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Review Article

Diuretic Activity of Psidium Guajava Pulp Extract on Albino Rats

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ABSTRACT

There is increasing interest in the health and wellness benefits of herbs and botanicals. Natural medicine is a precious resource of therapeutically active components compounds and has increasingly attracted the attention of researchers; further many studies have reported that herbal diuretics might be a useful tool in the treatment of hypertension and chronic kidney diseases. There are a growing number of studies purporting diuretic effects with traditional medicines. Diuretics drugs increase the rate of urine flow and adjust the volume and composition of body fluids. Drug-induced diuresis is beneficial for the treatment of many maladies such as congestive heart failure (CHF), chronic renal failure, nephritis, cirrhosis, hypertension and pregnancy-induced toxemia. Medicinal herbs are the significant source as Diuretics. Mono and poly-herbal preparations have been used as diuretics. In present study diuretic activity of Psidium Guajava Pulp Extract on Albino Rats was evaluated using modified LIPSCHITZ Test. Furosemide is used as model drug in present work. Observation shows that there is significant increase in increased the urine output along with an increase in elimination of Sodium, Potassium, and Chloride ions after administration of pulp extract of Psidium Guajava.

INTRODUCTION

There is increasing interest in the health and wellness benefits of herbs and botanicals. Natural medicine is a precious resource of therapeutically active components compounds and has increasingly attracted the attention of researchers; further many studies have reported that herbal diuretics might be a useful tool in the treatment of hypertension and chronic kidney diseases. There are a growing number of studies purporting diuretic effects with traditional medicines.

Diuretics drugs increase the rate of urine flow and adjust the volume and composition of body fluids. Drug-induced diuresis is beneficial for the

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treatment of many maladies such as congestive heart failure (CHF), chronic renal failure, nephritis, cirrhosis, hypertension and pregnancy-induced toxemia. Medicinal herbs are the significant source as Diuretics. Mono and poly-herbal preparations have been used as diuretics. According to one estimate, more than 650 mono and poly-herbal preparations in the form of decoction, tincture, tablets and capsules from more than 75 plants are in clinical use. The various methods for screening of diuretic agents provides useful tool to evaluate the safety and effectiveness of the drugs. It is also useful for determining the dose level of particular class of diuretic agents. [1 - 5]

High blood pressure represents an important risk factor to development of other cardiovascular diseases and constitutes one of the main causes of mortality in the world.[6] Diuretic compounds that stimulate the excretion of water are potentially useful in most of disorders including those exhibiting oedema such as congestive heart failure, nephritis, toxemia of pregnancy, premenstrual tension and hypertension.[7] Diuretic can also increase the elimination of electrolytes.[8] The modern era of diuretic therapy began in 1949 when sulphanilamide was discovered to possess diuretic and natriuretic properties.[9] The net excretory effect of diuretic agents causes changes in urine flow, pH, and ionic compositions of urine and blood.[10]

However, many of the diuretics currently used in clinical practice have been associated with a number of adverse effects, including electrolyte imbalance, metabolic alterations, the onset of diabetes, activation of the renin- angiotensin and neuroendocrine systems, and impairment of sexual function. Therefore, it is important to consider alternatives that have greater effectiveness and fewer side effects. Many of the herbs used in folk medicine have yet to be scientifically evaluated for their effectiveness and safety[11]

For thousands of years, humans have been using diuretics to reduce the water retention caused by some health conditions such as high blood pressure, heart diseases or pre-menstrual syndrome, among others. Although there is a wide therapeutic stock of synthetic drugs that belong to this pharmacological group, a considerable amount of decoctions and in fusions of medicinal plants are used to reduce fluid retention.But the diuretic effectiveness of this kind of medicinal plants needs to be experimentally proved, because diuresis could be influenced not only by the form of administration which implies the consumption of a great amount of liquids that can provoke an increase in the volume of urine excreted without a true evidence of a diuretic action, but also by the difficulty of obtaining reproducible data involving a larger number of animals.[11]

Table 1: Classification and adverse effects of diuretics [12,13]

Class of diuretic	Examples	Adverse Effects
Thiazides and related diuretics	Bendroflumethiazide Chlorthalidone Cyclopenthiazide Indapamide Metolazone Xipamide	Orthostatic hypotension. Decreased serum Na^+, K^+, Mg^+, and H^+. Modest increases in Ca^{2+}. Increases in serum uric acid, glucose, cholesterol, LDL, and triglycerides. Erectile dysfunction, impotence, and lithium accumulation.

