2]. Most diuretic drugs have the adverse effect on quality of life including impotence, fatigue, and weakness. Naturally occurring diuretics include caffeine in coffee, tea, and cola, which inhibit Na + reabsorption and alcohol in beer, wine inhibit secretion of ADH[2]. Many indigenous drugs

have been claimed to have diuretic effect in Ayurvedic system. Among the several plants, *Boerhaaviaverticillata*[3], *Urtica dioica*[4], *Aerva lanata*[5], *Spergularia purpurea*[6], Strychnos potatorum[7] , *Helichrysum bracteatumin*[8] , *Fabiana patagonica*[9], *Cocculus* hirsutus[10] have shown excellent diuretic activity.

Rumex vesicarius Linn. (Polyganaceae) is commonly called as Chukka kura in Telugu, Chukra in Hindi , Bladder Dock in English. It is a pale ,green ,dichotomously branched, succulent herb. Leaves are fleshy, sour, alternate, elliptic-ovate, broadly ovate,entire,acute (or) obtuse,cordate at base, long petiole. Flowers are white,monoecious in terminal and leaf opposed racemes.Perianth lobes 6,in 2 whorls of 3 each stamens 6,cornate at base to form a cup or tube.Ovary is trigonous,unilocular,stigmas are 4.Fruits are nutlets,seeds are erect and trigonous[11]. In literature review it was found that the parts of the plant are used as Diuretic, antiscorbutic, appetiser, astringent, carminative, laxative, stomachic and tonic, and for jaundice[11,12]. The leaves are eaten fresh and much appreciated for their acid taste; it can be added to salad. The plant is considered as excellent pasture to fatten updromedaries and goats. In Marrakech, the powdered seeds are used to treat liver diseases and also as a laxative. In Tissint the fresh leaves are used for jaundice, liver problems, and constipation. In general, the consumption of raw leaves is known to be tonic.In Egypt, the plant is known to be a laxative, stomachic, tonic and analgesic. *Rumex vesicarius* contains Flavonoids, C-glycosides: vitexin, isovitexin, orientin and iso-orientin and anthraquinones: emodin and chrysophanol, rumicine, lapathine, oxalic acid, tannins, mucilage, mineral salts and vitamin C[12,13] .Some workers have reported pharmacological activities like antibacterial and antioxidant activities. However, there are no reports on the diuretic activity of the plant. Hence, the present study was designed to verify the claims of traditional use of the plant.

EXPERIMENTAL SECTION

Plant Material

The Plant were collected from local market in Nalgonda. It was identified and authenticated by Mr. A. Lakshma Reddy, Retired Professor, Dept. of Botany, Nagarjuna Govt. College (Autonomous) Nalgonda. The plant herbarium was prepared and deposited in the Dept. of Pharmacognosy for further reference. The plant was identified as *Rumex vesicarius* Linn.

(Polygonaceae) under the voucher no: NCOPNLG/ph'cog/2010-2011/034.

Preparation of extracts

The collected aerial parts of the plant were washed and dried under the shade. Around 500 g of the coarsely powdered aerial parts of the plant was packed in a soxhlet apparatus and extracted with the benzene and then with ethanol. The extracts so obtained were concentrated under vacuum using rotary vacuum evaporator and dried in dessicator until use.

Animals

Male albino rats were obtained from NIN Hyderabad, India.The experiment was conducted as per the permission of Institutional animal ethical committee bearing Ref.NO: NCOP / IAEC / Approval / 34 / 2011.The animals are uniform in weight between 150 and 200 g were used in the experiments. The animals were placed randomly and allocated to different treatment groups. The animals were housed in polypropylene cages with paddy husk bedding at a temperature of 22 ± 2 5^{o} C and relative humidity of 65 ± 5%. All the animals were allowed free access to water and feed, the standard commercial pelleted chaw (Hindustan liver). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) .

Equipments and Chemicals
Beakers, Burettes, Metaboliccages, Flamephotometery, Silvernitrate, Potassiumchromate, Sodium chloride, Tween 80

Phytochemical analaysis[14,15,16,17]
The extracts of *Rumex vesicarius* were subjected to the phytochemical tests for the presence of carbohydrates, flavonoids, tannins, phenolic compounds, saponins, and steroids.

