AQUA LOSS
Product Highlights

- *Maximum Strength Natural Diuretic†*
- *Effective Same Day Water Loss†*
- *Helps Reduce Bloat and Enhance Definition†*
- *With Key Electrolytes for Healthy Fluid Balance†*
- *Safe & Effective Results†*
- *Works for Men & Women†*
- *Stimulant-Free*

AQUA LOSS is a fast-acting natural diuretic that helps to eliminate excess water from the body. It helps reduce bloat and enhance definition by supporting subcutaneous water loss (from beneath the skin). AQUA LOSS contains key electrolytes to help maintain muscle strength and fullness.†

WARNING: This product is intended to be used by normal healthy adults and should not be taken by anyone with any known medical conditions. Do not use if pregnant or nursing. **KEEP OUT OF REACH OF CHILDREN.**

Nutrex® RESEARCH

LIPO6

AQUA LOSS™

MAXIMUM STRENGTH WATER LOSS†

NATURAL DIURETIC†

REDUCES WATER & BLOAT†

Dietary Supplement
80 Capsules

SUPPLEMENT FACTS

Serving Size: 4 Capsules **Servings per Container: 20**

	Amount per serving	% DV
Magnesium (as Magnesium Taurate)	11mg	2%
Potassium (from Potassium Glycinate Complex)	63mg	<2%
Dandelion Root Extract	1g	*
Oxystelma Esxulentum (aerial parts)	750mg	*
Horsetail Extract (whole plant)	400mg	*
Uva-Ursi Leaf	375mg	*

*Daily Value not established.

OTHER INGREDIENTS: Hypromellose (Vegetable Capsule), Magnesium Stearate, Silica, Titanium Dioxide.

RECOMMENDED USE: Take 4 capsules twice a day with 8 oz of water, morning and afternoon. We suggest using this product for 5 consecutive days to achieve your desired loss of excess subcutaneous water. Drink at least 6-8 glasses of water a day. Do not exceed recommended dosage.

Regular exercise and proper nutrition are essential for achieving your weight loss & physique goals. As individuals vary so may results from using this product.

† These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.

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A Complete Review on *Oxystelma esculentum* R. Br.

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ABSTRACT

Many plants which are found commonly and are mentioned in texts of traditional Indian medicine have not been investigated thoroughly. It is necessary to conduct systematic evaluation, standardization, documentation and patenting of these plants. *Oxystelma esculentum* R. Br. (Family – Asclepiadaceae), commonly known as 'Jaldudhi', is one such plant which has not been studied sufficiently. It has many potential therapeutic uses which are of vital importance in curing the diseases of the modern world like cancer, hepatitis, kidney disorders, stress-related disorders and microbial infections. It contains two very important classes of phytoconstituents: cardenolides and pregnane glycosides, which are easily obtained from this plant and can act as precursors of many therapeutically important compounds. The study of this plant will be important in the future for bioactivity-guided fractionation of medicinal phytoconstituents, for conducting pre-clinical or clinical trials and for preparing formulations or semi-synthetic compounds. The present review, based on an extensive literature search of reputed books, scientific websites and high-impact journals, sheds light on the research done on this plant so far, thereby providing informative guidelines regarding the work that can be done in the future.

Key words: Asclepiadaceae, Cardenolides, Diuretic, Jaldudhi, *Oxystelma esculentum*, Pregnane glycosides

INTRODUCTION

Oxystelma esculentum R. Br. (Family – Asclepiadaceae), known as 'Jaldudhi', is a common Ayurvedic herb which has not been sufficiently explored. It is one of the few plants to contain cardenolides and pregnane glycosides, which are major classes of therapeutically important phytoconstituents. *O. esculentum* has been reported to possess good therapeutic action against many ailments of the current world. The present review, based on an extensive literature search of reputed books, websites and journals, remarks on the study done on this plant so far, thereby providing a direction for future research.

Synonyms

Oxystelma secamone Linn., *Periploca esculenta* Roxb., *Periploca secamone* Linn., *Sarcostemma secamone* Bennet, *Sarcostemma esculentum* Linn., *Asclepias rosea* R. Br.[1]

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Vernacular names[2]

Sanskrit	: Dugdhika, Tiktadugdha
Hindi	: Dudhlata
Gujarati	: Jaldudhi
Bengali	: Khirai, Dudhialata
Marathi	: Dudhani, Dudhika
Telugu	: Dudipala
Tamil	: Usipallai

Distribution[2]

Throughout plains and lower elevation areas of India, usually near water. Also found in Pakistan, Sri Lanka, Burma and extends to China and Indonesia.

Taxonomic classification[3]

KINGDOM	: Plant
DIVISION	: Phanerogams
SUBDIVISION	: Angiosperms

CLASS	: Dicotyledons
SUBCLASS	: Sympetalae/Gamopetalae
ORDER	: Gentianales
FAMILY	: Asclepiadaceae
GENUS	: *Oxystelma*
SPECIES	: *Oxystelma esculentum* R. Br.

