

**EVALUATION OF DIURETIC ACTIVITY OF POLYHERBAL FORMULATION**Vaijayanthimala Palanisamy<sup>2\*</sup>, Sureshkumar Shanmugam<sup>1</sup>, Sangameswaran Balakrishnan<sup>2</sup><sup>1</sup>J.K.K.Nattraja College of Pharmacy, Department of Pharmacognosy, Komarapalayam, Tamilnadu, India<sup>2</sup>SSM College of Pharmacy, Department of Pharmaceutical Chemistry, Erode, Tamilnadu, India**\*Corresponding author e-mail:** [vaijphd2014@gmail.com](mailto:vaijphd2014@gmail.com)**ABSTRACT**

The present study was carried out to investigate the diuretic effect of ethanol extract of the leaves of *Trichosanthes cucurmena* L. (EETC), *Cucumis sativus* L. (EECS) and fruits of *Corriandrum sativum* L. (EECRS) to make a polyherbal formulation (PHF) and were administered to experimental rats orally at the dose level of 150mg/kg and compared with standard drug Furosemide (20mg/kg). The diuretic effects of the extracts and PHF were evaluated by measuring the parameters like urine volume, sodium, potassium and chloride contents. The lipschitz method used in rat for the experiment purpose. The extract and PHF showed a marked level of increase in urine volume and electrolytes like Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> ion concentration. The moderate diuretic effect was observed and significant diuretic effect from PHF. PHF (150mg/kg) showed more diuretic effect than standard. This might be the first formal report on diuretic effects of these three plant combinations.

**KEYWORDS:** Diuretic activity, *Trichosanthes cucurmena* L, *Cucumis sativus* L, Furosemide, *Corriandrum sativum* L

**INTRODUCTION**

Since the instance immemorial our conventional system of medicine and folklore claiming that medicinal plants as a whole or their parts are being used in all types of sickness successfully. [1]

About 65% of world populations have right to use local medicinal plant knowledge system. [2] Traditional systems of medicine are popular in developing countries and up to 80% of people relies on traditional medicines for their primary health care needs. [3] India has about 45000 plant species and amongst them, more than several 1 thousands have been declared to possess medicinal properties. [4]

Diuretics are the agents which cause an enhanced in the urinary output and also increase the rate of urine, sodium excretion and are used to adjust the volume and composition of body fluids in a selection of clinical circumstances. Drug induced diuresis is valuable in many life threatening disease conditions like hypertension, congestive heart failure, cirrhosis,

nephritic syndrome, renal failure and pregnancy toxemia. [5]

The progress of a polyherbal formulation is a tough job because of the large number of different chemical compounds present in the different medicinal plants. [6] Hence, the entire herbal drug formulation is viewed as an active drug substance, apart from that whether constituents with defined therapeutic activity are known. But, the quality of a majority of them remains unknown. [7]

*T. cucumerina* (F. Cucurbitaceae) are used to treat kidney disorders. It is one of the ingredients in various Ayurvedic formulations used for the treatment of kidney disorders and also in other diseases. The leaves and stems are used for bilious disorders and skin diseases and as an emmenagogue. The leaf is alexiteric, astringent, diuretic and emetic. [8] *C. sativus* (F. Cucurbitaceae) is a well known plant also has a deep history in the treatment of kidney disease. The herbal formulation of the mixture of this Cucurbitaceae plant with fruit of *C. sativum* (Apiaceae) leads to cure the kidney disease. [8] *C.*

*sativum* is Diuretic in function and hence helps excrete out toxins and extra water from cells. This property makes *C. sativum* best for treating kidney related diseases as it makes kidneys pass out more urine and purifies the system. [9]

The present study was used to develop the polyherbal formulation (PHF) and to evaluate the diuretic effect of developed PHF in rat model using lipschitz method and compared with standard drug Furosemide (20mg/kg).

## MATERIALS AND METHODS

**Drugs and Chemicals:** All reagents used in the procedure were analytical grade. Furosemide ((Aventis Pharma Ltd) purchased from a drugstore.

**Plant collection:** Fresh leaves of *T. cucumerina*, *C. sativus* and fruits of *C. sativum* fruits were collected from field of Komarapalayam Voucher specimen (No: JKKNCP/0102/12, 13 and 14) has been deposited in the Department of Pharmacognosy, JKK Nataraja College of Pharmacy, Komarapalayam, Tamilnadu, India and authenticated by Dr.P. Satyanarayana, Scientist D & Head office in charge, Southern Regional Centre, TNAU campus, Coimbatore.

