Evaluation of Laxative Activity of *Oxystelma esculentum*

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ABSTRACT

Oxystelma esculentum is a perennial twiner growing near water-logged areas in the Indian subcontinent. It is used traditionally as a laxative. The present work deals with the investigation of laxative potential of various extracts of *O. esculentum.* The plant was successively extracted with solvents of varying polarities, which served as the test extracts. Laxative effect was checked in Wistar rats using different models. The petroleum ether extract was found to possess the most effective laxative activity, thereby supporting the traditional claim of the plant as a laxative. Phytochemical screening of this extract revealed the presence of important classes of compounds like cardenolides, flavonoids, phenolics, sterols and triterpenoids. This bioactivity-guided phytochemical screening can guide further therapeutic investigations and isolation of therapeutically important compounds from *Oxystelma esculentum*.

Key words: Laxative, *Oxystelma esculentum*, *Oxystelma secamone*, *Periploca esculenta*

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 laxative, 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 investigating the laxative activity of the plant, but also finding the most potent extract and performing its phytochemical screening, so as to guide further fractionation of therapeutically potent constituents from this plant.

MATERIALS AND 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.

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Preparation of extracts

Successive extraction of 1 kg 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 (Agar-agar), 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 12 h-light and 12 h-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 200-220 g were kept in individual cages during one week. Any rat producing wet feces was rejected. The rats were fasted for 12 h before dosing but were given water ad *libitum*. 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). Normal control group received normal saline (25 ml/kg). Standard control group received 300 mg/kg Agar-agar (Pharma Pvt. Ltd.) orally. 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 200 mg/kg and the remaining three animals from each of these groups received dose of 400 mg/kg body weight.[4] After administration of the test extracts, the feces were weighed upto 8h and 16h (Table 1, Graph 1).

The same animals were used after a washing period of 4 months for observing the laxative effects of the various extracts in constipated rats. The same procedure was repeated, but Loperamide (Pharma Pvt. Ltd., 5 mg/kg) was used to induce constipation after 1h of administration of each extract. Feces were weighed upto 8h and 16h (Table 2, Graph 2). The results were expressed as the mean (g) of total feces.[5]

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

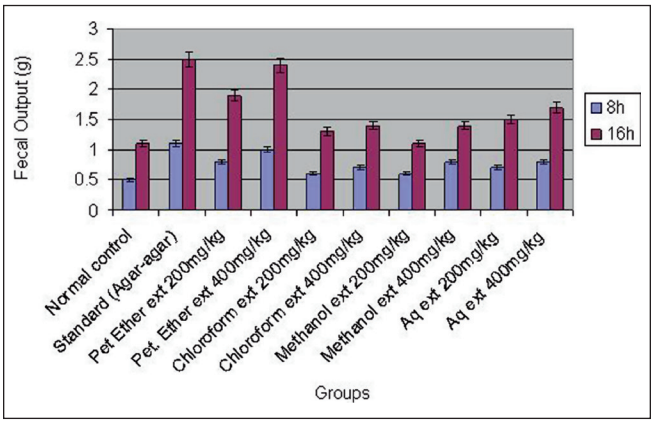
Petroleum ether extract was found to have the most potent and statistically significant laxative activity. This extract was subjected to phytochemical screening involving established methods for detecting various classes of phytoconsituents (Table 3).[6-11]

RESULTS

Table 1: Laxative activity of various extracts in rats

Groups	Fecal Output (g) At 8h	Fecal Output (g) At 16h
Normal control	0.5 ± 0.1	1.1 ± 0.1
Standard (Agar-agar)	1.1 ± 0.1	2.5 ± 0.1
Pet Ether ext 200 mg/kg	0.8 ± 0.1	1.9 ± 0.1
Pet Ether ext 400 mg/kg	1 ± 0.1	2.4 ± 0.1
Chloroform ext 200 mg/kg	0.6 ± 0.1	1.3 ± 0.2
Chloroform ext 400 mg/kg	0.7 ± 0.2	1.4 ± 0.2
Methanol ext 200 mg/kg	0.6 ± 0.2	1.1 ± 0.2
Methanol ext 400 mg/kg	0.8 ± 0.1	1.4 ± 0.2
Aqueous ext 200 mg/kg	0.7 ± 0.1	1.5 ± 0.1
Aqueous ext 400 mg/kg	0.8 ± 0.1	1.7 ± 0.2

