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Diuretic potential of *Cynodon dactylon, Emblica officinalis, Kalanchoe pinnata* and *Bambusa nutans*

Atul Sohgaura, Papiya Bigoniya and Birendra Shrivastava

Abstract
The study aims at enrichment of *C. dactylon, E. officinalis, K. pinnata* and *B. nutans* ethyl acetate fractions with its acute toxicity and diuretic potential exploration. Ethyl acetate fractions was separated from hydro-methanolic extract of *C. dactylon, E. officinalis, K. pinnata* and *B. nutans*. Acute toxicity was determined as per the guidelines of organization for Economic Co-operation and Development (OECD) guideline No. 423 and 420 followed by diuretic potential assessment by Lipschitz test. Acute toxicity limit test showed the LD_{50} to be greater than the test dose 2000 mg/kg for *C. dactylon, K. pinnata* and *B. nutans*, and 1000 mg/kg for *E. officinalis* ethyl acetate fractions. *C. dactylon, E. officinalis, K. pinnata* and *B. nutans* has showed high sodium (Na^+) and potassium (K^+) excretion potential where as high chloride (Cl^-) excretion ability was observed in *K. pinnata* followed by *C. dactylon* and *E. officinalis*. Most potential diuretic activity was observed in *K. pinnata* and *C. dactylon* ethyl acetate fraction that can be correlated to rich presence of flavonoids and polyphenols. The present study supports the traditional and Ayurvedic use of *K. pinnata, C. dactylon* and *E. officinalis* plants for diuretic potential.

Keywords: diuretic, *Bambusa nutans*, *Cynodon dactylon*, *Emblica officinalis*, flavonoid, *Kalanchoe pinnata*, polyphenol

Introduction
Plant-derived substances are of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs having application as traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al*., 2008) [23]. Extraction is the separation of medicinally active portions of plant using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or in dry powder after removing the solvent, that are intended for oral or external use. Such preparations are popularly called galenicals, named after Galen, the second century Greek physician (Remington, 2010) [29]. Pharmaceutically used extraction methods involves the separation of medicinally active components of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity (Ncube *et al*., 2008) [23]. The purpose of preparation of standardized extracts for crude drugs from medicinal plant is to attain therapeutically desired phytoconstituents and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract thus obtained, after standardization, can be used as medicinal agent in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form. These products contain complex mixture of many plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids etc. (Handa *et al*., 2008) [15].

Varieties of plants mentioned in Ayurvedic system of medicine are renowned to possess water pill properties. The plants selected for the study are *Kalanchoe pinnata* (Crassulaceae) leaf, *Emblica officinalis* (Euphorbiaceae) fruit, *Bambusa nutans* (Graminae) shoot and *Cynodon dactylon* (Poaceae) whole plant. *K. pinnata* grows as a succulent herb throughout India and cultivated in gardens and wild on the hills of North-Western India, Deccan and Bengal. *E. officinalis* enjoys a hallowed position in Ayurveda, which according to ancient Indian mythology, it is the first tree to be created in the universe and also grows in tropical and subtropical regions. Amla, the fruits of *E. officinalis* are widely used in the Ayurveda. *B. nutans* is a deciduous, clump-forming bamboo with fairly thick-walled culms and edible shoots. Several parts of bamboo plant has persisting medicinal uses and also as vegetable for its low fat, high fiber and rich mineral elements. *C. dactylon* is a perennial grass with underground rhizomes and on the ground runners, mostly growing on uncultivated ground, dry

places and roadsides throughout India. All these plants have well known traditional and Ayurvedic uses for their diuretic potential but mostly lacking well documented and scientifically proven data for diuretic activity.

Ethyl acetate fraction of *C. dactylon*, *E. officinalis*, *K. pinnata* and *B. nutans* showed 40.00, 53.75, 65.00 and 5.25% content of total flavonoid and 46.83, 66.37, 72.39 and 47.56% total polyphenol respectively (Sohgaura *et al*., 2018) [33]. Chen *et al*. (2014) [5] and Feng *et al*. (2013) [13] reported diuretic effect of ethyl acetate and n-butanol fraction of *Alismatis rhizome* and *Poria cocos*. Flavonoid and polyphenolic phytocompounds are known to have diuretic potential (Nayak *et al*., 2017; Păltinean *et al*., 2017; Compaoré *et al*., 2011) [22, 26, 8]. This study aims at assessment of diuretic potential of the enriched ethyl acetate extracts of *C. dactylon*, *E. officinalis*, *K. pinnata* and *B. nutans* with rich flavonoid and polyphenolic content on rats.