Diuretic activity[18]
Male albino rats weighing between 200-250g, deprived of water for 16-18hrs before the test drugs are administered. The animals were pre-treated with physiological saline (0.9% NaCl) at an oral dose of 0.15 ml/10 g body weight,to impose a uniform water and salt load.Four animals were kept in each group.

Group 1: Treated with Normal saline
Group 2: Treated with Distilled Water
Group 3: Treated with control(Tween80)
Group 4: Standard(Furosemide 10mg/kg)
Group 5, 6: Ethanol extract of Rumex vesicarius at a dose 750 and 1000mg/kg
Group 7, 8: Benzene extract of Rumex vesicarius at a dose 750 and 1000mg/kg
Group 9: Placebo and Formulation

Immediately after administration of the drugs, animals were placed in metabolic cages individually, to allow the separation of urine and faeces and maintained at room temperature of 25±0.5° c throughout the experiment. During this period no water and food was made available to animals.

Parameters observed were:
- Urine volume
- Electrolyte excretion(Sodium,potassium and chlorine levels)
- Diuretic action: Urinary excretion of test group/urinary excretion of control group.
- Diuretic activity: Diuretic action of extract/Diuretic action of standard
- Saluretic index: mMoles/L of test group/mMoles of control group

The concentrations of Na^+ & K^+ were measured by flame photometry.The concentration of Cl^- was estimated by Argentometric titration with silver nitrate solution (N150) using 3 drops of 5% potassium chromate as indicator

Statistical analysis
Data were expressed as mean±S.E.M (standard error of mean). Statistical analyses were performed with one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Significant differences were set at P values less than 0.01.

Formulation[19]
16.67 gms of sugar was dissolved in sufficient water to get 25ml of concentrated simple syrup.To this concentrated syrup add 10gms of ethanolic extract and stirred to give uniform solution.Then the solution was filtered and stored in an amber coloured bottle in refrigerator for evaluation and diuretic screening

Table 1: Diuretic screening by ethanol and benzene extract of *Rumex vesicarius. Linn*

Groups	1hr	2hr	3hr	4hr	5hr	6hr	Diuretic action	Diuretic activity
Normal saline	0.44±0.018	0.87±0.017	1.16±0.015	1.21±0.01	1.26±0.0085	1.28±0.013	0.94	0.50
Distilled water	0.50±0.014	0.92±0.018	1.23±0.014	1.31±0.011$^{\#}$	1.31±0.011	1.34±0.011	0.99	0.53
Control	0.54±0.009$^{\#}$	0.95±0.015	1.25±0.012	1.32±0.012$^{\#}$	1.32±0.012	1.35±0.013		
Standard	0.97±0.004$^{\#*\wedge}$	1.86±0.006$^{\#*\wedge}$	2.39±0.081$^{\#*\wedge}$	2.5±0.084$^{\#*\wedge}$	2.53±0.091$^{\#*\wedge}$	2.53±0.077$^{\#*\wedge}$	1.86	
Benzene (750mg/kg)	0.67±0.018$^{\#*\wedge\$<>}$	0.83±0.034$^{\wedge\$<>}$	1.21±0.009$^{\$<>}$	1.26±0.0175$^{\$<>}$	1.32±0.012$^{\$<>}$	1.35±0.013$^{\$<>}$	1	0.53
Benzene (1000mg/kg)	0.7298±0.019$^{\#*\wedge\$<>}$	0.98±0.011$^{\#\$<>\&}$	1.31±0.025$^{\#\$<>}$	1.42±0.022$^{\#*\$<>\&}$	1.47±0.021$^{\#*\wedge\$<>\&}$	1.5±0.0193$^{\$<>}$	1.1	0.59
Ethanol (750mg/kg)	1.21±0.009$^{\#*\wedge\$}$	2.74±0.004$^{\#*\wedge\$}$	3.25±0.008$^{\#*\wedge\$}$	3.41±0.013$^{\#*\wedge\$}$	3.48±0.006$^{\#*\wedge\$}$	3.5±0.0091$^{\#*\wedge\$}$	2.59	1.39
Ethanol (1000mg/kg)	1.53±0.017$^{\#*\wedge\$<}$	2.82±0.02$^{\#*\wedge\$}$	3.75±0.047$^{\#*\wedge\$<}$	3.84±0.0178$^{\#*\wedge\$<}$	3.92±0.029$^{\#*\wedge\$<}$	4.2±0.147$^{\#*\wedge\$<}$	3.11	1.67
Placebo	0.42±0.017$^{\wedge\$<>\&@}$	0.64±0.021$^{\#*\wedge\$<>\&@}$	0.98±0.014$^{\#*\wedge\$<>\&@}$	1.01±0.010$^{\#*\wedge\$<>\&@}$	1.03±0.017$^{\#*\wedge\$<>\&@}$	1.05±0.021$^{\#*\wedge\$<>\&@}$	0.77	0.41
Formulation (syrup)	0.84±0.022$^{\#*\wedge\$<>\&@!}$	1.98±0.038$^{\#*\wedge\$<>\&@!}$	3.01±0.029$^{\#*\wedge\$<>\&@}$	3.24±0.047$^{\#*\wedge\$<>\&@!}$	3.52±0.024$^{\#*\wedge\$>\&@!}$	3.7±0.021$^{\#*\wedge\$\&@!}$	2.74	1.47