Description of the plant

The plant genus derives its name from two words: *Oxys* (sharp) and *Stelma* (crown), which describes the acute lobes of the corolla.[4] It is a twining herb or undershrub whose stem is cylindrical, glabrous, long, slender and much branched. Leaf is simple, opposite, dorsiventral, deciduous, usually 8cm x 0.5cm, linear lanceolate with acute apex and symmetrical base, having long and slender petiole. Inflorescence is racemose, subumbellate cyme or solitary. Flowers are widely open, white with purple veins, 2.5-3cm in diameter, drooping, pedunculate, lateral subumbellate or racemose flowered cymes. Calyx is pentasepalous, connate, glabrous, oblong-lanceolate, acute, glandular inside. Corolla is pentapetalous, connate, 2.5cm wide, glabrous, saucer-shaped, broadly rotate, lobed half-way down, having a densely pubescent corolline corolla, double corona. Corolla lobes are triangular, acute, ciliate, purple veined, valvate at base and shortly overlapping to the right. Androecium consists of five stamens, adnate near base of corolla, having connate filaments, anthers with inflexed membranous deltoid tips and waxy, pendulous, elongate-clavate, solitary pollen in each cell. Gynoecium is bicarpellary, with style apex truncate or convex, stigma depressed or sub-convex. Follicles are 4.5-7.5cm long, often solitary, ovate-lanceolate, glabrous, having acute apex and containing numerous black, broadly ovate seeds [Fig.1]. Other species of *Oxystelma*, which is rarely found in India, is *O. esculentum* var. *wallichii*. Its follicles are shorter, 2.5-4cm long, oblong and rounded at both ends. The only morphological difference between the two varieties lies in its follicles.[5]

Uses

The plant has the property of being *Ushna* (hot), *Guru* (heavy), *Ruksha* (dry) and *Katu* (bitter). It is a diuretic, laxative, spermatogenetic, antitussive, anthelmintic and antileprotic. It increases *Vatta* and stimulates female fertility.[6] Entire plant is used as diuretic, laxative, antiseptic, 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. Root is used ethnomedicinally in jaundice by the people of Orissa, India. Fruit is bitter tonic, expectorant, anthelmintic. Fruit juice is used in muscle pain, gonorrhoea, cough and leucoderma, and given to children as astringent. [7,8]

Fig.1 *Oxystelma esculentum* R. Br.

PHYTOCHEMICAL REVIEW

General chemical analysis

Researchers first carried out the chemical analysis of *O. esculentum* which revealed the presence of water, fibers, proteins, lipids and carbohydrates. [9]

Isolation of pregnane glycosides

A group of researchers isolated a pregnane ester glycoside Oxystine from the roots of *O. esculentum*. Powdered roots were extracted with solvents of different polarities. Repeated column chromatography of the diethyl ether extract over silica gel using chloroform: methanol (96:4) as eluent afforded oxystine, which was found to be a tetraglycoside of 12-*O*-cinnamoyl desacylmetaplexigenin.[10] Another pregnane glycoside Oxysine was isolated from the roots. Column chromatography of the chloroform extract using chloroform: methanol (24:1) as eluent afforded oxysine, a triglycoside of calogenin.[11] A pregnane glycoside Esculentin was also isolated from the roots. Column chromatography of the methanolic

extract using chloroform: methanol (24:1) as eluent afforded esculentin, a triglycoside of sarcogenin.[12] Researchers isolated polyhydroxypregnane glycosides Alpinosides A, B and C from the leaves of *O. esculentum*. Dried aerial parts were exhaustively extracted with ethanol: water (7:3) in a Soxhlet apparatus. The extract was condensed under reduced pressure to a syrupy consistency. Crude extract was dissolved in methanol: water (2:1) and transferred into a separator funnel. The extract was shaken with hexane, chloroform and n-butanol respectively till exhaustion. The chloroform fraction was loaded on silica gel column. Fractions eluted with chloroform: methanol yielded three compounds of kidjolanin: Alpinoside A (pentaglycoside), Alpinoside B (tetraglycoside) and Apinoside C (pentaglycoside).[13]

Isolation of cardenolides

Three cardenolides, Oxystelmine, Oxyline and Oxystelmoside, have so far been isolated from the roots of *O. esculentum*. Oxyline was found to be a tetraglycoside of 3-epi-uzarigenin, oxystelmoside is a diglycoside of uzarigenin whereas oxystelmine is a diglycoside of periplogenin. [14,15]

PHARMACOLOGICAL REVIEW

Diuretic activity

Considering the claims in the traditional medicinal texts, researchers studied the effects of methanolic extract of leaves of *O. esculentum*. on diuresis in male Wistar albino rats. Urinary output and excretion of electrolytes (Na^+, K^+, Ca^{2+} and Cl^-) were measured. The methanolic extract significantly increased the urine output and had a significant effect on the electrolyte balance in a dose dependent manner, indicating that *O. esculentum* is an effective hypernatremic, hyperkalaemic, hypercalcemic and hyperchloremic diuretic.[16]

Antioxidant activity

A group of researchers performed the evaluation of antioxidant and free radical scavenging activities of methanolic extracts of leaves of *O. esculentum* in various *in vitro* models. It was discovered that the total antioxidant activity increased with increasing concentration. The reducing capability and free radical scavenging activity in DPPH also increased in a dose dependent manner. The methanolic extract was found to scavenge the superoxide generated by PMS/NADH/NBT system. Moreover, the extract was found to inhibit the nitric oxide radical generated from sodium nitroprusside. The extract was also found to inhibit the hydroxyl radical generated by Fe^{3+}/ascorbate/EDTA/water system. The extract scavenged the hydrogen peroxide in a dose dependent manner. These results give a clear indication that *O. esculentum* has a strong antioxidant activity and can be used as a natural antioxidant.[17]

Anticancer activity

Antineoplastic activities of methanolic leaf extracts of *O. esculentum* on Swiss albino mice bearing Ehrlich's ascites carcinoma were studied. Decrease in tumor volume, packed cell volume, and viable cell count were observed in methanolic extract-treated mice. The extract also decreased the body weight of the EAC-bearing mice. Hematological profiles indicated decrease in white blood cells, increase in red blood cells and increase in Hemoglobin content. The methanolic extract restored all the parameters of hematological profiles to normal. Treatment with methanolic extract decreased the levels of LPO and increased the levels of GSH, SOD and CAT. These data indicate that the methanolic extract of leaves of *O. esculentum* exhibits significant antitumor activity.[18]