Materials and Methods

Collection and identification of plant

The *K. pinnata* leaf was collected in the month of August, 2014, from Rewa District (M.P.). *E. officinalis* fruit was collected in April, 2015, from local wander of Bhopal (M.P.). *B. nutans* shoot was collected in August, 2014, from Bhim Baithaka, Raisen (M.P.), and *C. dactylon* whole plant was collected in October, 2014, from Bhopal (M.P.). Herbarium was prepared for all the four plants. Herbarium samples of the plants were identified and authenticated by Dr. Zia Ul Hasan, Prof and Head, Herbarium Department, Department of Botany, Saifia College of Science Bhopal (M.P.). Voucher specimen no. 456/Bot/saifia/14 was allotted.

Extraction of Plant Material

Drying, processing and enrichment of ethyl acetate fraction from hydro-methanolic extract was performed as described previously (Sohgaura *et al*., 2018) [33].

Experimental Animal

In-vivo study was performed with due permission from Institutional Animal Ethical Committee (IAEC Approval No.: IAEC/RCP/JUN 2014/05). Laboratory breed adult Swiss Albino mice (25-30 gm) and Wistar rat (100-150 gm) of either sex was used. The animals were housed in polypropylene cages with paddy husk bedding maintained in hygienic condition at 22±2°C temperature and 12 hr light-dark cycle. The animals were fed with standard pallet balanced diet and water *ad libitum*. All experimental procedures were conducted in accordance to the ethical guidelines of CPCSEA, New Delhi.

Acute toxicological study

LD_{50} was determined as per the guidelines of organization for Economic Co-operation and Development (OECD) following the Up and Down method (OECD guideline No. 423) and Fixed Dose method (OECD guideline No. 420). Based on these guidelines a limit test was performed to categorize the toxicity class (LD_{50}) of the compound (OECD, 2000). Literature search reveals that *C. dactylon*, *E. officinalis*, *K. pinnata* and *B. nutans* are commonly used plant in Indian traditional system of medicine and most likely to be nontoxic. The limit test was performed at 2000 mg/kg, p.o. for ethyl acetate fraction of *C. dactylon*, *K. pinnata* and *B. nutans*. Bhattacharya *et al*. (1999) [4] reported use of 5 and 10 mg/kg, IP dose of *E. officinalis*, hydrolysable tannin fraction for *in vivo* antioxidant activity study, based on this literature limit test of *E. officinalis* was performed at 1000 mg/kg orally.

In-vivo screening of diuretic potential

The 75 rats were divided into fifteen groups comprising five animals in each group. Each group had been treated as per the protocol to assess the effect of ethyl acetate fraction of *C. dactylon* (EACD), *E. officinalis* (EAEO), *K. pinnata* (EAKP) and *B. nutans* (EABN) following the method of Lipschitz *et al*. (1943) [19] and Jayasree and Kishore (2011) [17].

GROUP 1: Fed with Normal saline10 ml/kg (Vehicle control)
GROUP 2: Treated with Furosemide (10 mg/kg, p. o)
GROUP 3: Treated with Cystone (750 mg/kg, p.o)
GROUP 4: Treated with EACD (100 mg/kg, p.o)
GROUP 5: Treated with EACD (300 mg/kg, p.o)
GROUP 6: Treated with EACD (500 mg/kg, p.o)
GROUP 7: Treated with EAEO (25 mg/kg, p.o)
GROUP 8: Treated with EAEO (50 mg/kg, p.o)
GROUP 9: Treated with EAEO (100 mg/kg, p.o)
GROUP 10: Treated with EAKP (25 mg/kg, p.o)
GROUP 11: Treated with EAKP (50 mg/kg, p.o)
GROUP 12: Treated with EAKP (100 mg/kg, p.o)
GROUP 13: Treated with EABN (25 mg/kg, p.o)
GROUP 14: Treated with EABN (50 mg/kg, p.o)
GROUP 15: Treated with EABN (100 mg/kg, p.o)

