DIURETIC POTENTIAL OF VARIOUS EXTRACTS OF *OXYSTELMA ESCULENTUM* AND ITS PRELIMINARY PHYTOCHEMICAL SCREENING

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Summary

Oxystelma esculentum is a perennial twiner growing near water-logged areas in the Indian subcontinent. Diuresis is one of its traditional uses. The present work deals with the investigation of diuretic potential of various extracts of *O. esculentum*. The plant was successively extracted with petroleum ether, chloroform, methanol and water, which served as the test extracts. Various parameters of diuresis like Lipschitz value, urinary excretion, diuretic action, diuretic activity, saluretic activity and natriuretic activity were measured using appropriate animal models. The petroleum ether extract was found to possess the most effective diuretic activity, thereby supporting the traditional claim of the plant as a diuretic. Phytochemical screening of this extract revealed the presence of important classes of phytoconstituents like cardenolides, flavonoids, phenolics, sterols and triterpenoids. This bioactivity-guided phytochemical screening can pave the way for further therapeutic investigations and isolation of therapeutically important compounds from *Oxystelma esculentum*.

Key words: *Oxystelma esculentum*, *Oxystelma secamone*, *Periploca esculenta*, Asclepiadaceae, diuretic.

Introduction

Oxystelma esculentum R. Br. syn. *Oxystelma secamone* Linn., *Periploca esculenta* Roxb., *Periploca secamone* Linn., *Sarcostemma secamone* Bennet, *Sarcostemma esculentum* Linn., *Asclepias rosea* R. Br., is a perennial twiner found throughout the plains of the Indian sub-continent near water-logged areas [1]. The plant is used as diuretic, laxative, antiseptic, depurative, anthelmintic, antiulcer, aphrodisiac, hepatoprotective and useful in leucoderma and bronchitis. Decoction of plant is used in ulcer, sore-throat and itches. Milky juice is used as galactogogue, antiperiodic, antiulcer and as a vulnerary. Leaves are used as antiperiodic. Its root is prescribed in jaundice. Fruit is bitter, tonic, expectorant, anthelmintic. Fruit juice is used in muscle pain, gonorrhoea, cough and leucoderma, and given to children as astringent [2,3]. The present work deals with not only ascertaining the diuretic effect of the plant, but also finding the most potent extract and performing its phytochemical screening, so as to guide further fractionation and isolation of therapeutically potent phytoconstituents from this plant.

Methods

Collection and authentication

Oxystelma esculentum in flowering & fruiting stage was collected from Barda Hills near Porbandar, Gujarat, India, in October 2008. Herbarium of the collected sample was prepared and deposited in Department of Pharmacognosy, RK College of Pharmacy (No. RKCP/COG/01/2008). Authentication was done by Dr. N. R. Sheth, Head of Department of Pharmaceutical Sciences, Saurashtra University.

Preparation of extracts

Successive extraction of 1kg powder of the entire plant was carried out using four solvents in the decreasing order of their polarity index: petroleum ether, chloroform, methanol and distilled water. Complete extraction of the powder with each solvent was carried out in round-bottom flask at a temperature <50°C. The yield of the dried extracts was found to be 10.1%w/w, 8.5%w/w, 7.5%w/w and 14.1%w/w respectively. Their concentrations were adjusted in the solvents according to their dose.

For investigation of each activity, the experimental animals were divided into six groups, with six animals in each group: Normal control , Standard (Furosemide), Petroleum Ether extract, Chloroform extract, Methanol extract, Aqueous extract.

Pharmacological study

The pharmacological study was approved by the Institutional Animal Ethics Committee (RKCP/COG/RP/10/06) and carried out according to CPCSEA guidelines.

All animals were maintained under environmentally controlled conditions of 24±1°C and 12h-light and 12h-dark cycle. The animals were acclimatized to laboratory conditions for 1 month before starting the pre-clinical trials. All studies were performed under standard conditions of temperature, light, humidity and noise.