Antimicrobial activity

Antibacterial activity of leaves of *O. esculentum* against some hospital isolated human pathogenic bacterial strains was studied. From the results it is clear that leaves of *O. esculentum* are effective in controlling both gram positive and gram negative bacterial pathogens. The most effective crude extracts were ethyl acetate and methanolic fractions. Aqueous extract also showed sensitivity against all test organisms. Petrol and benzene extracts of the leaves showed weak antimicrobial action.[19] Antimicrobial activity of methanolic extract of leaves *O. esculentum* was studied further. The antibacterial studies confirmed that the methanolic extract had a zone of inhibition, but the MIC in two fold serial dilution method ensured no prominent action on the tested bacterial strains. The antifungal studies confirmed that methanolic extract had an effective zone of inhibition against *C. albicans* and *C. neoformans*. In MIC studies, the methanolic extract had more effect on *C. albicans*, thus giving a lead for further *in vivo* anticandidal studies. In future, the active constituent can be formulated into a topical dosage form.[20]

DISCUSSION

Oxystelma esculentum is one of the few plants to contain cardenolides and pregnane glycosides, which can be obtained by simple and inexpensive methods from this plant. Also, these phytoconstituents can act as precursors of many other therapeutically important compounds. Due to the changing climate and lifestyle, health disorders like cancer, hepatitis, stress-related disorders, urinary disorders and bacterial infections have emerged as serious global issues. This plant has been reported to possess good therapeutic action against

many of such diseases. The present review can pave a way for a thorough evaluation, standardization, documentation and patenting of this plant. An exhaustive pharmacognostical, phytochemical, pre-clinical, clinical and formulation-based research on this plant can prove to be very fruitful for mankind.

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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

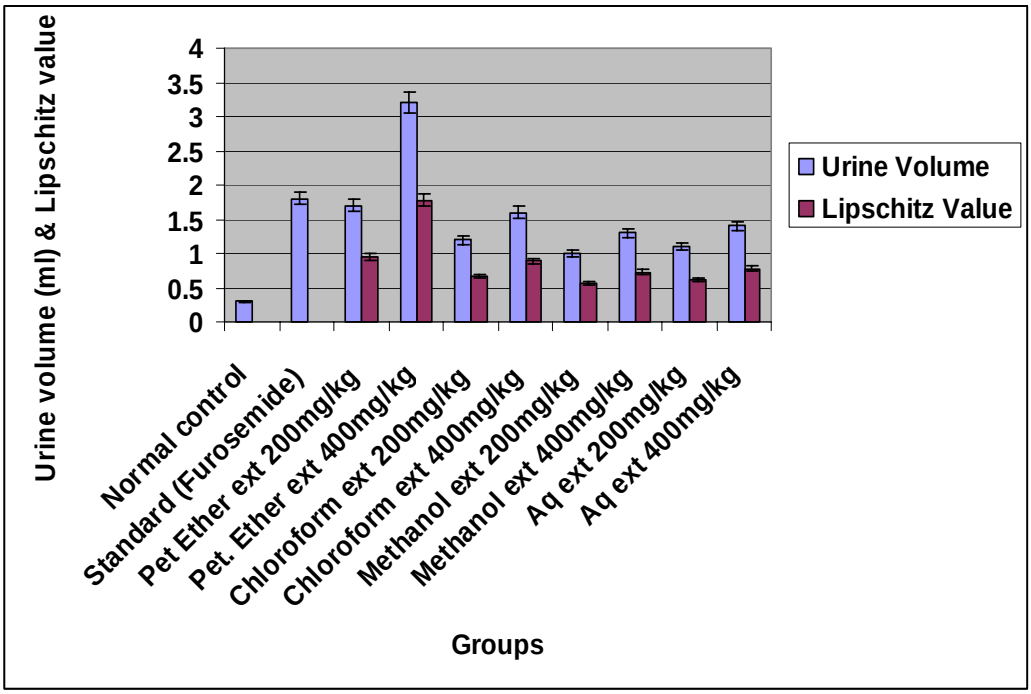

Fig. 1. Comparison of diuretic potential of various extracts (at 5h)