Collection and analysis of urine: Animals were fasted for 18 hr prior to the experimentation but with free access to water only. All the rats received priming dose of normal saline 25 ml/kg orally. Immediately after administration of vehicle, standard drug and different doses of test substance according to body weight all the rats were placed in metabolic cages (group wise) specially designed to separate urine and faeces at room temperature of 25±0.5°C. Urine was collected in a graduated cylinder for 6 hours. During this period no food and water was made available to animals. Cumulative urine excretion was calculated in relation to body weight and expressed as ml/kg body weight and pH was determined. The urine samples were stored at 4°C after adding a drop of concentrated hydrochloric acid. Urine samples were analyzed for level of sodium, potassium and chloride with the help of diagnostic kits (Span Diagnostics Ltd, India) using auto analyzer Star 20 (Rapid Diagnostic Pvt. Ltd., Delhi) and expressed as mmol/L/kg body weight.

Statistical analysis

The values were expressed as Mean ± SEM. Statistical comparison was performed using one way analysis of variance ANOVA to assess the Statistical significance, followed by Dunnett multiple comparison test. A P value of less than 0.05 was considered as statistically significant.

Result

Acute toxicity

C. dactylon at 2000 mg/kg dose did not show sign of adverse effect on physiological responses of animals. *E. officinal* 1000 mg/kg dose treated animals become sluggish with depression of locomotion and gait, whereas righting reflux was normal with depression of arousal and wakefulness. *B. nutans* at 2000 mg/kg dose showed respiratory depression without any sign of pain, distress. Animals treated with *K. pinnata* 2000 mg/kg dose was free of any sign of toxicity but showed hyperactivity. From the outcome of the acute toxicity limit test, LD_{50} is considered to be greater than the test dose 2000 mg/kg for *C. dactylon*, *K. pinnata* and *B. nutans*. The limit

dose for *E. officinalis* ethyl acetate fraction was 1000 mg/kg. Dose range selected for *E. officinalis*, *K. pinnata* and *B. nutans* are 25, 50 and 100 mg/kg, p.o., and 100, 300 and 500 mg/kg for *C. dactylon* to explore the *in vivo* pharmacological activity study.

Table 1: Diuretic potential of *C. dactylon* ethyl acetate fraction on rats

Treatment Group (mg/kg, P.O)	Urine content					Na^+/K^+ ratio	% increase in Na^+ excretion
	Volume (ml)	pH	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)		
Vehicle control	9.80 ± 0.39	5.72 ± 0.32	102.33 ± 9.68	52.24 ± 2.72	82.14 ± 3.66	1.96	-
Frusemide (10)	15.36 ± 0.92***	5.61 ± 0.82ns	176.56 ± 10.72**	92.51 ± 4.49**	130.26 ± 8.58**	1.91	72.53
Cystone (750)	13.66 ± 0.72**	5.85 ± 0.54ns	146.34 ± 9.66*	79.62 ± 3.53**	97.51 ± 5.82ns	1.84	43.00
EACD (100)	11.98 ± 0.62ns	5.90 ± 0.75ns	132.42 ± 8.74ns	64.15 ± 4.62ns	86.72 ± 3.44ns	2.06	29.40
EACD (300)	12.66 ± 0.57ns	6.33 ± 0.43ns	146.23 ± 10.54*	78.14 ± 4.63**	98.34 ± 4.74ns	1.87	42.90
EACD (500)	14.55 ± 0.84**	6.25 ± 0.35ns	169.16 ± 8.81**	93.18 ± 6.76**	110.13 ± 7.83*	1.82	65.30

All values are M ± SEM of 6 animals in each group. *P<0.05, **P<0.01, ***P<0.001 and ns = not significant compared to vehicle control group. EACD = ethyl acetate fraction of *C. dactylon*.

Diuretic potential

C. dactylon showed significant (P < 0.5-0.01) increase in Na^+ and K^+ elimination at 300 and 500 mg/kg dose whereas increase in total urine volume and Cl^- excretion was significantly (P < 0.5) higher at 500 mg/kg dose only compared to vehicle control. Effect on pH was non-significant at all three doses (Table 1).