Wistar rats of either sex weighing 200–220g were fed with standard diet and water *ad libitum*. Fifteen hours prior to the experiment, food and water were withdrawn. Three animals per group were placed in one metabolic cage (each cage is provided with a wire mesh at the bottom and a funnel to collect the urine; stainless-steel sieves are placed in the funnel to retain feces and to allow the urine to pass). Normal control group received normal saline (25ml/kg). Standard control group received 1g/kg Furosemide (Lasix, Aventis Pvt. Ltd.) orally [4]. Two groups of three animals were used for each dose of the test extract. Three animals of the test extract groups received orally a dose of 200mg/kg and the remaining three animals from each of these groups received dose of 400mg/kg body weight [5]. No food or water was given during this study. Urine excretion was recorded after 5h (Table 1, Fig. 1) and 24h (Table 2, Fig. 2). The urine was analyzed by flame photometry for sodium and potassium ions and argentometrically for chloride ions at 5h (Table 3, Fig. 3) and 24h (Table 4, Fig. 4) [6]. The instrument was calibrated with standard solutions containing different concentrations of sodium, potassium and chloride [7].

Following parameters were calculated for each test extract [5]:

1. Lipschitz Value: [Urine volume excreted by test / Urine volume excreted by standard]
2. Urinary excretion: [(Total urinary output / Total liquid administered) X 100]
3. Diuretic action: [(Urinary excretion in test / Urinary excretion in standard) X 100]
4. Diuretic activity: [(Diuretic action of test / Diuretic action of standard) X 100]
5. Saluretic activity: [$Na^+ + Cl^-$]
6. Natriuretic activity: [Na^+/K^+]
7. Carbonic anhydrase inhibition: [$Cl^- / (Na^+ + K^+)$]

Results were calculated as Mean ± Standard Deviation (SD). Statistical analysis of control and test data was performed by One-way ANOVA followed by Dunnett's test (Sigma-stat software). A probability value of $p<0.01$ was considered statistically significant.

Phytochemical screening

Petroleum ether extract was found to have the most potent diuretic activity. This extract was subjected to a detailed phytochemical screening involving established methods for detecting various classes of phytoconsituents (Table 5) [8-13].

Results

Table 1. Diuretic activity of various extracts of *O. esculentum* at 5h

Groups	UV (ml)	LV	UE	DA_1	DA_2
Normal control	0.3±0.1	--	2.00	--	--
Standard (Furosemide)	1.8±0.2	--	60.00	30.00	--
Pet Ether ext 200mg/kg	1.7±0.1	0.94 ± 0.13	113.33	56.67	1.89
Pet Ether ext 400mg/kg	3.2±0.1	1.78 ± 0.23	106.67	53.33	1.78
Chloroform ext 200mg/kg	1.2±0.2	0.67 ± 0.08	80.00	40.00	1.33
Chloroform ext 400mg/kg	1.6±0.3	0.89 ± 0.11	53.33	26.67	0.89
Methanol ext 200mg/kg	1±0.2	0.56 ± 0.07	66.67	33.33	1.11
Methanol ext 400mg/kg	1.3±0.3	0.72 ± 0.09	43.33	21.67	0.72
Aqueous ext 200mg/kg	1.1±0.1	0.61 ± 0.08	73.33	36.67	1.22
Aqueous ext 400mg/kg	1.4±0.1	0.78 ± 0.10	46.67	23.33	0.78

Values are expressed as mean ± SD
Number of animals (n) = 6

Table 2. Diuretic activity of various extracts of *O. esculentum* at 24h

Groups	UV (ml)	LV	UE	DA_1	DA_2
Normal control	1.5±0.1	--	10.00	--	--
Standard (Furosemide)	5.5±0.3	--	183.33	18.33	--
Pet Ether ext 200mg/kg	5.5±0.2	1.00 ±0.06	366.67	36.67	2.00
Pet Ether ext 400mg/kg	9.5±0.2	1.73 ±0.10	316.67	31.67	1.73
Chloroform ext 200mg/kg	4±0.4	0.73 ±0.04	266.67	26.67	1.45
Chloroform ext 400mg/kg	4.5±0.5	0.82 ±0.05	150.00	15.00	0.82
Methanol ext 200mg/kg	3.5±0.4	0.64 ±0.03	233.33	23.33	1.27
Methanol ext 400mg/kg	4±0.6	0.73 ±0.04	133.33	13.33	0.73
Aqueous ext 200mg/kg	3.7±0.5	0.67 ±0.04	246.67	24.67	1.35
Aqueous ext 400mg/kg	4.2±0.6	0.76 ±0.05	140	14.00	0.76