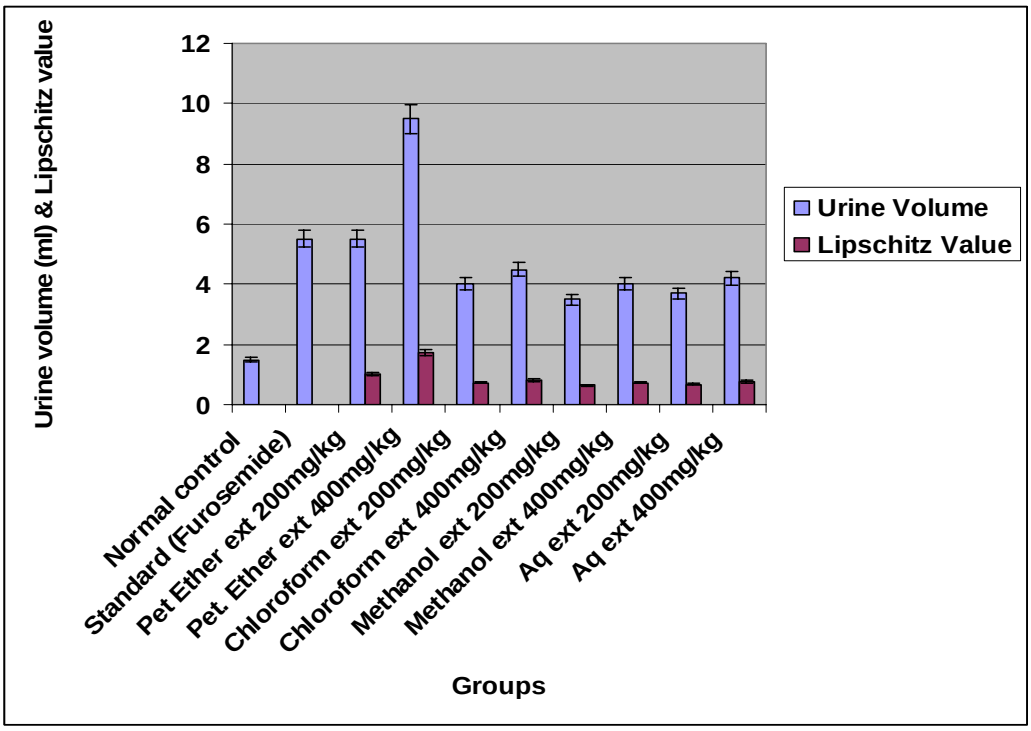

Fig. 2. Comparison of diuretic potential of various extracts (at 24h)

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

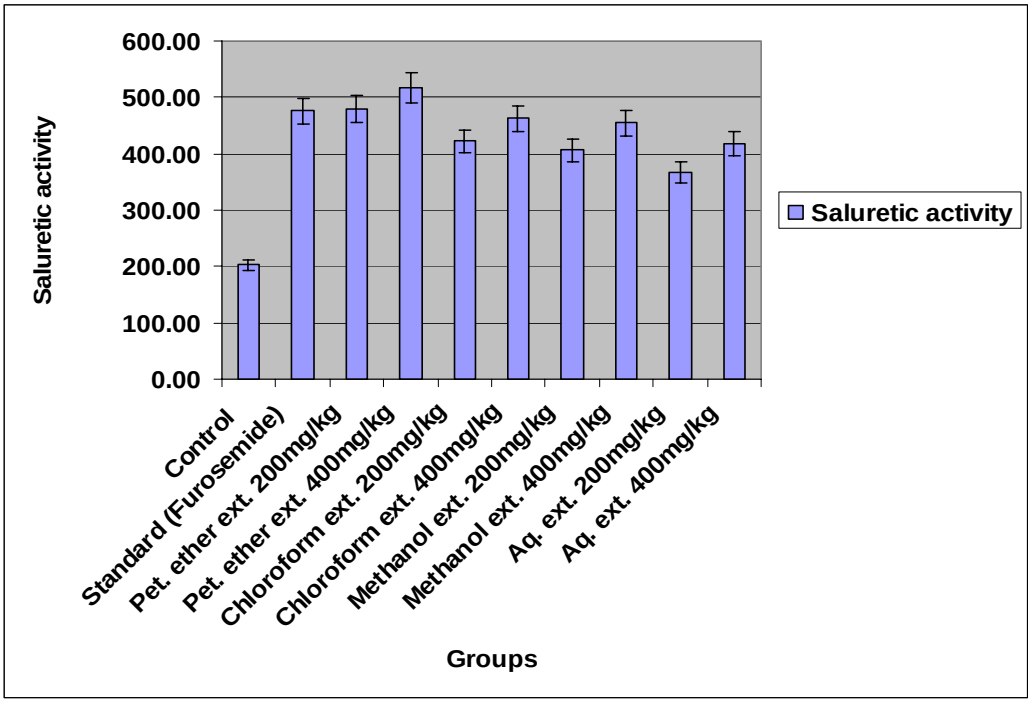

Fig. 3. Comparison of Saluretic activity (at 5h)

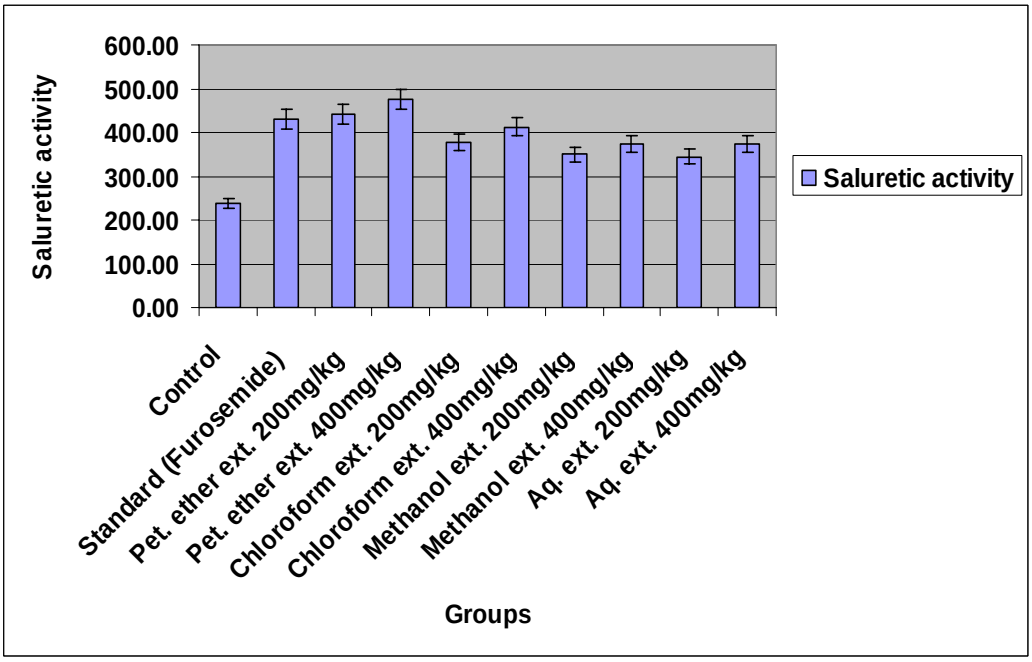

Fig. 4. Comparison of Saluretic activity (at 24h)

