

**EVALUATION OF DIURETIC ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF
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ABSTRACT

The present study was carried out to evaluate the Diuretic activity of the leaves of the plant *Marsilea quadrifolia* Linn based on the traditional use of the plant. The plant leaves were collected, dried and then subjected to Soxhlet extraction with various solvents from non-polar to polar. The extraction was carried out for 72 hrs with petroleum ether and ethanol. The aqueous extraction was carried out by cold maceration process. Then the extracts were subjected to preliminary phytochemical screening. Based on the preliminary phytochemical studies, the ethanolic extract was selected for the evaluation of Diuretic activity by *in-vivo*, Lipschitz test method using Frusemide as the standard drug. The ethanolic extract of *M. quadrifolia* showed a dose-dependent increase in both water (urine) excretion and excretion of Na^+ , K^+ , Cl^- ions. From the results, both the doses of ethanolic extract (200mg/kg & 400mg/kg) showed significant diuretic activity by increasing the total urine output and increasing the excretion of Na^+ , K^+ , Cl^- salts. Thus this plant can be used as a diuretic drug source and it seems to have lesser side effects when compared to that of the synthetic drugs.

KEYWORDS: Diuretics, *Marsilea quadrifolia* Linn, Soxhlet extraction, Lipschitz test, Frusemide.**1. INTRODUCTION**

The plant *Marsilea quadrifolia* Linn. is traditionally claimed to be useful in the treatment of various diseases like Hypertension, Diabetes, Sleep disorders and Head ache. Based on the ethnomedicinal information, the juice of the leaves possess the anti-inflammatory, Diuretic, anti-bacterial, anti-diabetic and analgesic depurative and refrigerant property. The phytoconstituents reported on *Marsilea quadrifolia* Linn. such as Marsilin, β -sitosterol, Flavonol-o-n-mono and diglycoside.

2. Collection and authentication of plant material^[1,4]

The leaves of *Marsilea quadrifolia* linn. were collected locally from Pochampalli wetlands, Krishnagiri district in the month of April-2016. The plant has been taxonomically identified and authenticated by the Botanist Dr. A. Balasubramanian. The authenticated plant were used for preparation of extract.

Preparation of the extract

The leaves of *Marsilea quadrifolia* linn. was collected and air dried under shade and then coarsely powdered with the help of mechanical grinder. The powder was passed through sieve no.40 and stored in an airtight container for the extraction.

The collected, cleaned and powdered leaves of *Marsilea quadrifolia* linn. was used for the extraction purpose 500 gms of powdered material was evenly packed in the

Soxhlet apparatus. It was then extracted with various solvents from non-polar to polar such as petroleum ether and ethanol. The solvents used were purified before use. The extraction method used was continuous hot percolation and carried out with various solvents, for 72 hours. The aqueous extraction was carried out by cold maceration process.

Method of extraction

- Continuous hot percolation process.

Requirements

- Shade dried coarse powder of leaves of plant *Marsilea quadrifolia* linn.
- Soxhlet apparatus

Solvents used

- Petroleum ether. (60-80°C)
- Alcohol 90% v/v. (75-78°C)
- Distilled water

3. Preliminary phytochemical studies

The extracts obtained (Pet. ether, ethanol and aqueous) was subjected to the following preliminary phytochemical studies.

4. Evaluation of diuretic activity

Introduction^[5]

Adult male albino rats weighing between 150-350 gms. were used in groups of four. Food and water was left available till the experiment started. During the experiment which lasted for five hours, no food or water was allowed. Each time at least one group of rats received urea and others received theophylline, acetazolamide or mersalyl. Ten groups of rats were crossed over to receive another during next experiment with a minimum rest period of four days. Each rat was primed by administering 10 ml/kg. of 0.9% saline intraperitoneally. Drug administered was dissolved in the saline injected for priming the animal. Each group of four rats was kept in a metabolic cage and urine collected free from feces. Urine volume was recorded at the end of five hours. With mersalyl 24 hour urine volume was taken into account and control urea volume was also recorded for 24 hours when mersalyl was used. Ten groups of rats were used each with mersalyl, acetazolamide, theophylline and urea. Five or 24 hour urine volume was expressed as percentage of initial hydration. Urea was taken as control and results were expressed as percentage increase over control (urea) run simultaneously.