Values are expressed as mean ± SD
Number of animals (n) = 6

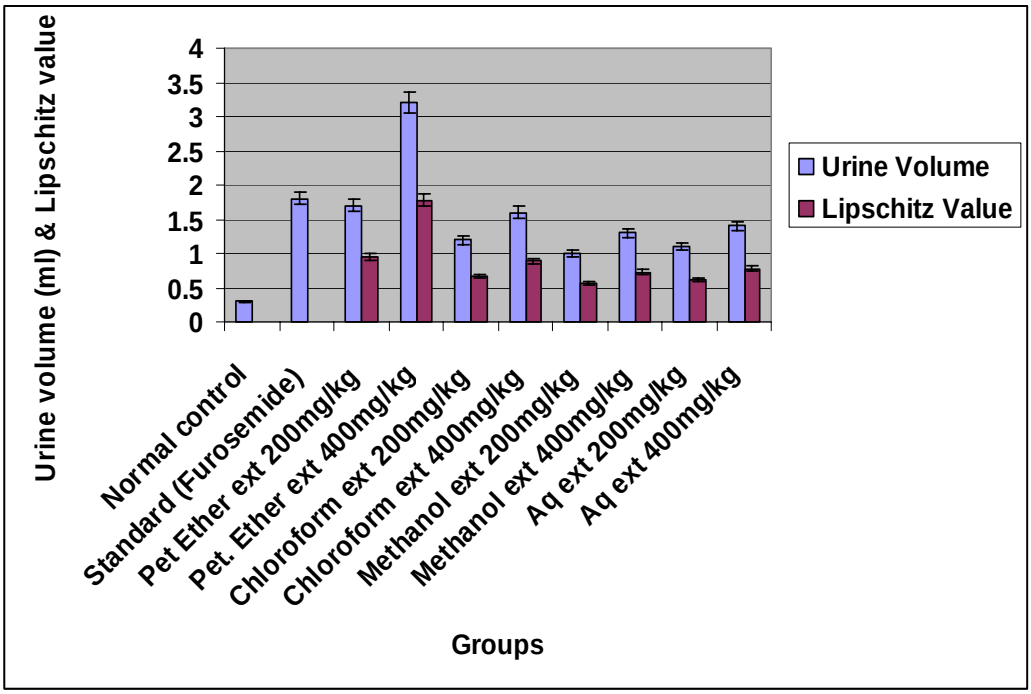

Fig. 1. Comparison of diuretic potential of various extracts (at 5h)