The fruits of *C. sativum* were dried and then crushed into fine powder by using laboratory Homogenizer then stored for further use.

**Preparation of Plant Extracts:** All the three crude drugs were extracted with alcohol. and then alcoholic extract of each plant was subjected to solvent extraction.

Ethanol extract of *T. cucumerina* and *C. sativum* (EETC) & (EECS): Fine powdered Leaves of *T. cucumerina* were extracted with ethanol (60-80° C) using soxhlet apparatus. The extract was filtered and evaporated, then separate solvent and residue. The semisolid residue obtained was stored in desiccator until further use.

Ethanol extract of *C. sativum*: (EECRS): Fine powdered fruits of *C. sativum* were extracted successively with petroleum ether and ethanol (60-80° C) using soxhlet apparatus in muslin cloth packed column. The extract was filtered and evaporated to separate solvent and residue. The semisolid residue thus obtained was stored in desiccator until further use.

**Formulation of PHF:** Each tablet of PHF contains 150 mg of active ingredients. It was powdered and suspended in 0.5% carboxyl methyl cellulose (CMC)

in distilled water, and then administered to the animal with the help of an oral gavage needle.

Each tablet contains *T. cucumerina* 50mg, *C. sativus* 50mg, *C. sativum* 50 mg and adequate amount of disintegrator, super disintegrator and diluents added then congestive mixture was prepared and passed through the sieve. The prepared granules are dried. The dried granules pass through a tablet compression machine before compression the granules parameters viz. Angle of repose, Tapped bulk density, Loose bulk density and compressibility were evolved. Poly herbal formulation is prepared by different combination and of Disintegrator and diluents and named as PHF I, PHF II and PHF III. The tablet characteristics (Average weight, Hardness, Friability and Disintegration time) were evolved. Finally PHF III was selected to diuretic activity.

**Animals:** Albino rats either sex weighing between 150 ± 25gm were used in this evaluation. These rats were procured from animal house located in JKK Nataraja college of pharmacy, Komarapalayam. They were housed in well ventilated stainless-steel cages at room temperature (24±2°C) in hygienic condition under natural light and dark schedule and were fed on a standard laboratory diet. Food and water were given ad libitum.

### Experimental protocol:

**Acute oral toxicity study:** The acute oral toxicity study was followed by using OECD GUIDELINES - 423 (Organization of Economic Co-operation and Development) - Fixed dose procedure (FDP).

Acute toxicity study was performed for ethanol extract of *T. cucumerina* (EETC), *C. sativus* and *C. sativum* extracts according to the acute toxic classic method as per OECD (423) guidelines, albino rats were used for acute toxicity study. The animals were kept in fasting condition for overnight providing only water, then the extracts were administered orally at the dose of 5, 50, 300 and 2000 mg/kg and observed for 16 days. If death was observed in 2 out of 3 animals, then the dose administered was concluded as toxic dose. Animals aren't shown signs of toxicity including mortality; nature, severity, and duration of effects up to the dose level of 2000 mg/kg for all the three extracts.

**Diuretic Activity:** Male rats (albino strain) weighing 150±25 gm were maintained under standard condition of temperature and humidity. The method of Lipschitz was employed for the assessment of diuretic activity. 5 groups of six rats in each and were fasted and deprived of water for eighteen hours prior to the experiment.

Group I (control): Received **Normal saline** (0.9%) orally at a dose of 10 ml/kg b.wt.

Group II (standard): Received **Furosemide** orally at a dose of 20 mg/kg b.wt.

Group III (Test 1): Received **EETC** at a dose of 150 mg/kg b.wt.

Group IV (Test 2): Received **EECS** at a dose of 150 mg/kg b.wt.

Group V (Test 3): Received **PHF** at a dose of 150 mg/kg b.wt.