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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RESEARCH ARTICLE

Effects of Methanolic Extract of *Oxystelma esculentum* on Diuresis and Urinary Electrolytes Excretion in Rats

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ABSTRACT

The diuretic activity of methanol extract of *Oxystelma esculentum* aerial parts (MEOE) was studied in male Wister albino rats at 5h and 24h intervals. The animals were divided into 5 groups: control, urea, furosemide, 200mg/kg and 400mg/kg MEOE. The MEOE was administered intraperitoneally (i.p) and all animals were pretreated with saline before commencing the experiment. The urine volume (in mL) and electrolytes excretion (Na^+, K^+, Ca^{2+} and Cl^-) at 5h and 24h duration were measured. The urine output increased significantly in urea, furosemide and both MEOE groups (p<0.001). MEOE increased the urine volume and electrolytes balance in a dose dependent manner. The results indicate that MEOE is an effective hypernatramic, hyperkalaemic, hypercalcemic and hyperchloremic diuretic, which supports the traditional claim about the *Oxystelma esculentum* being used as a diuretic.

Keywords: *Diuretic activity, Methanol extract, Oxystelma esculentum, Urine volume, Electrolytes excretion.*

Plant medicine was commonly used for traditional treatment of some renal diseases and a lot of plants were reported to show significant diuretic activity [1]. Many investigators demonstrated that studies of herbal plant used in traditional medicine as diuretics, were in progressive elevation in the last decades [2], and might be a precious tool used in human disease treatment.

Oxystelma esculentum R. Br. (Asclepiadaceae) is a perennial twining herb. It is distributed throughout the plains, on hedges and among bushes usually near water and lower hills of India, Ceylon and Java [3,4]. The decoction of the plant is used as gargle in aphthous ulcerations of mouth and in sore throat. The root is considered specific for jaundice and the milk sap is used as a wash for ulcers [5-7]. In Ayurveda, the plant is a diuretic, aphrodisiac, anthelmintic and anti-bronchitis, is useful in leucoderma and the fruit is expectorant, anthelmintic. The fruit juice is used in gonorrhoea and pain in muscles [8]. A cardenolide tetraglycoside, oxyline isolated from roots and polyhydroxy pregnane glycosides, alpinoside A, B and C from aerial parts of the plant were reported [9,10]

Drug induced diuresis are beneficial in many life threatening disease conditions such as congestive heart failure, nephritis, hypertension and pregnancy toxemia [11]. Urine/electrolyte excretion is regulated by the $[HCO_3^-/Cl^-]$; $[HCO_3^+ /H^+]$ and the $[Na^+/H^+]$ exchanges. These are major intracellular/extra cellular pH regulators mediated by carbonic anhydrase, carbonic hydrogenase and phosphorylase [12]. The purpose of this study was to evaluate the diuretic activity and its possible mechanism of action in the methanol extract from *O.esculentum* in animal models.

MATERIALS AND METHODS

Collection of Plant Material

The aerial parts of *Oxystelma esculentum* R. Br. used in this study were collected in Srirangapatnam, Near Mysore, Karnataka. They were identified by H.O.D, Department of Botany, Kuvempu First Grade College, Channapatna, Karnataka, India. A voucher specimen No DAKJU-02/2005 has been deposited in our laboratory, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India for future reference.

Preparation of Extract

The collected aerial parts were air dried, pulverized in to a coarse powder and sieved. The dried powdered material was defatted with petroleum ether ($60\text{-}80^0$) and