Table 2: Screening of diuretic potential of *E. officinalis* ethyl acetate fraction on rats

Treatment Group (mg/kg, P.O)	Urine content					Na^+/K^+ ratio	% increase in Na^+ excretion
	Volume (ml)	pH	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)		
Vehicle control	9.80 ± 0.39	5.72 ± 0.32	102.33 ± 9.68	52.24 ± 2.72	82.14 ± 3.66	1.96	-
Frusemide (10)	15.36 ± 0.92***	5.61 ± 0.82ns	176.56 ± 10.72**	92.51 ± 4.49**	130.26 ± 8.58**	1.91	72.53
Cystone (750)	13.66 ± 0.72*	5.85 ± 0.54ns	146.34 ± 9.66*	79.62 ± 3.53**	97.51 ± 5.82ns	1.84	43.00
EAEO (25)	11.64 ± 0.77ns	5.92 ± 0.42ns	152.39 ± 8.35**	71.80 ± 2.41**	79.22 ± 2.71ns	2.12	48.92
EAEO (50)	13.98± 0.84*	6.19 ± 0.62ns	167.40 ± 8.34**	84.97 ± 2.39**	84.33 ± 2.66ns	1.97	63.58
EAEO (100)	14.10± 0.95*	6.08 ± 0.73ns	169.57 ± 7.62**	98.68 ± 3.49**	104.53 ± 4.83*	1.72	65.70

All values are M ± SEM of 6 animals in each group. *P<0.05, **P<0.01, ***P<0.001 and ns = not significant compared to vehicle control group. EAEO = ethyl acetate fraction of *E. officinalis*.

E. officinalis had non-significant effect on pH at all three doses, but significantly (P < 0.01) increased urine Na^+ and K^+ content of at 25, 50 and 100 mg/kg doses whereas increase in Cl^- excretion was significantly (P < 0.5) only at 100 mg/kg dose. Urine volume was high at 50 and 100 mg/kg doses (Table 2). *K. pinnata* has increased Na^+ and K^+ urinary elimination significantly (P < 0.01) at dose range of 50 and 100 mg/kg, but urine volume and Cl^- excretion was significantly (P < 0.01) higher only at 100 mg/kg dose (Table 3). *B. nutans* has significantly (P < 0.01) increased K^+ elimination at 50 and 100 mg/kg dose and Na^+ elimination along with urine volume only at 100 mg/kg dose. The observed value indicated non-significant changes in pH and Cl^- level as compared to vehicle control (Table 4).

Table 3: Screening of diuretic potential of *K. pinnata* ethyl acetate fraction on rats

Treatment Group (mg/kg, P.O)	Urine content					Na^+/K^+ ratio	% increase in Na^+ excretion
	Volume (ml)	pH	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)		
Vehicle control	9.80 ± 0.39	5.72 ± 0.32	102.33 ± 9.68	52.24 ± 2.72	82.14 ± 3.66	1.96	-
Frusemide (10)	15.36 ± 0.92***	5.61 ± 0.82ns	176.56 ± 10.72**	92.51 ± 4.49**	130.26 ± 8.58**	1.91	72.53
Cystone (750)	13.66 ± 0.72*	5.85 ± 0.54ns	146.34 ± 9.66*	79.62 ± 3.53**	97.51 ± 5.82ns	1.84	43.00
EAKP (25)	10.32± 0.85ns	6.37 ± 0.80ns	136.44 ± 9.37ns	64.22 ± 3.64ns	83.65 ± 4.74ns	2.12	33.33
EAKP (50)	12.34± 0.72ns	6.17± 0.39ns	152.35 ± 8.76**	77.66 ± 5.67**	91.38 ± 4.58ns	1.96	48.88
EAKP (100)	14.00± 0.78**	6.27 ± 0.45ns	160.33 ± 9.68**	89.54 ± 4.71**	107.90 ± 7.74**	1.79	56.67

All values are M ± SEM of 6 animals in each group. *P<0.05, **P<0.01, ***P<0.001 and ns = not significant compared to vehicle control group. EAKP = ethyl acetate fraction of *K. pinnata*.