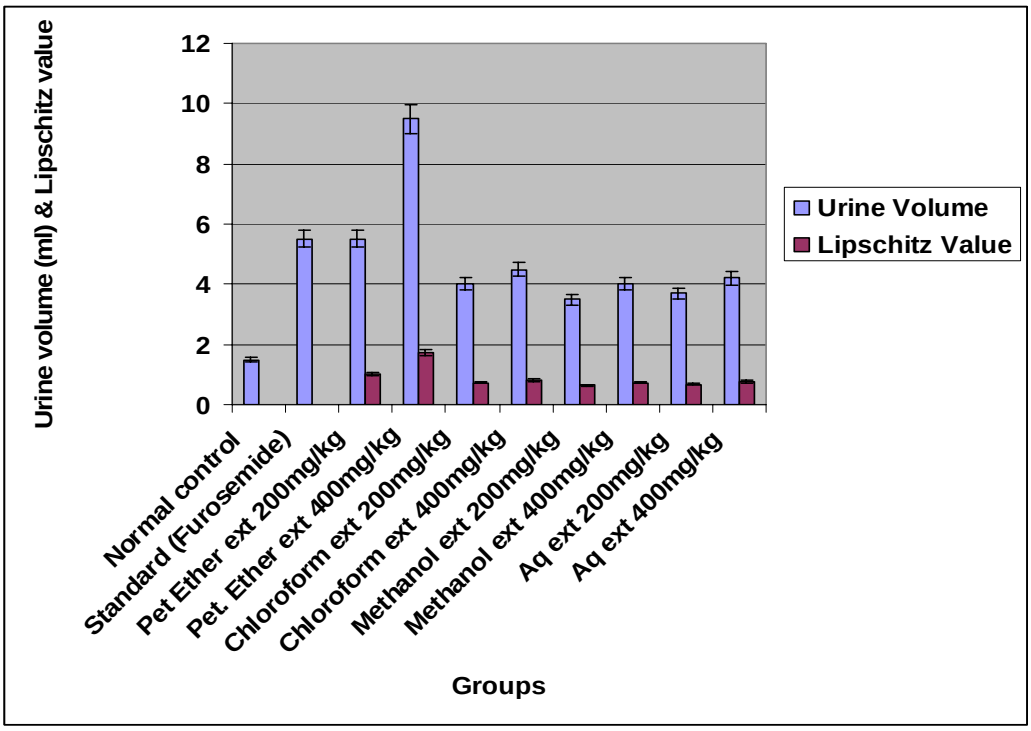

Fig. 2. Comparison of diuretic potential of various extracts (at 24h)

Table 3. Electrolytes excretion, saluretic & natriuretic activity at 5h

Groups	Na^+	K^+	Cl^-	SA	NA	CAI
Normal control	61.1 ±1.5	48.7 ±0.9	141.3 ±1.5	202.4 ±3.0	1.25 ±0.01	1.29 ±0.01
Standard (Furosemide)	204.5 ±1.4	85.5 ±1.1	271.1 ±1.5	475.6 ±2.9	2.39 ±0.02	0.93 ±0.01
Pet Ether ext 200mg/kg	205.8 ±1.5	80.9 ±1.1	272.7 ±1.3	478.5 ±2.8	2.54 ±0.02	0.95 ±0.01
Pet Ether ext 400mg/kg	219.7 ±1.5	86.1 ±1.3	297.5 ±1.3	517.2 ±2.8	2.55 ±0.01	0.97 ±0.01
Chloroform ext 200mg/kg	181.2 ±2.1	85.2 ±1.9	240.7 ±2.2	421.9 ±4.2	2.13 ±0.02	0.90 ±0.01
Chloroform ext 400mg/kg	198.3 ±2.1	93.4 ±1.9	264.4 ±2.1	462.7 ±4.2	2.12 ±0.02	0.91 ±0.01
Methanol ext 200mg/kg	175.4 ±1.8	77.7 ±1.6	230.8 ±1.5	406.2 ±3.3	2.26 ±0.02	0.91 ±0.01
Methanol ext 400mg/kg	194.7 ±1.7	86.1 ±1.7	259.5 ±1.8	454.2 ±3.5	2.26 ±0.01	0.92 ±0.01
Aqueous ext 200mg/kg	163.4 ±1.6	69.7 ±1.5	203.4 ±1.5	366.8 ±3.1	2.34 ±0.02	0.87 ±0.01
Aqueous ext 400mg/kg	182.3 ±1.4	77.6 ±1.5	235.8 ±1.5	418.1 ±2.9	2.35 ±0.01	0.91 ±0.01