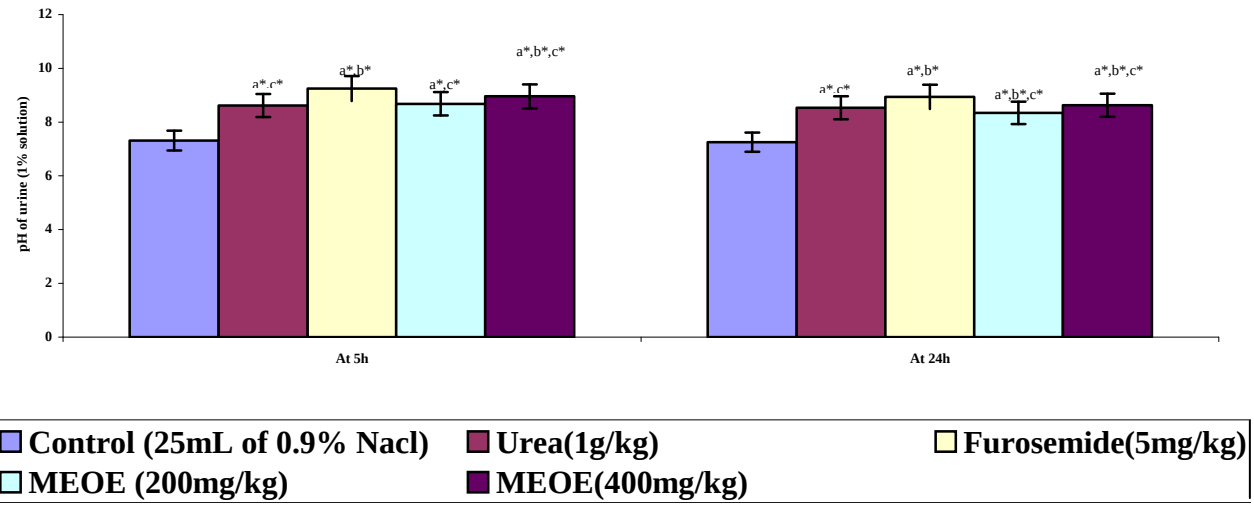

Fig 1. Effect of methanol extract of *Oxystelma esculentum* (MEOE) on pH in normal rats at 5th and 24th hour by intraperitoneal administration.
Values are expressed as mean ± SEM (Number of animals, n=6); a and a* indicates $p<0.05$ and $p<0.001$vs. Control; b and b* indicates $p<0.05$ and $p<0.001$vs. Urea; c and c* indicates $p<0.05$ and $p<0.001$vs. Furosemide.

was further extracted with methanol in a soxhlet apparatus. The extract was filtered and the solvent was removed by distillation under vacuum. The chemical constituents of the extract were identified by qualitative analysis [13]. The dried MEOE was suspended in distilled water and used for further studies.

Experimental Animals

Male Wister albino rats weighing 150-180g body weights were obtained from Indian Institute of Chemical Biology, Kolkata. All animals were maintained under environmentally controlled conditions of 24 ± 1^0C and 12 h light –12h dark cycle. The animals had free access to water and food and were acclimatized to laboratory condition at least 1 week before starting the experiments. The experiment was performed under standard conditions of temperature, light, humidity and noise.

Chemicals

Petroleum ether was obtained from Merck Limited, Mumbai; Methanol and urea were from Sisco Research Laboratories Pvt Ltd, Mumbai, Furosemide (Lasix) was obtained from Aventis pharma limited, Thane. All other chemicals used were of reagent grade.

Pharmacological Evaluation

Experimental design

Five groups of six male Wister albino rats each weighing between 150-180g/kg, b.w were used. All the animals received normal saline (25mL/kg, b.w) orally, prior to start of the experiment. Group I which received normal saline was treated as control. Group II received urea (1kg/kg). Group III received furosemide (5mg/kg). Group IV and V received the methanol extract at the dose of 200mg/kg and 400mg/kg body weight respectively. Immediately after administration of the drug, the rats were each placed in metabolic cages, specially designed to separate urine and faecal matter and observed at room temperature. The animals were denied food and water during the experiment. The urine volume was collected after 5h and 24h of the intraperitoneal administration [14,15]. The urine volume (mL/day) was measured and then assayed for Na^+, K^+, Ca^{2+} and Cl^- concentrations [16-18]. Na^+, $K^+$and Ca^{2+} were measured by aflame photometric method (Chemito 1020) while Cl^- was measured by titration with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate solution as an indicator [19]. The pH was determined using a pH meter (Mettler Toledo, Seven Easy) [20]. The instrument was calibrated with standard solutions containing different concentrations of sodium, potassium and calcium [21].

Diuretic Activity and Urinary Volume Excretion

The volume of the urine excreted after 5h and 24h of study by control, urea, furosemide and MEOE (200mg and 400mg/kg, b.w) was expressed as percent of the liquid administered giving rise to a measure of "Urinary excretion" (U.E)-independent of group weight [22], thus

$$\text{Urinary Excretion} = \frac{\text{Total urinary output}}{\text{Total liquid administered}} \times 100$$

The ratio of (U.E) in test group and control group was denoted. Diuretic action, which was used as the measure of degree of diuresis [23].

$$\text{Diuretic action} = \frac{\text{Urinary excretion in test group}}{\text{Urinary excretion in control group}} \times 100$$

$$\text{Diuretic activity} = \frac{\text{Diuretic action of drug}}{\text{Diuretic action of urea}} \times 100$$

$$= \frac{D}{U}$$

Table 1. Dose response diuretic activity of methanol extract of *Oxystelma esculentum* (MEOE) in normal rats at 5th and 24 th hour by intraperitoneal administration.

Groups	At 5h After Drug Administration				At 24h After Drug Administration			
	Urine volume (mL)	Urinary Excretion $(V_0/V_1)X100$	Diuretic Action (UE_t/UE_c)	Diuretic Activity (DA_t/DA_u)	Urine volume (mL)	Urinary Excretion $(V_0/V_1)X100$	Diuretic Action (UE_t/UE_c)	Diuretic Activity (DA_t/DA_u)
Control (25mL of 0.9% Nacl)	0.66±0.01	18.11	_	_	2.24±0.01	60.27	_	_
Urea(1g/kg)	0.83±0.02^{a*,c*}	21.56	1.19	_	2.50±0.01^{a*,c*}	64.94	1.08	_
Furosemide (5mg/kg)	2.13±0.01^{a*,b*}	54.34	3.00	2.52	4.33±0.01^{a*,b*}	110.46	1.83	1.69
MEOE (200mg/kg)	1.17±0.01^{a*,b*,c*}	32.50	1.79	1.50	2.90±0.01^{a*,b*,c*}	80.56	1.34	1.24
MEOE (400mg/kg)	2.17±0.01^{a*,b*}	55.64	3.07	2.58	4.40-±0.01^{a*,b*,c}	112.82	1.87	1.73

Values are expressed as mean ± SEM (Number of animals, n=6); V_0= Total urinary output; V_1= Total fluid input; UE_t = Urinary excretion in test group; UE_c=Urinary excretion in control group; DA_t= Diuretic action of the test sample; DA_u=Diuretic action of the Urea; a and a* indicates $p<0.05$ and $p<0.001$vs. Control; b and b* indicates $p<0.05$ and $p<0.001$vs. Urea; c and c* indicates $p<0.05$ and $p<0.001$vs. Furosemide

Table 2. Electrolytes excretion (mMol/L), saliuretic and natriuretic activity of methanol extract of *Oxystelma esculentum* (MEOE) in normal rats at 5th hour by intraperitoneal administration.