Lipschitz Method^[6,19]

The diuretic activity of EEMQ and frusemide was carried out by using *in-vivo*, Lipschitz test method. The rats were divided into 4 groups of 5 animals each and deprived of food and water for 5 hours. All the rats received priming dose of normal saline (10ml/kg) orally. Both the extracts and frusemide (Standard) were dissolved in a normal saline. Group I served as control in which only normal saline (10ml/kg) was administered through intraperitoneal route. Group II served as standard received frusemide (100mg/kg). Rest of the groups served as treated groups. Group III and Group IV received EEMQ at the dose levels of 200 and 400 mg/kg i.p., respectively. Immediately after administration, the rats (one in each cage) were placed in metabolic cages specially designed to separate urine and faeces and kept at room temperature of $25 \pm 0.5^\circ\text{C}$. The urine was collected in a measuring cylinder upto 5 h. During this period, no food or water was made available. The volume of urine collected was measured for all the groups. The parameters taken for each individual rat were body weight before and after test period, urine volume (concentrated for water intake during the test period), concentration of Na^+ and K^+ in urine. The content of Na^+ and K^+ in the urine was estimated by digital flame photometer and Chloride was estimated by schales and schales method.

Animals

Animals	:	Wistar albino rats
Sex	:	Both sexes
Weight	:	150-180g
Animals per group	:	5
Number of groups	:	4

Grouping and treatment protocol

Group I - Normal control received only Normal saline.
 Group II - Standard Frusemide (100 mg/kg)
 Group III - Ethanolic Extract of *Marsilea Quadrifolia linn.* (200mg/kg)
 Group IV - Ethanolic Extract of *Marsilea Quadrifolia linn.* (400mg/kg)

Statistical analysis

All the results are expressed as mean+ standard error. The data was analyzed statistically using ANOVA followed by Dunnett's Multiple Comparison Test.

RESULTS AND DISCUSSIONS

The dried leaves of *Marsilea quadrifolia linn.* On successive extraction were extracted with solvent of increasing polarity by soxhlet apparatus.

The percentage yield of the dried leaves of *Marsilea quadrifolia linn* was found to be 2.6%, 5.8% and 8.24% with pet ether, ethanol and water respectively.

The percentage yield of Aqueous extract of dried leaves of *Marsilea quadrifolia linn* was found to be greater 8.24% than other extract.

The phytochemical examination of the selected extracts showed the presence of various constituents. From that ethanolic and aqueous extracts showed maximum phytoconstituent especially flavonoids, tannins and phenolic compounds.

Based on the phytochemical studies, the ethanolic extract was selected for the Pharmacological studies.

Diuretic Activity

Diuretics relieve pulmonary congestion and peripheral edema. These agents are useful in reducing the syndrome of volume overload, including orthopnea and paroxysmal nocturnal dyspnoea. They increase plasma volume and subsequently venous return to the heart. This decreases cardiac work load, oxygen demand and plasma volume, thus decreasing blood pressure. Thus diuretics play an important role in hypertensive patients. On the basis of the results of the present investigations, we can conclude that the ethanolic and aqueous extracts are potent natriuretic but weak diuretic. That means the natriuretic effect of lower dose may not be sufficient to induce diuresis.

According to previous survey carried out the leaves of *Marsilea quadrifolia linn.* largely used for the treatment of hypertension and renal disease, but to the best of our knowledge, no previous pharmacological or clinical study has been carried out to test the diuretic activity of this plant. Ethanolic extract of *Marsilea quadrifolia linn.* showed a dose-dependent increase in urine excretion, (100mg/kg) showed an increase of 44% grouping urine volume. Thus the diuretic effect of extract indicated by increase in both water excretion and excretion of

Sodium, Potassium, Chloride ion. Ethanolic extract (100mg/kg) showed significant result in excretion of water & sodium ion, which proved it strong diuretic agent.

From the results both the doses of ethanolic extract show significant diuretic activity by increasing the total urine output and increased excretion of Sodium, Chloride and Potassium salts, comparable to the standard drug Frusemide. The results were presented in table no 1.2 and figure no 1.2.