*# Normal saline, * Disstilled water ,^ Control,$ Std,< Ethanol 750,> Ethanol 1000,& Benzene 750,@ Benzene 1000,! Placebo; n=4,P<0.01,Data expressed in Mean±S.E.M performed with ANOVA followed by Tukey's multiple comparison test .*

Table 2 : Effect of ethanolic, benzene extracts and formulation on Urinary electrolyte excretion in rats

Groups	**Na^+**	**K^+**	**Cl^-**	**Saluteric index**			**Na/k**
				Na^+	**K^+**	**Cl^-**	
Normal saline	0.014±0.0008	0.0053±0.00008	235.7±0.85	1	0.92	0.84	0.84
Distilledwater	0.012±0.0007	0.0054±0.00016	45.7±0.85$^{\#}$	0.857	0.94	0.16	2.22
Control	0.014±0.0008	0.0057±0.0001	277.7±0.85$^{\#*}$				2.482
Standard	0.0173±0.0007	0.007±0.000060	299.2±3.14$^{\#*}$	1.209	1.215	1.074	2.444
Benzene(750mg/ml)	0.00326±0.000085$^{\#\wedge\$<>}$	0.0051±0.00008$^{<>}$	490.2±0.85$^{\#*\wedge\$<>}$	0.227	0.888	1.765	0.636
Benzene(1000mg/ml)	0.01413±0.0007$^{>\&}$	0.0038±0.000087$^{\$<>}$	405±1.29$^{\#*\wedge\$<>\&}$	0.988	0.667	1.45	3.673
Ethanol (750mg/ml)	0.023±0.0012	0.01±0.0006$^{\#*\wedge\$}$	289.2±24.7$^{\#*}$	1.610	1.875	1.04	2.132
Ethanol(1000mg/ml)	0.029±0.0012$^{\#*\wedge\$}$	0.014±0.0008$^{\#*\wedge\$<}$	323.7±0.85$^{\#*\wedge}$	2.048	2.479	1.165	2.051
Placebo	0.0069±0.000064$^{<>}$	0.0024±0.0000085$^{\#*\wedge\$<>\&}$	59.2±1.10$^{\#\wedge\$<>\&@}$	0.482	0.429	0.213	2.78
Formulation(syrup)	0.02±0.0067$^{*\&!}$	0.014±0.000075$^{\#*\wedge\$<\&@!}$	148.5±0.64$^{\#*\wedge\$<>\&@!}$	1.39	2.51	0.534	1.379

*# Normal saline, * Disstilled water ,^ Control,$ Std,< Ethanol 750,> Ethanol 1000,& Benzene 750,@ Benzene 1000,! Placebo; n=4,P<0.01,Data expressed in Mean±S.E.M performed with ANOVA followed by Tukey's multiple comparison test*

The syrup was evaluated for :

- Colour
- Odour
- PH
- Specific gravity
- Viscosity

RESULTS AND DISCUSSION

Phytochemical screening:

The qualitative phytochemical analysis of *Rumex vesicarius* shows the presence of Steroids, Flavonoids, Anthraquinones, C-glycosides, Saponins.

