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Abstract

In this study physico-chemical nature and diuretic activity was evaluated to establish the purity and diuretic activity by comparing with the standard acetazolamide. Pulp of *Cucurbita maxima* is a common cost effective Indian dish, rich in nutrients. Physico-chemical parameters like ash values, extractive values and loss on drying were performed to find the purity. The hydro-alcoholic extract was prepared by extracting the powder in soxhlet apparatus for 36 hours. The laboratory qualitative analysis was done and constituents like carbohydrates, proteins, lipids, flavonoids, alkaloids and vitamin C were found. The two doses of extract (150 and 300 mg/kg) were given to the rats. Group I served as control, Group II served as standard and Group III and IV served as test. Electrolytes and urine volume was measured after 5 hours of treatment and the same was compared with the control and the standard drug acetazolamide. Physico-chemical parameters were in the limit and the extract shows significant ($p < 0.01$) diuretic activity at the dose of 300 mg/kg when compared with control.

Introduction

Medicinal plants can be important sources of unknown chemical substances with potential therapeutic effects. Besides, 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 health-care needs (Farnsworth et al., 1985). Herbal formulations involve use of fresh or dried plant parts. Correct knowledge of such crude drugs is very important aspect in preparation, safety and efficacy of the herbal product (Modi et al., 2010). In developed countries due to lack of documentation and stringent quality control, herbal drugs were not used much. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy (Thomas et al., 2008). Standardization is essential measure for quality,

purity and sample identification.

Diuretics are drugs that increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations. Drug-induced diuresis is beneficial in many life-threatening disease conditions such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, hypertension, and pregnancy toxemia. Most diuretic drugs have the adverse effect on quality of life including impotence, fatigue and weakness (Vanamala et al., 2012).

The plant *Cucurbita maxima* Duchesne (commonly known as pumpkin) belongs to the family of Cucurbitaceae is widely cultivated throughout the world for use as vegetable as well as medicine. Both fruits and the aerial parts are commonly consumed as vegetable. It is a large climbing herb, annual or perennial. Its aerial part consists of flexible succulent stem with trifoliate



leaves (Kirtikar et al., 2003). Traditionally it is used in most countries as anti-diabetic, antitumor, antihypertensive, anti-inflammatory, immunomodulatory and antibacterial agents (Caili et al., 2006).

Materials and Methods

Collection of pulp

The pulp of *C. maxima* was collected along with plant and authenticated in botanical survey of India, Coimbatore. The inner pulp was scrapped and dried in shade.

Determination of ash values (Divakar, 2005)

The ash remaining following the ignition of medicinal plants is determined by three different methods which measures, total ash, acid-insoluble ash, sulfated ash and water soluble ash.

Determination of extractive values

Extractive values are useful to evaluate the nature of constituents present in the crude drug. Water soluble extractive and alcohol soluble extractive values were calculated as per standard procedures.

Determination of moisture content (Loss on drying)

1.5 g of powder was taken in weighed porcelain dish and heated for 100 or 105°C. cooled and the difference in the weight was calculated as moisture.

Preparation of extract

The coarsely powdered pulp was packed in soxhlet apparatus and boiled for 36 hours with 1:1 ratio of alcohol, water, and then the extract was filtered, concentrated, weighed and stored in desiccators for further studies (Harbone, 1998).

Preliminary phyto-chemical analysis (Sahu et al., 2010)

The hydro-alcoholic extract was subjected to the preliminary phytochemical investigation for the detection of carbohydrates, proteins, lipids, flavonoids, alkaloids, vitamin C and vitamin E (Hancock et al., 2003; Tadmor et al., 2005).

Animals

Twenty four healthy adult albino rats weighing 150-220 g were used. Animals were divided into 4 groups comprised six animals (n = 6). They were housed in standard environmental conditions. The rats were fed with standard diet and water *ad libitum*. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee and were in accordance with the Institutional ethical guidelines.

Diuretic activity

Fifteen hours prior to the experiment food and water

were withdrawn. Diuretic activity was measured by collecting total excreted urine (0-5 hours) of rat kept in metabolic cage. The cages together with the funnel and measuring cylinder used in the studies were coated with liquid paraffin before each experiment to facilitate the collection of urine with a minimum loss. Each animal was placed in metabolic cage provided with a wire mesh at the bottom and a funnel to collect the urine. Stainless steel sieves were placed in the funnel to retain feces and to allow the urine to pass. Rats were placed in metabolic cages individually as soon as the treatments started. The urine sample was collected for a total period of 5 hours.

Group I: Control group received 5 mL of 0.9% NaCl solution per kg body weight; Group II: Reference control group received acetazolamide (45 mg/kg body weight in 5 mL of 0.9% NaCl solution); Group III: Test group received *C. maxima* extract (150 mg/kg body weight in 5 mL of 0.9% NaCl solution suspending in 1% carboxy methyl cellulose); Group IV: Test group received *C. maxima* extract (300 mg/kg body weight in 5 mL of 0.9% NaCl solution suspending in 1% carboxymethyl cellulose).

The total volume of urine in 5 hours after the drug treatment was measured and the urine was subjected to estimation of total concentration of Na⁺, K⁺, and Cl⁻. Na⁺, K⁺ concentrations were measured by Flame photometry and Cl⁻ concentration was estimated by titration with silver nitrate solution (N/50) using three drop of 5% potassium chromate solution as indicator (Yar et al., 2009).