Table no: 1 Diuretic activity of Ethanolic Extract of *Marsilea Quadrifolia linn* and Frusemide on urine volume.

Group	Drug treatment	Dose(mg/kg B.W)	Urine volume
I	Control (Normal saline)	10ml/kg	4.63±0.7
II	Std (Frusemide)	100mg/kg	4.06±0.7**
III	Test (EEMQ)	200mg/kg	2.63±0.4**
IV	Test (EEMQ)	400mg/kg	3.21±0.5**

Table no: 2: Diuretic activity of Ethanolic Extract of *Marsilea Quadrifolia linn* urine electrolytes concentration.

Group	Drug treatment	Dose(mg/kg)	Na ⁺ (mEq/l)	K ⁺ (mEq/l)	CL(mEq/l)	PH
I	Control (Normal saline)	10ml/kg	107±1.13**	47±0.45**	77±0.56**	7.0±0.05
II	Std (Frusemide)	100mg/kg	188±1.23**	81±1.14**	127±1.25**	7.0±0.05
III	Test (EEMQ)	200mg/kg	124±1.26**	64±0.14**	88±1.03**	6.2±0.07
IV	Test (EEMQ)	400mg/kg	132±1.11**	70±0.17**	94±1.14**	6.6±0.09

Statistical analysis by ANOVA and Dunnet's Multiple comparison Test. Results are expressed as mean ± standard error, n = 5 in each group. *Significantly difference compared to control group at p < 0.05.

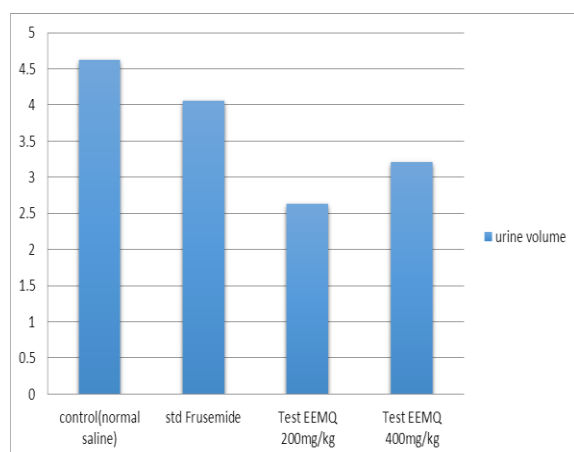


Fig no: 1: Diuretic activity of Ethanolic Extract of *Marsilea Quadrifolia linn* and Frusemide on urine volume.

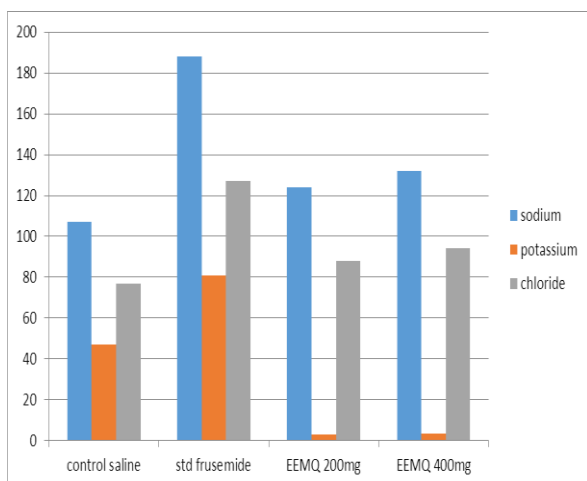


Fig no: 2: Diuretic activity of Ethanolic Extract of *Marsilea Quadrifolia linn* urine electrolytes concentration.

CONCLUSION

The ethanolic extract of leaves of *Marsilea quadrifolia linn.* showed significant diuretic activity. Further investigation experiment are required to prove the mechanism of action of the plant extract and isolation of active substance responsible for its biological action are necessary. this result of this study revealed the ethanolic extract of *Marsilea quadrifolia linn.* contains pharmacologically active substances.

Therefore the crude extract of *Marsilea quadrifolia linn.* leaf could be new source of development of new plant based therapy for management of several disease.

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