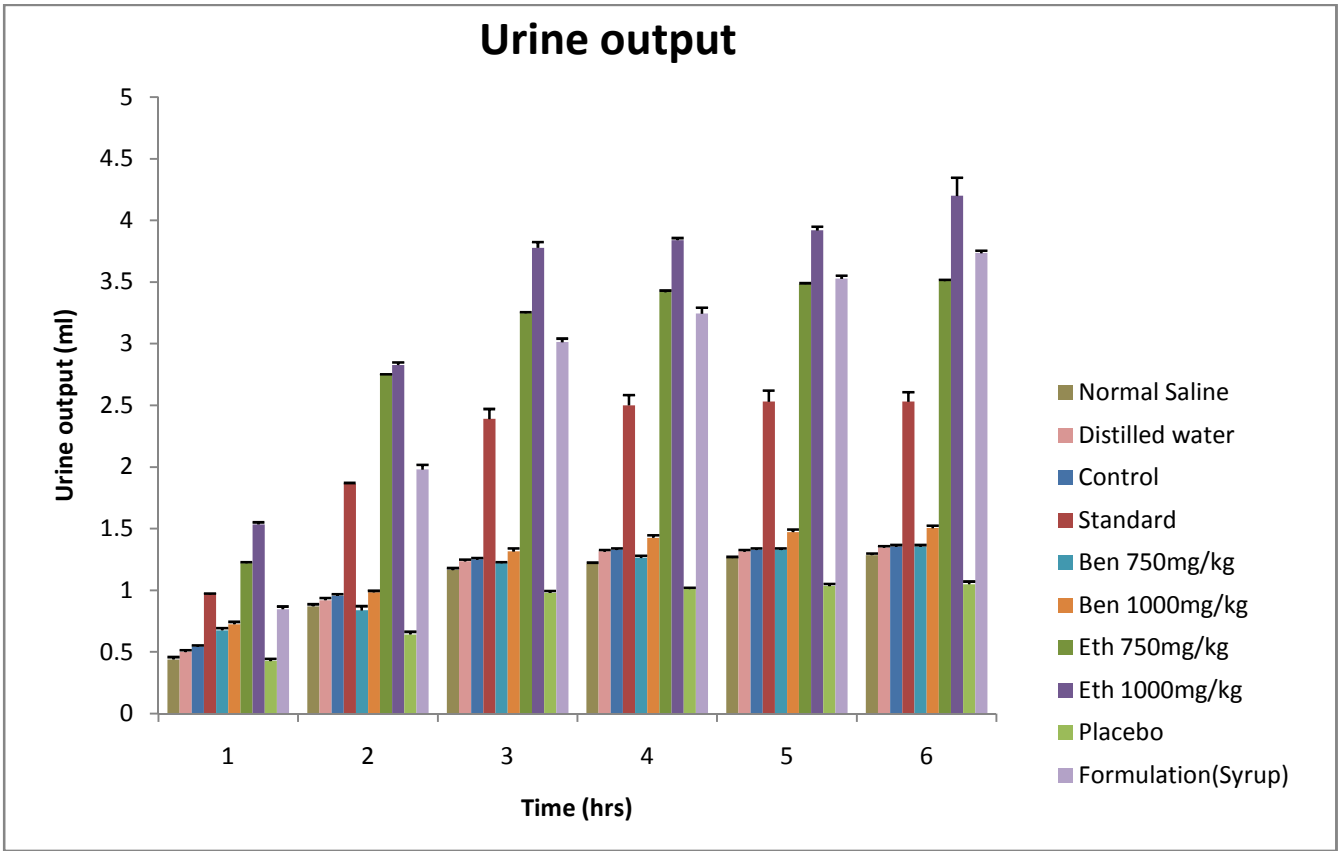

Figure 1 : Urinary excretion by different extracts and formulation of *Rumex vesicarius*. Linn