Table 4: Screening of diuretic potential of *B. nutans* ethyl acetate fraction on rats

Treatment Group (mg/kg, P.O)	Urine content					Na^+/K^+ ratio	% increase in Na^+ excretion
	Volume (ml)	pH	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)		
Vehicle control	9.80 ± 0.39	5.72 ± 0.32	102.33 ± 9.68	52.24 ± 2.72	82.14 ± 3.66	1.96	-
Frusemide (10)	15.36 ± 0.92***	5.61 ± 0.82 ns	176.56 ± 10.72**	92.51 ± 4.49**	130.26 ± 8.58**	1.91	72.53
Cystone (750)	13.66 ± 0.72**	5.85 ± 0.54 ns	146.34 ± 9.66*	79.62 ± 3.53**	97.51 ± 5.82ns	1.84	43.00
EABN(25)	10.98± 0.41 ns	5.95± 0.46 ns	122.55 ± 8.66ns	64.81 ± 3.55ns	74.56 ± 4.66ns	1.89	19.75
EABN (50)	11.66± 0.54 ns	5.88 ± 0.40 ns	137.18 ± 8.65ns	78.52 ± 3.64**	80.19 ± 2.84ns	1.75	34.05
EABN (100)	12.78± 0.56*	6.27 ± 0.58 ns	159.39 ± 9.74**	89.93 ± 5.73**	102.33 ± 5.75ns	1.77	55.76

All values are M ± SEM of 6 animals in each group. *P<0.05, **P<0.01, ***P<0.001 and ns = not significant compared to vehicle control group. EABN = ethyl acetate fraction of *B. nutans*.

All the plants *C. dactylon, E. officinalis, K. pinnata* and *B. nutans* has showed high sodium (Na^+) and potassium (K^+) excretion potential where as high chloride (Cl^-) excretion ability was observed *K. pinnata* followed by *C. dactylon* and *E. officinalis*. Significant effect on total urine volume was observed in all extracts but *C. dactylon* and *K. pinnata* ethyl acetate fraction had shown most prominent response. As per the observed diuretic profile the plants can be graded descending as *K. pinnata*, *C. dactylon*, *E. officinalis* and *B. nutans* for diuretic potential. The potential of the observed results showed that the most effective diuretic plant is *K. pinnata* and *C. dactylon* (Figure 1).

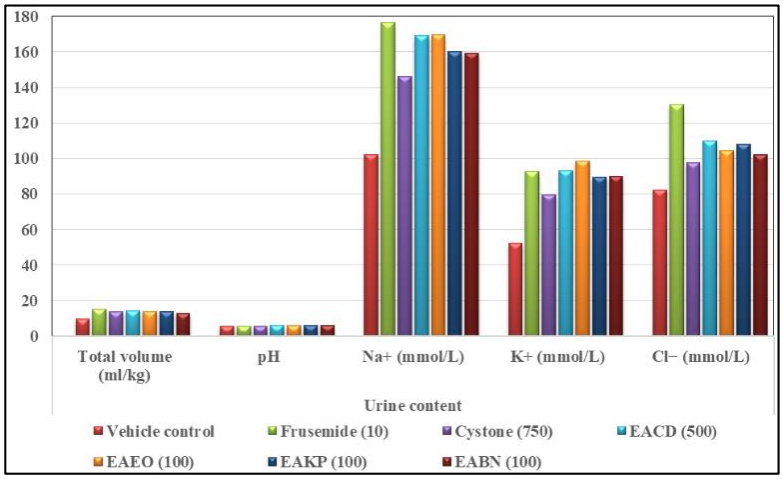

Fig 1: Comparative diuretic potential of *C. dactylon, E. officinalis, K. pinnata* and *B. nutans* ethyl acetate fraction at high dose level

Discussion

Diuretics enhance the amount of water and ion excretion in urine to maintain the balance and composition of body fluids in variety of clinical condition. Drug-induced diuresis is useful in several life-threatening conditions like internal organ failure, nephritic syndrome, cirrhosis, failure, toxemia of physiological condition, premenstrual tension and high blood pressure (Pandya *et al*., 2012) [27]. The currently available thiazides and loop diuretics exhibit many adverse effects like electrolytes imbalance and metabolic alterations. Medicinal plants derived diuretics are scientifically verified to be very helpful for the treatment of mild to moderate high blood pressure. Diuretics relieve congestion and peripheral puffiness in patients having cardiovascular complications. These agents are also helpful in reducing volume over load and relieve dyspnea (Hullatti *et al*., 2011) [16]. Diuresis has two major effects i.e., to increase urine excretion volume and to induce a net loss of electrolytes in the urine. In this study, urine volume and electrolytes excreted were measured to judge the diuretics potential of the plant extracts. Animals were pretreated with water as in previous studies on diuretic agents have found it to be advantageous to 'pre-treat' or 'prime' the animals. Since diuretics are utilized clinically for the treatment of fluid retention, it will be extremely vital to demonstrate effectiveness in presence of electrolytes and water (Nedi *et al*., 2004) [24].