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

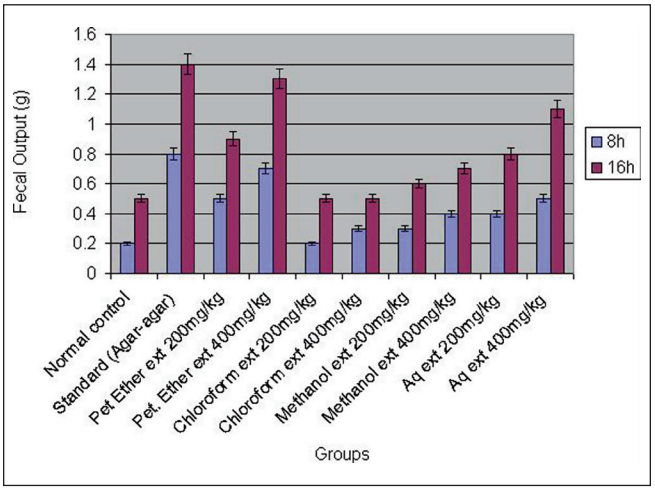


Graph 1: Comparison of laxative potential of various extracts

Table 2: Laxative activity of various extracts in Loperamide-induced constipation model

Groups	Fecal Output (g) At 8h	Fecal Output (g) At 16h
Normal control	0.2 ± 0.05	0.5 ± 0.05
Standard (Agar-agar)	0.8 ± 0.05	1.4 ± 0.05
Pet Ether ext 200 mg/kg	0.5 ± 0.05	0.9 ± 0.05
Pet Ether ext 400 mg/kg	0.7 ± 0.05	1.3 ± 0.05
Chloroform ext 200 mg/kg	0.2 ± 0.1	0.5 ± 0.10
Chloroform ext 400 mg/kg	0.3 ± 0.1	0.5 ± 0.15
Methanol ext 200 mg/kg	0.3 ± 0.08	0.6 ± 0.07
Methanol ext 400 mg/kg	0.4 ± 0.1	0.7 ± 0.15
Aqueous ext 200 mg/kg	0.4 ± 0.06	0.8 ± 0.10
Aqueous ext 400 mg/kg	0.5 ± 0.08	1.1 ± 0.10

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



Graph 2: Comparison of laxative potential of various extracts in Loperamide-induced constipation model

DISCUSSION

The present study shows that the petroleum ether extract of *Oxystelma esculentum* has the most potent, statistically significant and dose-dependent laxative activity amongst all extracts, comparable with agar-agar (Standard) at the dose of

Table 3: 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

400 mg/kg. Agar-agar exerts its laxative action by accumulation of water in the intestinal loop, increasing the bulk of the stools and stimulating the gastrointestinal motility. Also, loperamide abolishes diarrhea by acting on intestinal motility and consequently reducing the water and stools entering the colon.[12,13] The laxative activity of petroleum ether extract is comparable to agar-agar, indicating a mechanism of action similar to it, thereby overcoming loperamide-induced constipation. This proves the traditional claims of this plant as a potent laxative drug. Phytochemical screening of petroleum ether extract revealed the presence of cardenolides, flavonoids, phenolics, sterols and triterpenoids, which may be responsible for the laxative effect. This bioactivity-guided phytochemical screening can serve as a gauge for further study of therapeutic effects and isolation of therapeutically important compounds from *Oxystelma esculentum*.

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