Carbonic anhydrase inhibitors	Acetazolamide	Volume depletion, hypokalemia, hyperchloremic metabolic acidosis, light-headedness, weakness, and confusion
Loop diuretics	Furosemide Bumetanide Torasemide	Volume depletion, decreased serum K^+, Na^+, Mg^+, and H^+. Increased uric acid, glucose, cholesterol, LDL, and triglycerides. Nausea, ototoxicity, and allergic interstitial nephritis.
Osmotic diuretics	Mannitol	Low volume, K^+, and H^+. CHF, headache, nausea, vomit, fever, confusion, and lethargic state.
Potassium-sparing diuretics	Amiloride Triamterine	Increase serum K^+, Cl^-, & H^+. Nausea, flatulence & skin rash with amiloride or triamterene, nephrolithiasis with triamterene. Gynecomastia& decreased libido in men with spironolactone
Potassium-sparing diuretics and aldosterone antagonists	Spironolactone Eplerenone	

PLANTS EVALUATED FOR DIURETIC ACTIVITY:

Human beings utilize many species of flora for food and medicine. It is also expected that the traditional and modern medicine uses about 50,000 - 70,000 species of plants.[8] Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness.[4]Medicinal plants can be significant sources of undiscovered chemical substances with potential therapeutic effects. In fact, the World Health Organization has estimated that over 75% of the world's population still relies on plant-derived medicines, usually obtained from traditional healers, for basic healthcare needs.[14] Regardless of their specific pharmacological effects, most medicinal plants have been described as having a more or less pronounced diuretic effect. However, very few studies have examined the mechanisms of action of inducing renal excretion.[6] Some of the diuretics are derived from medicinal plants and a vast number of medicinal plants mentioned in Ayurvedic system of medicine are known to possess diuretic properties such as Abelmoschusesculentus, Bacopamonnieri, Barbara vulgaris and Cissampelospareira.[15]

Number of species and genuses reporting diuretic effects. of these, the most promising, at the present time, are the speciesFoeniculumvulgare, Fraxinus excelsior, Hibiscus sabdariffa, Petroselinumsativum and Spergulariapurpurea, and species from the genusesCucumis (Cucumismelo and Cucumistrigonus), Equisetum (Equisetum bogotense, Equisetum fluviatile, Equisetum giganteum, Equisetum hiemale var. affine and Equisetum myriochaetum), Lepidium (Lepidiumlatifolium and Lepidiumsativum), Phyllanthus (Phyllanthusamarus,Phyllanthuscorcovadensis and Phyllanthussellowianus) and Sambucus (Sambucusmexicana and Sambucusnigra).

So, apart from this *Psidium guajava,* Guava is highly nutritious and a good source of calcium, iron, and phosphorus. The vitamin C content of guava fruit is 2–5 times that of citrus. The plant has many medicinal properties mainly due to its bioactive phytoconstituents. This plant finds applications for the treatment of diarrhea, dysentery, gastroenteritis, hypertension, diabetes, caries and pain relief and for improvement in locomotors coordination. Its fruit is rich in vitamins A, C, iron, phosphorus and calcium and minerals. It contains high content of organic and inorganic compounds like secondary metabolites

e.g. antioxidants, polyphenols, antiviral compounds, anti-inflammatory compounds. The phenolic compounds in guava help to cure cancerous cells and prevent skin aging before time. The presence of terpenes, caryophyllene oxide and *p*-selinene produces relaxation effects. Guava leaves contain many compounds which act as fungistatic and bacteriostatic agents. Guava has a high content of important antioxidants and has radio-protective ability.