Groups	Electrolytes excretion in mMol/L				$Na^+ + Cl^-$	Na^+/K^+	$Cl^-/Na^+ + K^+$
	Na^+	K^+	Ca^{++}	Cl^-			
Control (25mL of 0.9% Nacl/kg)	133.43±0.04	55.51±1.15	59.66±0.34	105.33±0.66	238.76±0.70	2.40±0.05	0.5575±0.001
Urea (1g/kg)	150.26±3.08^{a,c*}	67.50±2.82^{a,c*}	68.79±0.54^{a,c}	146.66±1.76^{a*,c}	296.93±4.84^{a*,c*}	2.23±0.05^{a,c}	0.6740±0.010^{a*,c}
Furosemide (5mg/kg)	216.76±3.78^{a*,b*}	89.98±0.93^{a*,b*}	78.12±3.24^{a*,b}	176.33±3.17^{a*,,b}	393.09±6.95^{a*,b*}	2.40±0.01^{b}	0.5748±0.001^{b*}
MEOE (200mg/kg)	217.58±1.03^{a*,b*}	90.99±1.35^{a*,b*}	81.97±0.98^{a*,b*}	169.66±1.45^{a*,b}	387.24±2.49^{a*,b*}	2.39±0.02^{b}	0.5498±0.001^{b*,c}
MEOE (400mg/kg)	227.98±1.89^{a*,b8}	97.86±1.63^{a*,b*}	97.96±0.99^{a*,b*}	190.33±0.88^{a*,b}	418.31±2.77^{a*,b*}	2.33±0.02	0.5842±0.003^{a,b*}

Values are expressed as mean ± SEM (Number of animals, n=6) a and a* indicates $p<0.05$ and $p<0.001$vs. Control, b and b* indicates $p<0.05$ and $p<0.001$vs. Urea c and c* indicates $p<0.05$ and $p<0.001$vs. Furosemide

Saliuretic, Natriuretic and Carbonic Anhydrase Inhibition

The sum of $Na^+$and Cl^- excretion was calculated as a parameter of saliuretic activity. The ratio Na^+/K^+ was calculated for natriuretic activity. The ratio $Cl^-/Na^+ + K^+$(ion quotient) was calculated to estimate carbonic anhydrase inhibition [24].

Statistical Analysis

Results are mean ± S.E.M. Statistical analysis of control and test data was determined by ANOVA (SPSS computer software). Simple one-way analyses of variance were used for different doses with in a group. A probability value of $p<0.001$ and $p<0.05$ was considered statistically significant.

RESULTS

Phytochemical Analysis

The powdered materials were green in colour. The petroleum extract was dark green and had a musty odor while the methanol extract was blackish green and had a smelling flavor. The % yields were 5.22 and 14.60 for the petroleum ether and methanol extract respectively. The preliminary phytochemical screening of powdered *O.esculentum* revealed the presence of Glycosides, carbohydrates, flavonoids, phenolic compounds (Tannins), triterpenoids, saponins and steroids.

Effects on Urine Output and Diuretic Activity

The total urine volume over the period of 5h and 24 h were measured for the extracts, (200mg and 400mg/kg, b.w), standard diuretics (urea and furosemide) and control. Urea, furosemide and 200mg and 400mg/kg of MEOE increased the urine flow significantly at 5th h ($p<0.001$) and 24th h ($p<0.001$) when compared with control. The high dose excreted more than two fold the volume of urine as compared to control. MEOE increased urine flow in a dose dependent manner.

From the result it appears that MEOE exhibited diuretic activity at both doses (200mg and 400mg/kg, b.w) like furosemide at 5th h and 24th h and its effect was dose dependent (Table 1). The diuretic activity of a drug is considered to be good if it is above 1.50, moderate if it is within 1.00-1.50, little if it is between 0.72-1.00 and nil if it is less than 0.72. In this respect after the drug administration, MEOE showed good diuretic activity.

Effects on Electrolyte Excretion

The diuretic responses with its electrolyte excretion potency of the extract were highly moderate in comparison with control animals. The extract at doses of 200mg

Table 3. Electrolytes excretion (mMol/L), saliuretic and natriuretic activity of methanol extract of *Oxystelma esculentum* (MEOE) in normal rats at 24th hour by intraperitoneal administration