Results and Discussion

The physico-chemical values of the powder shows the purity (Table I). Moisture is an inevitable component of

Table I	
Physico-chemical parameters of <i>C. maxima</i> powder	
Parameters	Inference
Appearance	Powder
Colour	Yellowish white
Taste	Slight sweetish starchy
Odor	Characteristic
Total ash	16.6% w/w
Sulphated ash	26% w/w
Water soluble ash	4% w/w
Acid-insoluble ash	6.5% w/w
Water soluble extractive	5.8% w/w
Alcohol soluble extractive	19.2% w/w
Loss on drying	10% w/w

Table II

Effect of *C. maxima* extract on urinary output in rats

Treatment groups	Dose	Total urinary output	Normal saline intake	%Urinary excretion	Diuresis	
					Diuretic action	Diuretic activity
Control	1 mL/kg body weight in 5 mL of 0.9% NaCl	4.7 ± 0.2	4.9 ± 0.2	95.1	1.0	0.5
Acetazolamide	10 mg/kg body weight in 5 mL of 0.9% of NaCl	9.4 ± 0.2 ^b	4.8 ± 0.3	193.8	2.0	1.0
<i>C. maxima</i>	150 mg/kg body weight in 5 mL of 0.9% of NaCl	6.3 ± 0.3 ^a	5.0 ± 0.3	125.1	1.3	0.6
<i>C. maxima</i>	300 mg/kg in 5 mL of 0.9% of NaCl	9.6 ± 0.3 ^b	5.0 ± 0.2	192.7	2.0	1.0

Values are in mean ± SEM; ^ap<0.05 Vs control; ^bp<0.01 Vs Control; Urinary excretion = Total urinary output /total liquid intake x 100; Diuretic action = Urinary excretion of treated group/urinary excretion of control group; Diuretic activity = Diuretic action of treated group/diuretic action of standard group

Table III

Effect of *C. maxima* extract on urinary electrolytes excretion in rats

Treatment groups	Dose	Electrolytes (meq/L)			Na ⁺ / K ⁺
		Na ⁺	K ⁺	Cl ⁻	
Control	1 mL/kg body weight in 5 mL of 0.9% NaCl	192.7 ± 17.4	4.4 ± 0.2	113.3 ± 3.2	43.6
Acetazolamide	10 mg/kg body weight in 5 mL of 0.9% NaCl	254.5 ± 5.5 ^b	6.1 ± 0.2 ^b	155.2 ± 5.2 ^b	41.5
<i>C. maxima</i>	150 mg/kg body weight in 5 mL of 0.9% of NaCl	203.5 ± 6.7 ^a	4.3 ± 0.2	131.0 ± 3.8 ^a	33.5
<i>C. maxima</i>	300 mg/kg body weight in 5 mL of 0.9% of NaCl	257.2 ± 10.2 ^b	6.1 ± 0.1 ^b	148.0 ± 2.2 ^b	42.3

Values are in mean ± SEM; ^ap<0.05 Vs control; ^bp<0.01 Vs control

crude drugs, which must be eliminated as far as practicable. Deterioration time of the crude drugs depends upon the amount of water present in formulation. If the water content is high, the crude drugs can be easily deteriorated due to fungus and the moisture content of the crude drugs was found to be 10% w/w which signifies that the material was absorb water from the moisture due to the presence of carbohydrate, so it should be properly dried and properly stored. Pulp contains carotenoids and vitamin C (Nawirska-Olszańska and Biesiada, 2011) the compound which can be affected by light so it should be stored without exposing to the sunlight. The total ash value was found to be 16.6% w/w which includes physiological and non-physiological ash. Total ash usually consists of phosphates, carbonates, silicates and silica.

Water soluble ash was 4% w/w, sulphated ash was 26% w/w and acid-insoluble ash was 6.5% w/w. More value of sulfated ash indicates the presence oxides and carbonates which are converted into sulfates (Chaudhri, 1996).

Water soluble extractive value and alcohol soluble extractive values were found to be 5.8 and 19.2% w/w respectively indicates the nature and solubility of the chemical constituents.

The diuretic activity of *C. maxima* was measured in rats by administered the extract in two doses (150 mg/kg and 300 mg/kg). Hydro-alcoholic extract of *C. maxima* at 150 mg/kg showed less significant (p<0.05) diuretic activity and 300 mg/kg showed more significant (p<0.01) diuretic activity when compared with control (Table II).

Diuretic action of the extract at 300 mg/kg was as potent as the standard drug acetazolamide. When compared the electrolyte excretion efficiency of extract with the standard, 300 mg/kg shows more excretion of Na⁺, Cl⁻ and K⁺. But 150 mg/kg shows less excretion (Table III).

These two processes are happened by the suppression of renal tubular reabsorption of electrolytes, water and low molecular weight organic compounds into the blood stream and a consequence; promote the forma-

tion of urine (De Stevens, 1963). This activity of *C. maxima* hydro-alcoholic extract was due to the presence of glycosides, flavonoids, polyphenols (Parial et al., 2009) and alkaloids (Chandra Kalyan et al., 2011). The activity of the extract could be dose dependent one. Further isolation and characterization needs to explore the diuretic potency of hydro-alcoholic extract of *C. maxima*.

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