Animals were kept at room temperature of  $25 \pm 0.5^{\circ}\text{C}$  throughout the experiment. The urine was collected in measuring cylinder up to 5hrs after dosing. During this period, food or water was not supplied to the animals. The total volume of urine collected and measured for both control and treated groups. The parameters taken for individual rat were body weight (before and after test period) urine sodium, total urine volume and potassium concentrations were calculated by flame photometry and Chlorine concentration was estimated by titration with silver nitrate solution (0.02N) using 1 drop of 5% potassium chromate solution as indicator.[10,11]

The sum of  $\text{Na}^+$  and  $\text{Cl}^-$  excretion was calculated as a parameter of saluretic activity. The ratio of  $\text{Na}^+/\text{K}^+$  was calculated for Natriuretic activity. Diuretic Index was calculated by volume of urine in test group/volume of urine in the control group.

**Statistical Analysis:** The results were expressed as a mean  $\pm$  S.E.M. The differences were compared using One Way Analysis of Variance (ANOVA) and subsequently followed by Dunnett Multiple Comparisons Test.

## RESULTS AND DISCUSSION

**Effect of aqueous and ethanol extracts on urine output in rats:** Urine volume in rats treated with

aqueous extract of EETC at 150mg/kg doses was 5.83 ml/kg. Urine volume in rats treated with EECS at 150 mg/kg 5.33 ml/kg and the urine volume of PHF at 150mg/kg was found to be 8.4 mg/kg and it is comparable to standard.

**Effect of aqueous and ethanol extracts on electrolyte excretion in rats:** The concentrations of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  in rats treated with aqueous extracts of EETS at 150mg/kg are 60.04mEq/L, 62.03mEq/L, and 64.28mEq/L, . The concentrations of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  in rats treated with EECS at a dose level of 150mg/kg are 59.10mEq/L, 58.91mEq/L, 72.21mEq/L. The concentrations of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  in rats treated with PHF at a dose level of 150mg/kg are 109.34mEq/L, 105.61mEq/L, 132.30mEq/L and it was more than that of standard value.

## CONCLUSION

The present study revealed that apart from renal protection, the extracts of EETC and EECS possessed moderate diuretic activity at a dose level of 150mg/kg. The PHF possessed more diuretic activity when compared to the standard (Furosemide). A combination of both the extracts with EECRS was showed potent diuretic effect. A significant increase in the sodium ions in the urine supports that the extracts can be used to treat hypertension. The mechanism involved might be an increase in the Glomerular Filtration Rate and decreased tubular reabsorption.

The EETC and EECS both Extract have produced a moderate diuretic activity individually, and in their combination with EECRS in polyherbal tablet formulation to produce significant than the individual extract. So that it can be used to produce diuresis during edema and also to treat hypertension.

**TABLE I: DIURETIC ACTIVITY**

Treatment group	Urine volume (ml/Kg)	Concentration of Excreted Ions		
		Na+ (mEq/L)	K+ (mEq/L)	Cl- (mEq/L)
Control Water 10ml/kg	3.15 $\pm$ 0.86	55.96 $\pm$ 4.45	53.74 $\pm$ 5.94	53.65 $\pm$ 32.99
Frusamide 20mg/kg Water 10ml/kg	7.91 $\pm$ 1.88**	102.84 $\pm$ 11.49**	97.13 $\pm$ 9.51**	129.08 $\pm$ 89.80**
EETC 150mg/kg	5.38 $\pm$ 0.83†	60.04 $\pm$ 8.81†	62.03 $\pm$ 5.10†	64.28 $\pm$ 93.31 †
EECS 150mg/kg	5.33 $\pm$ 0.83†	59.10 $\pm$ 8.91 †	58.91 $\pm$ 11.84 †	72.218 $\pm$ 98.31†
PHF 150mg/kg	8.4 $\pm$ 2.93 †	109.34 $\pm$ 9.03 †	105.61 $\pm$ 8.36 †	132.30 $\pm$ 98.50 †

Values are expressed as mean  $\pm$  S.D. of six animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. N= 6. \*P < 0.01 as compared with control, †P < 0.01 as compared with standard, ns = non significant.

**Table 2: Comparison of Saluretic, Natriuretic and Diuretic Index of Extracts with Standard Drug Furosemide**

TREATMENT GROUP	SALURETIC INDEX		NATRIURETIC INDEX [Na+/K+]	DIURETIC INDEX
	Na +	K +		
Control Water 10ml/kg	----	----	1.04	----
Furosemide 20mg/kg	1.83	1.80	1.05	2.51
EETC 150mg/kg	1.07	1.15	0.96	1.70
EECS 150mg/kg	1.05	1.09	1.00	1.69
PHF 150mg/kg	1.95	1.96	1.03	2.66

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