So, keeping the above all the things in view, it was found that there were no research conducted on the diuretic activity of Psidium guajava and also due to presence of the antioxidant and polyphenolic constituents, the present study is planned to evaluate the Diuretic potential of Psidium guajava.[15]

PSIDIUM GUAJAVA L. is common guava, yellow guava, or lemon guava. *Psidium guajava* is considered a native to Mexico which further developed in South America, European, Africa and Asia**.** Historically this has been widely used in Peru since pre-Columbian times.[18] These are characterized by a low content of carbohydrates (13.2 %), fats (0.53 %), and proteins (0.88 %) and by a high water content (84.9 %), Food value per 100 g is: Calories 36–50 kcal, moisture 77–86 g, crude fibre 2.8–5.5 g, ash 0.43–0.7 g, calcium 9.1–17 mg, phosphorus, 17.8–30 mg, iron 0.30–0.70 mg , vitamin A 200–400 I.U., thiamine 0.046 mg, riboflavin 0.03–0.04 mg, niacin 0.6–1.068 mg, ascorbic acid 100 mg, vitamin B3 40 I.U. Manganese is also present in the plant in combination with phosphoric, oxalic and malic acids. Hexanal (65.9 %), butyrolactone (7.6%), (E)-2-hexenal (7.4 %), (E,E)-2,4-hexadienal (2.2%), (Z)-3-hexenal (2 %), (Z)-2-hexenal (1 %), (Z)-3-hexenyl acetate (1.3 %) and phenol (1.6 %) were reported from fresh white-flesh guayaba fruit oil. 3-caryophyllene (24.1 %), nerolidol (17.3%), 3-phenylpropyl acetate (5.3 %) and caryophyllene oxide (5.1 %) were isolated from essential oil extracted from the fruits. Subsequently, the active aromatic constituents in pink guava fruit the 3-penten-2-ol and 2-butenyl acetate were isolated. The fruit also contains glykosen 4.14 %, saccharose 1.62 %, and protein 0.3 %. The unripe fruit is high in tannins, is astringent and has a tendency to cause constipation, but it is sometimes employed in diarrhoea.The main traditional use known is as an anti-diarrhoeal. Other reported uses include gastroenteritis, dysentery, stomach, antibacterial colic pathogenic germs of the intestine. [16]

Table 2: Pharmacological Study [16]

Plant Part	Activity	Preparation(s)
Ripe fruit, flowers, and leaves	Anorexia, cholera, diarrhoea, digestive problems, dysentery, gastric insufficiency, inflamed mucous membranes, laryngitis, mouth (swelling), skin problems, sore throat, ulcers, vaginal discharge	Mashed, Decoction
Shoots, leaves, bark and leaves mixed, rip fruits	Febrifuge, expel the placenta after childbirth, cold, cough hypoglycaemic, affections of the skin, caries, vaginal haemorrhage, wounds, fever, dehydration, respiratory disturbances.	Decoction, poultice
Leaf, bark, unripe fruit, roots	Astringent, ulcers, wounds, diarrhea	Decoction and poultice

Plant Part	Activity	Preparation(s)
Leaves, roots, ripe fruit	Diarrhoea, coughs, stomach-ache, dysentery, toothaches, indigestion, constipation	Juice, the leaves are pounded, squeezed in salt water
Leaves	Cough, diarrhoea, stomach ache, hypertension, antiseptic, diabetes mellitus, Febrifuge, antispasmodic, rheumatism, convulsions, astringent, Anti-inflammatory	Decoction or infusion
Flower buds	Heart and constipation, conjunctivitis, cough, diarrhoea, digestive problems, dysentery, oedema, gout, haemorrhages, gastroenteritis, gastritis, lung problems, shock, vaginal discharge, vertigo, vomiting, worms	Infusion or decoction
Shoots, roots	Shoots, roots	Infusion or decoction
Whole plant, shoots	Skin tonic, painful menstruation, miscarriages, uterine bleeding, premature labour in women, wounds	Infusion or decoction, paste

DIURETICS drugs increase the rate of urine flow and adjust the volume and composition of body fluids. Drug-induced diuresis is beneficial for the treatment of many maladies such as congestive heart failure (CHF), chronic renal failure, nephritis, cirrhosis, hypertension and pregnancy-induced toxemia. [17]

FUROSEMIDE [17, 18]