Values are expressed as mean ± SD
Number of animals (n) = 6

Table 4. Electrolytes excretion, saluretic & natriuretic activity at 24h

Groups	Na^+	K^+	Cl^-	SA	NA	CAI
Normal control	70.4 ±1.5	58.1 ±1.4	166.3 ±1.5	236.7 ±3.0	1.21 ±0.01	1.29 ±0.01
Standard (Furosemide)	180.6 ±1.2	85.1 ±1.3	250.1 ±1.5	430.7 ±2.7	2.12 ±0.01	0.94 ±0.01
Pet Ether ext 200mg/kg	182.1 ±1.7	82.1 ±1.5	259.7 ±1.8	441.8 ±3.5	2.22 ±0.02	0.98 ±0.01
Pet Ether ext 400mg/kg	196.7 ±1.7	87.1 ±1.4	278.8 ±1.7	475.5 ±3.4	2.26 ±0.01	0.98 ±0.01
Chloroform ext 200mg/kg	163.3 ±2.1	87.3 ±1.9	213.2 ±1.8	376.5 ±1.9	1.87 ±0.01	0.85 ±0.01
Chloroform ext 400mg/kg	177.7 ±2.2	95.8 ±1.9	234.2 ±2.0	411.9 ±4.2	1.85 ±0.01	0.86 ±0.01
Methanol ext 200mg/kg	155.6 ±1.6	79.3 ±1.6	194.3 ±1.8	349.9 ±3.4	1.96 ±0.02	0.83 ±0.01
Methanol ext 400mg/kg	167.1 ±1.6	86.2 ±1.5	207.1 ±1.7	374.2 ±3.3	1.94 ±0.01	0.82 ±0.01
Aqueous ext 200mg/kg	153.1 ±1.3	75.6 ±1.1	191.1 ±1.5	344.2 ±2.8	2.03 ±0.02	0.84 ±0.01
Aqueous ext 400mg/kg	163.9 ±1.3	80.2 ±1.3	208.5 ±1.6	372.4 ±2.9	2.04 ±0.02	0.85 ±0.01

Values are expressed as mean ± SD
Number of animals (n) = 6

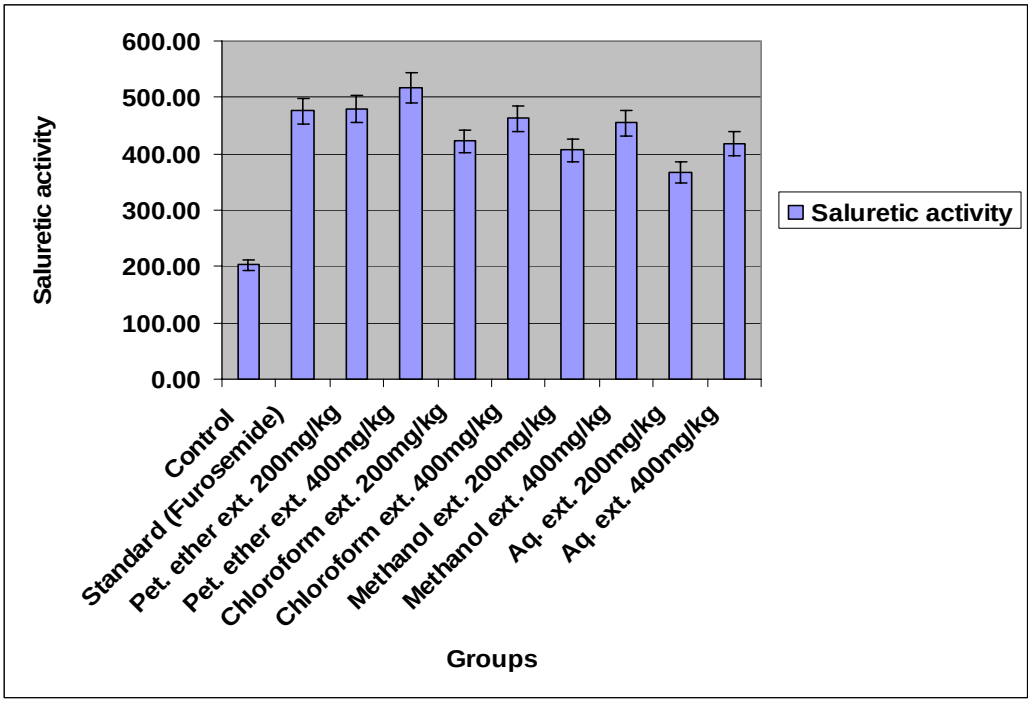

Fig. 3. Comparison of Saluretic activity (at 5h)