Diuresis has two different connotations, increase in urine volume per se and net loss of solute and water. This involves suppression of renal tubular reabsorption of electrolytes, water and low molecular weight organic substances into blood stream and consequently promoting the urine formation (Milton *et al*., 1970) [20]. *K. pinnata* and *C. dactylon* ethyl acetate fractions considerably increased urine volume acting as strong kaliuretic and natriuretic. The 6 hrs cumulative urine output induced by the *K. pinnata* (100 mg/kg), *C. dactylon* (500 mg/kg) and the standard drug were statistically high significant compared to control. *K. pinnata* induced brisk and significant diuresis by increasing both Na^+ and Cl^- level in urine. *E. officinalis* and *B. nutans* has good Na^+ and K^+ clearance ability but *E. officinalis* has moderate effect on Cl^- and urine volume where in *B. nutans* showed minimal increase in urine volume with no effect on Cl^- level in urine. The data presented in the study indicate that *K. pinnata* and *C. dactylon* ethyl acetate fractions contained phytochemicals that mediates diuretic effect by increasing the rate of urine output as well as electrolyte excretion primarily potassium. Quantitative phytochemical evaluation of *C. dactylon*, *E. officinalis*, *K. pinnata* and *B. nutans* ethyl acetate fraction for total flavonoid and polyphenol showed that *K. pinnata* had the highest content followed by *E. officinalis* and *C. dactylon* and *B. nutan* (Sohgaura *et al*., 2018) [33].

Hydroalcoholic extract of *K. pinnata* leaves were administered to male Wistar rats by oral and intraperitoneal route at the doses of 100, 300, 500 and 800 mg/kg. The effect on urine output was determined by comparing the urine volume collected by keeping individual animal in metabolic cages. Antiurolithiatic effect was determined by comparing urinary electrolyte levels, biochemical parameters and kidney histology with control and standard drug treated animals. Plant extract was found to exert significant diuretic and antiurolithitic activity (Patil *et al*., 2008) [28]. The reduced oxalate excretion in urine causes the formation of calcium oxalate stones. Fresh juice extracted from the leaves of *K. pinnata* was administered to patients having kidney stones. Regular intake of the juice effectively dissolved the stones regardless of its position, nature and previous treatments. There was an increase in the quantity of urine excreted, thus showing the diuretic nature of the juice. It also facilitated the decrease in oxalate excretion, while increasing citrate excretion. This study suggests that the juice may have antilithiatic properties (Gahlaut *et al*., 2012) [14]. Regular intake of the *B. pinnatum* leaves aqueous extract effectively dissolved the stones despite its position, nature and former treatments. This study suggests that the *B. pinnatum* leaf juice have diuretic properties (Gahlaut *et al*., 2012; Shukla *et al*., 2014) [14, 32].

In traditional system of medicine *C. dactylon* plant is used as diuretic in cases of dropsy as an astringent in cases of chronic diarrhea and dysentery. The oral administration of aqueous extract of *C. dactylon* root stalk has shown significant

increase in the urine volume at 100, 250, 500 and 750 mg/kg dose levels, clearly indicating diuretic activity in Albino rats (Shivalinge *et al*., 2009) [31]. Diuretic activity of *C. dactylon* has been investigated following oral administration of different concentrations of its extracts (125, 250 and 500 mg/kg body weight) along with the reference drug furosemide (15 gm/kg) to hydrated male Wistar rats. Furthermore, researchers also studied the toxicological effect of the same plant. The results showed that *C. dactylon* at 500 mg/kg dose showed significant increase in urinary output and electrolytes excretion. No lethality was observed among animals when *C. dactylon* was administered up to 1000 mg/kg, but caused 50% death of rats indicating LD_{50} at 4500 mg/kg. Aqueous extract administered at the dose of 500 gm/kg induced highly significant effect on urinary output of water and electrolytes and justified its use as diuretic remedy in traditional medicine (Sadki and Atmani, 2010) [30]. In recent study, *C. dactylon* crude extract at 2.5 ml/kg body weight dose possessed nearly similar diuretic effect as that of standard drug providing a quantitative evidence that *C. dactylon* has potential diuretic activity (Aruna *et al*., 2013a) [3]. Aruna *et al*. (2013b) [2] evaluated the diuretic activity of *C. dactylon* extract in guinea pigs and observed that administration of crude extract increase urine output compared to control group.