Time dependent increase in urinary excretion was seen in all the groups. And among the all groups screened ethanol extract(500and1000mg/ml)exhibited high urinary excretion more than the standard(Furosemide drug). The formulation exhibited significant urinary excretion with that of the ethanol extract. Placebo does not effect the urinary excretion.The diuretic action exhibited more in ethanol extract of *Rumex vesicarius*(1000mg/ml) than the standard furosemide.

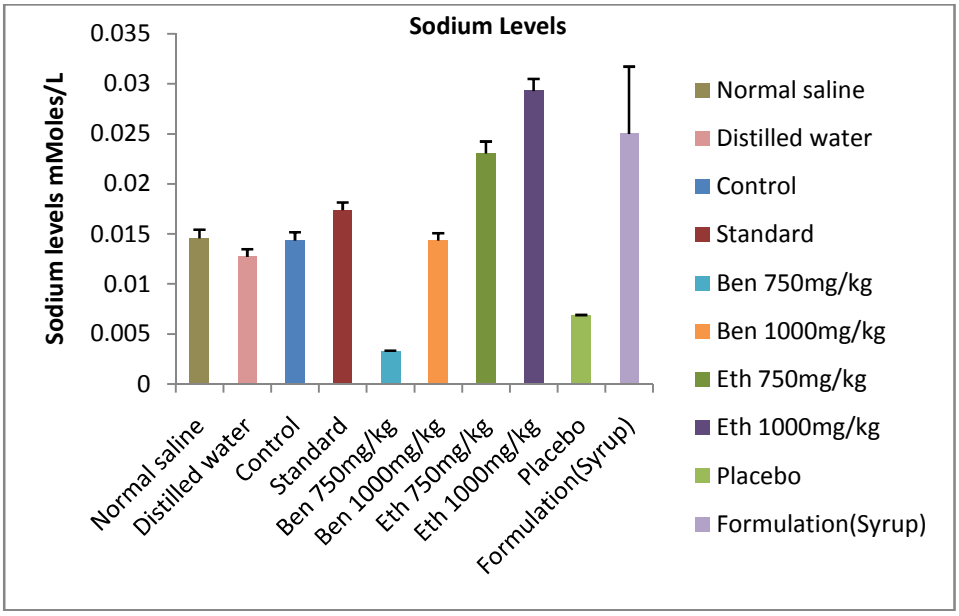

Figure 2 : Sodium excretion in the urine by different extracts and formulation of *Rumex vesicarius*. Linn