Furosemide is a loop diuretic used to treat hypertension and edema in congestive heart failure, liver cirrhosis, renal disease, and hypertension. It is an anthranilic acid derivative. Furosemide promotes diuresis by blocking tubular reabsorption of sodium and chloride in the proximal and distal tubules, as well as in the thick ascending loop of Henle. This diuretic effect is achieved through the competitive inhibition of sodium-potassium-chloride co-transporters expressed along these tubules in the nephron, preventing the transport of sodium ions from the lumenal side into the basolateral side for reabsorption. *Dose:For edema* the usual initial dose of is 20 to 80 mg given as a single dose. The dose may be carefully titrated up to 600 mg/day in patients with clinically severe edematous states. *For Hypertension* the usual initial dose is 80 mg, usually divided into 40 mg twice a day.

DIURETIC ACTIVITY IN RATS (LIPSCHITZ Test) [19 – 25]

The Lipschitz test has been proven to be a standard method and a very useful tool for screening of potential diuretics. A method for testing diuretic activity in rats has been described by Lipschitz et al. (1943). The test is based on water and sodium excretion in test animals and compared to rats treated with a high dose of urea. A method using rats for estimation of antidiuretic potency was described by Burn in 1931. This method or a modification of it has been used for diuretic assays by most of the subsequent workers. In 1943, Lipchitz et at. described a method suitable for diuretic assay using several commonly used diuretics. This method by itself is a modification of Burn's method. Since 1943, most of the workers have used the method of Lipschitz with some Modifications.

MATERIALS AND METHODS

ANIMALS:

Albino rats (Wistar) weighing 150-200 g were used in the study. Animals were acquired from Dr. V. V. P. F's College of Pharmacy, Ahmednagar. Animal house and were acclimatized for one week under laboratory conditions. The animals were

housed in groups of six in polypropylene cages and maintained at an ambient temperature under 12 hours of light/ dark cycle. They were provided with commercial food pellets and tap water was provided. Ethical clearance for handling the animals was obtained from the institutional animal ethics committee before the beginning of the project work. (1670/PO/ReBiBt/S/12/CPCSEA).

METHOD FOR EVALUATION OF DIURETIC ACTIVITY[26, 27]

The Lipchitz method was employed for the assessment of the Diuretic activity. 24 hours fasted rats were used for the Diuretic activity. The rats were divided into 6 groups of 6 animals each.

Group I served as a control and received normal saline orally (25ml/kg). Group II animals served as Standard and received the same amount of normal saline intraperitoneally in which Furosemide at a dose of 20mg/kg b.w. was dissolved. The other groups received normal saline orally (25ml/kg) in which Psidium guajava pulp extract were dissolved. Group III animals received 200mg/kg p.o.of pulp extract, Group IV animals received 400mg/kg p.o. of pulp extract,

All the drugs were freshly prepared prior to administration. Each of these preparations were given in such manner so that the fluid intake was the same in all cases. After dosing, different groups of animals were placed into different metabolic cages. These metabolic cages are specially designed to separate urine and feces. The volume of urine was collected and measured at the end of the 5 and 24 hours respectively. During this period, no water and food was made available to animals.

ANALYSIS OF URINE:

The amount of concentration of Na +, K+ and cl- in the urine sample were determined with the help of flame photometer.

Computation of Diuretic Parameters[25]:

a. Diuretic Index: Vt/ Vc
b. Lipschitz Value: Vt/ Vr
c. Saluretic Index: Ct/Cc
d. Na+/ K+ ratio :Cn/ Ck

Where,

Vt : urine volume of test group.
Vc : urine volume of control group.
Vr : urine volume of the reference group.
Ct : concentration of electrolyte in urine of test group.
Cc : concentration of electrolyte in urine of control group.
Cn : concentration of sodium ion in urine of group &
Ck : concentration of potassium in urine of same group

STATISTICAL ANALYSIS:

Arithmetic mean of the values of readings wear calculated for each experiment the result obtained was used for statistical analysis using INTA software. The data obtained from various models of diuretic activity of rats' experiments were subjected to analysis of variance (ANOVA) followed by Dunnett'-test using INTA software. The value of $p<0.001$ was considered statistically significant

RESULTS

EVALUATION OF DIURETIC ACTIVITY (LIPSCHITZ MODEL)

The diuretic activity was assessed by determination of Lipschitz value using the standard diuretic drug Furosemide. A significant

increase in the urinary excretion of electrolytes (Na^+ and K^+) over 24 hours in furosemide treated group. Furosemide treated group showed maximum diuretic effect (diuretic index 2.53; Lipschitz value 2.05) lasting over 24 h.