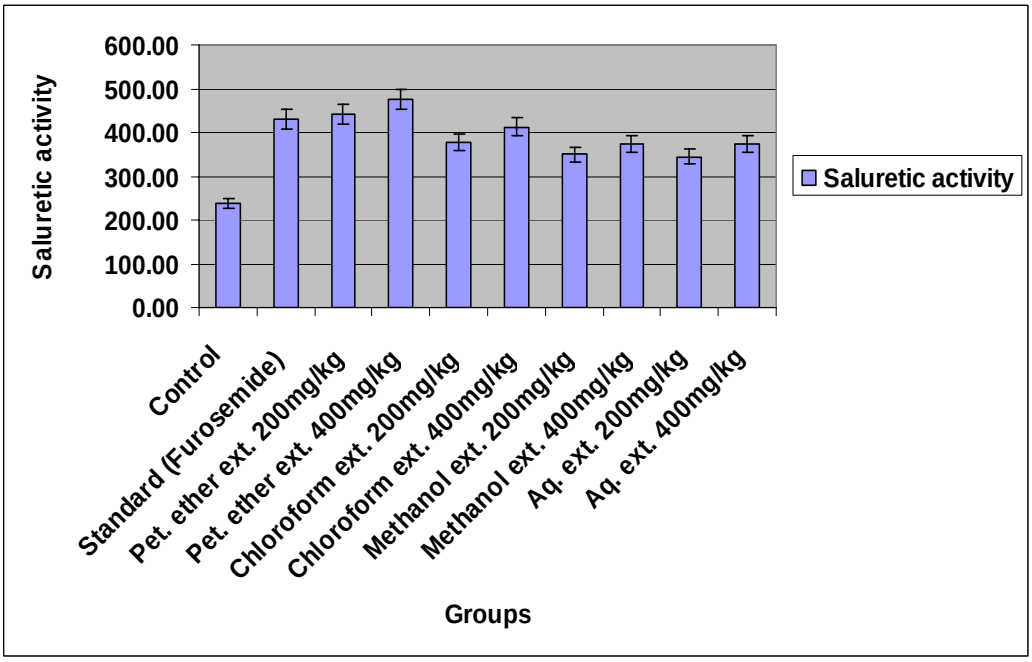

Fig. 4. Comparison of Saluretic activity (at 24h)

Table 5. Phytochemical screening of petroleum ether extract

Phytoconstituent	Test	Result
Alkaloids	Dragendorff's test	-ve
	Wagner's test	-ve
	Hager's test	-ve
	Mayer's test	-ve
Flavonoids	Shinoda test	+ve
	Fluorescence test	+ve
Phenolics	Ferric chloride test	+ve
	Folin ciocalteu test	+ve
Sterols and triterpenoids	Libermann Burchardt test	+ve
	Salkowski test	+ve
Carotenoids	Antimony trichloride test	-ve
Cardenolides	Kedde's test	+ve
	Baljet's test	+ve
	Legal's test	+ve

Discussion

The present study shows that all extracts of *Oxystelma esculentum* have diuretic potential in comparison with furosemide, of which the petroleum ether extract has the most potent diuretic activity. The petroleum ether extract caused a significant increase in urine and electrolytes excretion. Lipschitz value of more than 1.0 indicates it to be a good diuretic agent, whereas Lipschitz value of around 1.8 of pet. ether extract dose 400mg/kg indicates a potent saluretic effect. $[Na^+/K^+]$ values of more than 2.0 indicate a potent natriuretic effect. $[Cl^- / (Na^+ + K^+)]$ values are greater than 0.8, thereby eliminating the possibility of carbonic anhydrase inhibition. Excretion of electrolytes indicates that the plant can be used for the treatment of edema, congestive heart failure & hypertension [14]. The data was found statistically significant compared with control. This proves the traditional claims of this plant as a potent diuretic drug. Phytochemical screening of petroleum ether extract revealed the presence of cardenolides, flavonoids, phenolics, sterols and triterpenoids, which may be responsible for the diuretic effect. This bioactivity-guided phytochemical analysis can serve as a vital guide for further study of therapeutic effects and isolation of therapeutically important compounds from *Oxystelma esculentum*.

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