Amla fruits are acrid, cooling, astringent, diuretic and laxative as per traditional literature (Ambasta, 1996; Chopra *et al*., 2002) [9, 7]. The fruit of *E. officinalis* have diuretic effect (Anonymous, 2006) [1]. The crude powder, liquid extract and dry extract of *Phyllantbus niruri* commonly known as Bhuianla or Jaramla were found to have diuretic activity in rats (Devi *et al*., 1986) [11]. *E. officinalis* have potent nephroprotecive effect on renal dysfunction involved in oxidative stress during the aging process (Yokozawa *et al*., 2007) [38]. Amla is reported to possess potent free radical scavenging, antioxidant, anti-inflammatory, anti-mutagenic, immunomodulatory activities, which are efficacious in the prevention and treatment of various diseases like cancer, atherosclerosis, diabetes, liver and heart diseases (Dasaroju and Gottumukkala, 2014) [10].

Diuretic activity of *B. nutans* has not been reported earlier though in Ayurveda, the leaves, stem and roots of *Bambusa aurundinacea* Retz. a plant of bamboo spices is used as astringent, laxative and as diuretic (Mohan and Gopal, 1981) [21]. *Bambusa vulgaris* resin (tabasheer, banshalochan) is consider to have astringent, expectorant, cardiotonic, haemostatic, aphrodisiac and diuretic properties. Bamboo shoots are one of the best sources of phenolic compounds in the plants and acts as a natural diuretic and helps to get rid of excess salts. (Nirmala *et al*., 2014). The leaves of *B. nutans* and *B. vulgaris* are rich sources of phenolic compounds and natural antioxidants (Tripathi *et al*., 2015) [36].

Recent study on the alkaloids of thalictrum species signifies its potential diuretic activity, based on data indicating increased Na^+ and K^+ urinary elimination signifying furosemide like activity. Alkaloids of benzyl isoquinoline type also showed hypotensive potential (Erdemgil *et al*., 2001) [12]. Some flavonoids were found to increase urinary elimination of Na^+ and K^+ significantly binding with adenosine A1 Receptor which is closely associated with diuretic mechanism (Yuliana *et al*., 2009) [39]. The phytoconstituents like terpenoids, polyphenols and flavonoids have been reported to possess potent diuretic activity (Thambi *et al*., 2008) [34]. Flavonoids promote high levels of Na+ and K+ excretion in urine. There is a direct relationship between the volume of urine and the concentration of Na+, and through this mechanism diuretic effect is produced due to decreased re-absorption of Na+ ion in renal tubule (Vishal *et al*., 2012) [37]. *Nigella sativa* (Black Cumin) and *Nigella damascena* (Lady-in-a-Mist) seeds were found to contain phenolic compounds with antioxidant and diuretic effects. *N. sativa* extract exhibited a higher natriuretic than kaluretic effect and similar uricosuric effect and *N. damascene* showed decreased Na+ excretion (Toma *et al*., 2015) [35]. Flavonoid content of *Marchantia convoluta* dried leaves possessed distinct antimicrobial, anti-inflammatory and diuretic effect in mice (Jianbo *et al*., 2005) [18].

The diuretic potential of studied plants *K. pinnata*, *C. dactylon* and *E. officinalis* ethyl acetate fractions may have occurred through any of these possible mechanisms as rich presence of flavonoids and polyphenols was reported earlier. The precise site, molecule and cellular mechanisms of the diuretic action of these fractions remain to be elucidated. The present study supports the use of *K. pinnata*, *C. dactylon* and *E. officinalis* plants for diuretic potential in traditional Ayurvedic medicine practice.

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