Animals were divided in total of three groups (n = 6 in each group). All animals were deprived of food and water 18 h prior to the experiment. On the day of experiment, the dosing was scheduled as follows.

Immediately after the dosing, animals were placed in metabolic cages and urine was collected up to 5 hours and 24 hours after dosing. Room temperature was maintained up to 25± 0.5 ^{0}C.During this period no water or food was made available to the animals.

Table 3: Urinary excretion data

Group	Dose (mg/kg)	5 h			24 h		
		Urine excretion (ml/100 g)	Diuretic index	Lipschitz value	Urine excretion (ml/100 g)	Diuretic index	Lipschitz value
Control (Group I)	Normal saline orally (25ml)	0.254 ± 0.02	1.00	--	1.201± 0.45	--	--
Furosemide (Group II)	20 i.p.	0.510 ± 0.025***	2.00	1.00	3.010± 0.6	2.50	1.00
Pulp extract (Group III)	200 p.o.	0.331± 0.03***	1.30	0.64	2.102± 0.45	1.75	0.58
Pulp extract (Group IV)	400 p.o.	0.502± 0.03***	1.97	0.98	2.915± 0.55	2.42	0.80

N = 6, Mean ± S.E.M. Significant, *** P < 0.001 Vs. Control

Table 4 Urinary excretion of electrolytes (5 h)

Group	Dose (mg/kg)	Concentration of ions (mEqu/100 ml/100 g)			Saliuretic index			Na^+/K^+
		Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	
Control (Group I)	Normal saline orally (25ml)	0.40 ± 0.06	0.15± 0.01	0.59± 0.03	1.00	1.00	1.00	2.66
Furosemide (Group II)	20 i.p.	0.78 ± 0.05***	0.33± 0.02***	1.11± 0.04***	1.95	2.2	1.88	2.36
Pulp extract (Group III)	200 p.o.	0.66± 0.04***	0.24± 0.01**	0.88± 0.05***	1.65	1.6	1.49	2.75
Pulp extract (Group IV)	400 p.o.	0.72± 0.06***	0.33± 0.03***	0.95± 0.06***	1.8	2.2	1.61	2.18

Values are the mean ± S.E.M. of six rats / treatment. Significant **P < 0.05, *** P < 0.001 Vs. Control.

Table 5: Urinary excretion of electrolytes (24 h)

Group	Dose (mg/kg)	Concentration of ions (mEqu/100 ml/100 g)			Saliuretic index			Na^+/K^+
		Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	
Control (Group I)	Normal saline orally (25ml)	2.91± 0.09	1.02± 0.02	3.55± 0.1	1.0	1.0	1.0	2.85
Furosemide (Group II)	20 i.p.	5.55± 0.2**	1.52± 0.02 **	5.35± 0.06**	1.91	1.49	1.51	3.65

Group	Dose (mg/kg)	Concentration of ions (mEqu/100 ml/100 g)			Saliuretic index			Na^+/ K^+
		Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	
Pulp extract (Group III)	200 p.o.	4.81± 0.08**	1.15± 0.03 **	4.78 ± 0.08**	1.65	1.13	1.35	4.18
Pulp extract (Group IV)	400 p.o.	5.55± 0.09**	1.48± 0.04**	5.55± 0.1**	1.91	1.45	1.56	3.75

Values are the mean ± S.E.M. of six rats / treatment. Significant ** P < 0.001 Vs. Control.