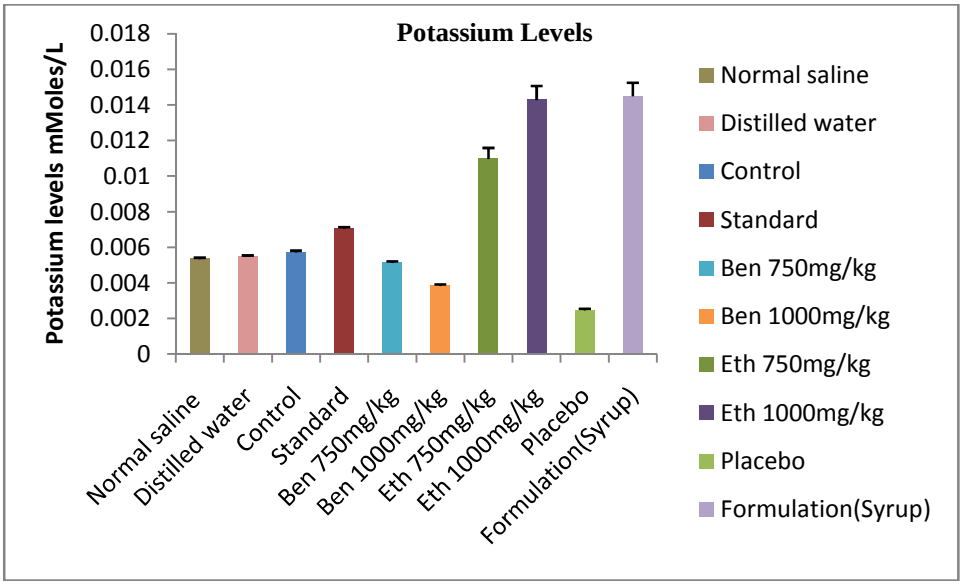

Figure 3 : Potassium excretion in the urine by different extracts and formulation of *Rumex* vesicarius. Linn

Dose dependent urinary excretion of Sodium and Potassium ions were seen with Benzene and Ethanol extract of *Rumex vesicarius* administration. But ethanol extract of *Rumex vesicarius* exhibited urinary Sodium and Potassium excretion in rats more than the standard.Whereas the formulation exhibited similar urinary sodium and potassium ions excretion as that of the ethanol extract of *Rumex vesicarius*.Linn at (1000mg/kg body weight).

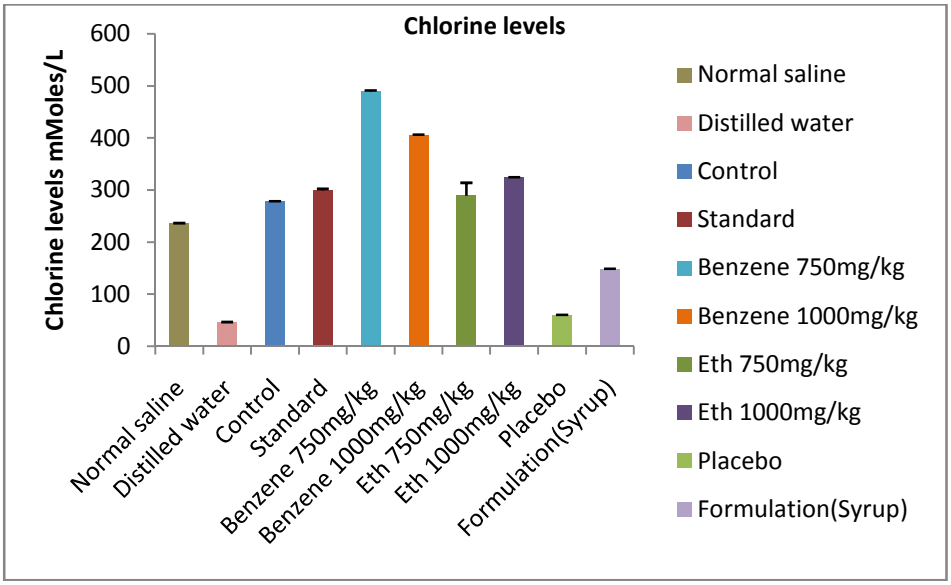

Figure 4 : Chlorine excretion in the urine by different extracts and formulation of *Rumex vesicarius*. Linn

Dose dependent urinary excretion of chlorine was seen in ethanol extract of *Rumex vesicarius*.Linn and more than the standard furosemide. Whereas in benzene extract of *Rumex vesicarius*.Linn the chlorine levels are higher than the ethanol extract it may be due to the chemical constituents. These chemical constituents may inhibit the reabsorption of chlorine with the benzene extract.

Evaluation of Syrup:

Table 3 : Evaluation of physical parameters for formulation syrup of *Rumex vesicarius*. Linn ethanol extract

Parameters	Formulated syrup
Specific gravity	1.06
PH	3
Viscosity	2.83cps
Colour	Brownish black
Odour	Charateristic