Groups	Electrolytes excretion in mMol/L				$Na^+ + Cl^-$	Na^+/K^+	$Cl^-/Na^+ + K^+$
	Na^+	K^+	Ca^{++}	Cl^-			
Control (25mL of 0.9% Nacl/kg)	146.57±0.50	88.58±1.20	96.71±1.02	188.00±1.15	334.57±1.65	1.65±0.01	0.7995±0.001
Urea (1g/kg)	168.22±1.31^{a*}	101.79±1.64^{a*,c}	123.93±0.83^{a*,c}	218.00±1.73^{a*,c*}	386.22±3.04^{a*,c*}	1.65±0.03^{a,c}	0.8074±0.007^{c*}
Furosemide (5mg/kg)	170.44±1.21^{a*}	110.51±1.28^{a*,b}	140.47±1.02^{a*,b*}	242.00±1.15^{a*,b*}	412.44±2.37^{a*,b*}	1.54±0.007^{a,b}	0.8614±0.003^{a*,b*}
MEOE (200mg/kg)	171.77±0.86^{a*}	113.51±1.69^{a*,b*}	142.12±1.00^{a*,b*}	244.00±1.15^{a*,b*}	415.77±2.00^{a*,b*}	1.51±0.02^{a,b}	0.8553±0.006^{a*,b*}
MEOE (400mg/kg)	186.52±1.21^{a*,b*}	116.58±1.27^{a*,b*}	150.37±1.09^{a*,b*}	262.00±1.73^{a*,b*}	448.52±2.56^{a*,b*}	1.60±0.007	0.8645±0.006^{a*,b*}

Values are expressed as mean ± SEM (Number of animals, n=6) a and a* indicates $p<0.05$ and $p<0.001$vs. Control, b and b* indicates $p<0.05$ and $p<0.001$vs. Urea c and c* indicates $p<0.05$ and $p<0.001$vs. Furosemide

and 400mg/kg showed increase in Na^+, Ca^{2+} and Cl^- excretion, accompanied by the excretion of K^+. The highest dose (400mg/kg) enhanced significantly the urine excretion of sodium ($p< 0.001$), potassium ($p< 0.001$), calcium ($p< 0.001$) and chloride ($p<0.001$) compared with that of control.

Effects on Urine pH

The urine pH after administration of MEOE, 200mg and 400mg/kg body weight were 8.68±0.03, 8.96±0.008 and 8.34±001, 8.63±0.01 at 5th h and 24th h. Urea increased the urine pH 8.62 ±0.01and 8.54±0.0 compared to control. Similarly, after furosemide treatment the urine pH was 9.25±0.02 and 8.94±0.02 at 5th h and 24th h respectively, thus making the urine more alkaline. All the values were compared with that of control, 7.31±0.02 and 7.25±0.01 at 5th h and 24th h (Fig 1).

Effects on Natriuretic, Saliuretic and Carbonic Anhydrase Inhibition

From the Table 2 and 3, the methanol extract of *Oxystelma esculentum* at both doses (200mg and 400mg/kg) showed natriuretic and potent saliuretic activity comparable to that of control. No carbonic anhydrase inhibition was detected [24,25].

DISCUSSION

This study shows that MEOE produced striking increase in total urine output over a period of 5h and 24h. It also increased the excretion of sodium, calcium, chloride accompanied with potassium significantly. Therefore MEOE has been shown to possess significant diuretic, natriuretic and kaliuretic effects, which may be one of the reasons of its therapeutic application in various ailments such as treatment of renal disorders, treatment of liver disorders, ulcers and pain in muscles. Diuretic activity may be very useful in a number of conditions like hypertension, hypercalciuria and cirrhosis of liver [26,27].

The diuretic activities of the extracts were highly potent when compared to control. However, there were significant differences in urinary excretion followed by diuretic action and diuretic activity. The extract caused increase urine elimination and increase in Na^+, K^+, Ca^{2+} and Cl^- excretion as compared to control (normal saline). The extract may possibly act by the synergistic action mechanism of the [HCO_3^-/Cl^-], [HCO_3^+/H^+] and the [Na^+/H^+] antiporter, to cause diuresis [28].

Furosemide acts by inhibiting electrolyte reabsortption in the thick, ascending limp of the loop of Henle [29]. Greger and Wangermann (1987) also found from micropuncture experiment that high ceiling diuretics enhanced Na^+, Ca^{2+} and Cl^- excretion; and microperfusion experiments revealed that there was a complete inhibition *in vitro* at luminal concentration of drugs in the range expected to occur *in vitro* [30]. The high ceiling diuretics may not affect K^+ loss [31-33].

At the 5th h and 24th h, the MEOE extracts showed change in urine output at both dose levels tested (200mg and 400mg/kg). The diuretic effect of the methanol extracts was significant at 5h and 24h. However, there is a slightly delayed effect at 24h. Even though, the diuretic activity at 24th h at both doses was significant. It showed the extracts acted in time and dose dependent manner which could have been as a result of absorption of the active principle(s) in the crude preparations or the extracts could have been stimulating *in vivo* a diuretic compound(s). Both doses of the methanol extract induced a significant increase in urine, Na^+, Ca^{2+} and Cl^-, accompanied by a significant excretion of K^+. This is a characteristic of high ceiling diuretic. No carbonic anhydrase inhibition was detected.

The 5h and 24h cumulative urine output induced by the extracts and standard drugs were statistically significant compared with control (saline treated). The high dose produced the highest urine volume and electrolyte output over the 5h and 24h period, and the low dose produced significant urine output and electrolyte excretion but it was less compare with furosemide and more or less similar to that of urea. It was reported by Nilve-

ses et al (1989) that an increment of the urine output in rats might result from high potassium content in the plant extract [34]. The pH values were also alkaline as compare with control.

The data reported in the present work indicate that the MEOE showed good diuretic activity, in comparison with furosemide high ceiling diuretic agent [35]. It can be observed that the methanol extract of *Oxystelma esculentum* is an effective hypernatramic, hyperchloremic, hypercalcemic and hyperkalaemic diuretic; which correlate well with the traditional use of the plant as a diuretic. From the observations showed, MEOE had similar diuretic spectrum to that of furosemide. Therefore, further researches are ongoing to find out the active principles responsible for the activities in our laboratory.

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