In the present study, pulp extract was procured and evaluated for pharmacological activity. Rats were selected in the present study because the urinary system of rats resembles that of humans.The parameters like urine volume, concentration of electrolytes in the urine such as sodium, potassium and chloride were measured to assess the diuretic potential of all the groups.Diuresis occurs by mainly two phenomena including, net increase in urine volume (water excretion) and elevated excretion of electrolytes (solutes) in the urine. These processes result from suppression of renal tubular reabsorption of water and electrolytes into the blood stream.

Furosemide showed significant diuresis in rats over a period of 24 h. significant rise in urine volume and electrolyte concentration was observed.

DISCUSSION

Diuretic activity:

URINARY EXCRETION DATA (at 5 h)

Extracts of *Psidium Guajava* pulp extract shows increased in urine volume and excretion of electrolytes. Results of extract at dose of 200 mg shows diuretic index of 1.30 and Lipschitz value 0.64 confirms presence of diuretic effect however its extent is less as compared with that of standard drug. Further increase in dose of extract to 400 mg shows diuretic index of 1.97 and Lipschitz value 0.98 confirms presence of diuretic effect which is comparable with that of standard drug. Results shows that increase in dose of extract from 200 mg to 400 mg shows increased in effect of diuresis.

URINARY EXCRETION DATA (at 24 h)

Extracts of *Psidium Guajava* pulp extract shows increase in urine volume and excretion of electrolytes. Results of *Psidium Guajava* pulp extract at dose of 200 mg shows diuretic index of 1.75 and Lipschitz value 0.58 confirms presence of diuretic effect however its extent is less as compared with that of standard drug. Further increase in dose of aqueous extract to 400 mg shows diuretic index of 2.42 and Lipschitz value 0.80 confirms presence of diuretic effect which is comparable with that of standard drug. Results shows that increase in dose of aqueous extract from 200 mg to 400 mg shows increased in effect of diuresis.

URINARY EXCRETION OF ELECTROLYTES

Urinary excretion of electrolytes as found in furosemide treated group and group treated with that of test (extract) shows similar excretion. However excretion in group treated with standard is more as compared with that of *Psidium Guajava* pulp extract at dose of 200 mg/kg and 400 mg/kg.

While, similar results obtained on electrolytic excretion by both the doses suggesting a difference in their comparative diuretic profile. Results showed a gradual rise in excretion of electrolytes (Na^+, K^+ and Cl^-) in a dose-dependent manner.

Higher dose of test (*Psidium Guajava* pulp extract) shows increase in urine excretion. Hence it is

observed that test (*Psidium Guajava* pulp extract) at 400 mg shows comparable results with that of standard.

CONCLUSIONS

In the present study, an attempt was made to evaluate diuretic activity of *Psidium Guajava* pulp extract using Wistar albino rats.

The pharmacological screening (diuretic activity) was carried out. The findings from present study support for use of *Psidium Guajava* pulp extract for its diuretic actions. *Psidium Guajava* pulp extract was selected depending on presence of Phytoconstituents. Two doses of the extracts were evaluated i.e. dose of 200 mg/kg and 400 mg/kg respectively.

Among these extract 400 mg dose shows more renal excretion as compared 200 mg dose. Based on the pattern of excretion of water and electrolytes, it is found that the *Psidium Guajava* pulp extracts has good diuretic activity when compared with that of Furosemide.

The main constituents of guava are vitamins, tanins, phenolic compounds, flavonoids, essential oils, sesquiterpene alcohols and triterpenoid acids. These and other compounds are related to many health effects of guava. [28-31] Some authors have found high concentrations of carotenoids (beta-carotene, lycopene, and beta-cryptoxanthin), vitamin C and polyphenols in guava pulp.[32-33] Lycopene has been correlated with the prevention of cardiovascular damage because of its positive effects on dyslipidemia.[33-34] Ascorbic acid is recognized for its important antioxidant effects.[68] However, the contribution oftannins, flavonoids, terpenes, proteins and polyphenolic compounds to diuretic effect cannot be ruled out because all these chemical constituents are known to be responsible for the diuretic activity.

In conclusion, it is evident from the above data that the evaluated parts of plant shows potential diuretic which is comparable with that of Furosemide supporting folklore uses of these plants as traditional medicine. Although, the mechanism underlying this effect is still unknown, but it is apparently related to significant diuretic effects.

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