Drug content in syrup

Table 4 : Drug content in 0.1N Hcl buffer

Absorbance (222nm)	Conc (µg/ml)	Amount(mg)
0.032	0.743	0.367
0.034	0.809	0.4

The amount of drug present in 1ml of syrup is 0.3835±0.0165

Table 5 : Drug content in PBS P^H 6.8

Absorbance (222nm)	Conc (µg/ml)	Amount(mg)
0.835	7.78	0.389
0.843	7.86	0.393

The amount of drug present 1ml of syrup is 0.3965±0.0075

Both the ethanol and benzene extract of *Rumex vesicarius*.Linn exhibited a dose-dependent and time dependent increase in urine excretion.With respect to the ethanol extract of *Rumex vesicarius*.Linn ,the maximum increase in urinary excretion was produced at 1000mg/ml with a

value of 4.2ml compared while the Benzene extract(1000mg/ml) showed 1.5ml at 6hrs.This is comparable with the loop diuretic furosemide.

The diuretic effect of the ethanol extract of *Rumex vesicarius*.Linn was generally high and qualitatively similar to that of furosemide,which clearly shows that the ethanol extract of *Rumex vesicarius*.Linn has a potential to induce diuresis markedly as those of known synthetic diuretic like furosemide.The ethanol extract of *Rumex vesicarius*.Linn induces the urinary output accompained by a corresponding increase in Na^+ ,K^+ ,Na^+ / K^+ ratio.Collectively these observations suggest that it is acting as an osmotic diuretic.Osmotic diuretic are usually given intravenously and are pharmacologically inert.

The *Rumex vesicarius*.Linn is orally active and contains flavonoids,anthraquinones and C-glycosides, Saponins which have other biological effects.ADH plays a vital role in the regulation of urinary output.The extract of *Rumex vesicarius*.Linn may stimulate diuresis by inhibiting ADH release or its action on the uriniferous tubules or it could produce diuresis by stimulating the release of endogenous natriuretic peptides,which promote sodium and water secretion.It promotes an increase in natriuresis and kaleuresis with its diuretic action[20].

Therefore the plant was acting as a loop diuretic.Loop diuretics are the most powerful of all diuretics and they inhibit the Na^+,K^+,$2Cl^-$ co-transporter system of the ascending limb of Henle's loop.The Ethanol extract is not associated with a reduction in urinary K^+ levels,unlike some plant extracts that have been reported to have an interesting K^+ saving effect,suggesting that this plant was not acting as potassium sparing diuretic[18].

In ethanol extract of *Rumex vesicarius*.Linn it should be pointed out that the water soluble salts are not present in the extract in sufficient amount as they have poor solubility in such solvent.Hence such water soluble solutes do not interfere with the urinary excretion.Thus the notable diuretic effect produced by the ethanol extract of *Rumex vesicarius*.Linn was reaffirimed that the diuretic activity of *Rumex vesicarius*.Linn was not due to its content of potassium salt rather it was due to intrinsic ability of the plants phytoconstituents to exert the effect.

Diuretic effect may be produced by stimulation of regional blood flow or initial vasodilation or by producing inhibition of tubular reabsorption of water and anions[18,20]. The increased sodium and water excretion activity also provide strong basis for its proved anti-hypertensive action.

All the physical evaluation parameters like viscosity,specific gravity,colour ,odour, PH were observed shown in the table 3.And the drug content for the syrup was observed in the stomach buffer(0.1N Hcl) and PBS buffer at PH 6.8.And the results are in Mean±SD given in tables 4 and 5 for formulation syrup were within ranges

Formulation exhibits the diuretic activity similar to that of the ethanol extract of *Rumex vesicarius*.Linn but slight decrease with the ethanol extract(1000mg/kg)it may be due to sugar solution base. Further scope must be kept on the suitable formulation development other than the syrups

CONCLUSION

Diuretic activity was performed for the Benzene and Ethanol extracts of *Rumex vesicarius*.Linn.The ethanol extract exhibited very good urine output, electrolyte excretion,

Diuretic action and Diuretic activity higher than that of the standard furosemide.It may be due to the presence of Flavonoids. Formulation (Simple syrup) exhibited better diuretic activity when compared with the standard.

Finally it was concluded that the plant *Rumex vesicarius*.Linn proved to be a potential nutraceutical diuretic.And further scope has to explored for suitable formulation other than the syrups.

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