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PHYTOCHEMICAL SCREENING AND DIURETIC ACTIVITY OF *ALLIUM SATIVUM* STEROIDAL AND TRITERPENOID SAPONIN FRACTION

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ABSTRACT

Allium sativum L. (Liliacea) is a perennial bulb with a tall, erect flowering stem. The bulb of the plant has been used in many parts of the world as a stimulant, carminative, antiseptic, expectorant, anthelmintic and diuretic. This study has been planned to assess the diuretic activity of fresh garlic bulb extract targeting the steroidal and triterpenoidal saponin content. The rats were randomly divided into 4 groups of 5 animal each as vehicle control (2 % tragacanth suspension), standard drug frusemide (20 mg/kg, p.o), and n-butanol extract (10 mg/kg and 20 mg/kg, p.o) treated. Urine was collected in a graduated cylinder and its volume was measured for next 5 hr. Na^+, K^+ and Cl^- concentrations were measured. Phytochemical analysis of *A. sativum* n-butanol fraction showed presence of steroids, triterpenoidal saponins and carbohydrates. At 20 mg/kg dose onset of diuresis and total volume of urine formed was significantly ($P<0.01$-0.05) higher. Fifth hour urine volume at 20 mg/kg dose was 9.3 ml as compared to 5.5 ml of control. Extract at 20 mg/kg dose produced 24.57% increase in Na^+ excretion against 132.65% increase by frusemide when compared to control signifying natriuretic and aquaretic response. The study confirmed the ethnopharmacological and Ayurvedic use of *A. sativum* as a diuretic agent.

INTRODUCTION: The medicinal plants have enormous commercial potential throughout the globe. In worldwide herbal boom, it is estimated that high quality phytomedicinals will provide safe and effective medication. In India, Ayurveda, Siddha, Unani etc. consist of large number of herbal remedies, being used from ancient times and having their potential therapeutic claims with quality control specifications. These medications, however, suffer from lack of standardization parameters and documentation based on scientific screening procedures. The evaluation of these herbal drugs is mostly based on phytochemical, pharmacological and allied approaches including various instrumental techniques.

With the emerging interest in the world to adopt and study the traditional system and to exploit their potentials based on different healthcare systems, the evaluation of the rich heritage of the traditional medicine is essential [1].

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Allium sativum L. (Liliacea) is a perennial bulb with a tall, erect flowering stem that grows to between 2 and 3 feet. The plant produces pink to purple flowers that bloom from July to September and the bulb is odiferecous. Garlic was valued as an exchange medium in ancient Egypt and its virtues were described in inscription on the Cheops pyramid. The folk uses of garlic have ranged from the treatment of leprosy in humans to managing clotting disorders in horses. Physicians prescribed the herb during the middle ages to cure deafness. The bulb of the plant has been used in many parts of the world as a stimulant, carminative, antiseptic, expectorant, anthelmintic and diuretic [2].

The bulbs contain an odorless sulphur containing amino acid called alliin (s-allyl-1-cysteine sulfoxide), which has no pharmacologic activity. The enzyme alliinase is released when the bulbs are grinded which results in the conversion of alliin to 2-propenessulforic acid, which dimerizes to form allicin. Allicin gives the pungent characteristic odour to crushed garlic and is believed to be responsible for some of the pharmacologic activity of the plant. Garlic's strong odour is largely due to sulphur-containing compounds (S-allylcysteine sulphoxide), which are also accounted for most of its medicinal properties [3]. Garlic contains a variety of effective compounds that exhibit anticoagulant (anti-thrombotic) [4, 5, 6], antioxidant [7], antidiabetic [8], antibiotic [9, 10, 11], hypocholesterolaemic [12] as well as cardioprotective [13].

Garlic is well known for its diuretic activity in traditional as well as in alternative therapy [14]. Pantoja et al. (1991, 1996, 2000) has done comprehensive research on diuretic activity of *A. sativum* (garlic) on anaesthetized dogs and rabbits. Administration of encapsuled garlic powder to anaesthetized dogs induced dose-dependent natriuretic and diuretic responses. Intravenous administration of chromato-graphically purified fractions of garlic targeting allicin to anaesthetized rabbits elicits diuretic-natriuretic responses. Purified fraction at 6 µg/kg, i.v. to anaesthesizeSd dogs showed biphasic diuretic and inhibitory effect on kidney Na, K-ATPase [15, 16, 17].

Many garlic preparations are commercially available, confusion remains because of the inconsistency of clinical-study results and the lack of scientific studies on individual products. Although not all of active ingredients of garlic are known, and allicin-like transient components are not directly active, research suggests that an allicin-free garlic preparation is also active and various effects of garlic may be attributed to it. Furthermore, various chemical constituents in garlic products, including nonsulfur compounds such as saponins, may contribute to the essential biological activities of garlic. The presence of steroid saponins has been detected in garlic extract by thin-layer chromatography [18]. Presence of alkaloids, steroids and triterpenes in aqueous extract of dried *A. sativum* are also reported [19].

Further studies are needed to confirm associated biological activities of *A. sativum* steroidal saponins. This study has been planned to assess the diuretic activity of fresh garlic bulb extract targeting the steroidal and triterpenoidal saponin content.

MATERIAL AND METHODS:

Extraction: *A. sativum* bulbs were purchased from local market. Two kg of air-dried bulbs of the garlic were cut into small pieces, dried and pulverized. The powdered bulbs were then soaked in hydroalcoholic solution (40:60) and heated at 40°C this was allowed to cool down. The recovered filtrate was then dried to concentrate the sample. The resultant yellowish colored crude extract was fractionated with n-butanol. The n-butanol fraction was separated, dried and used for the study.

Phytochemical Screening: The extract was subjected to different phytochemical tests to evaluate presence of major phytochemical constituents such as alkaloids, steroids, triterpenes, saponins, glycosides, tannins and carbohydrates [20, 21].

Experimental Animal: Animal study protocol was approved by Institutional Animal Ethical Committee of Radharaman College of Pharmacy, Bhopal. Laboratory breed Wistar Albino rats (150-200 gm) were grouped and housed in polycyclic cages with not more than six animals per cage and maintained under standard laboratory conditions of 12 hr. light and dark cycle with relative humidity 55±5% and at 25±2°C. They were allowed to free access for standard dry pallet diet (Trimurti Feeds, Bhopal) and water *ad libitum*.

Acute Toxicity study: Healthy adult nulliparous and non-pregnant female Wistar Albino rats (150-200 gm), 8-12 weeks old were employed. Test substance was suspended in 2% tragacanth in distilled water, freshly prepared, the volume administered was 1 ml/100 gm body weight. The animals were fasted overnight with free access to water, weighed before dosing and test substance administered. After drug administration food were withheld for 3-4 hours. Animals were observed individually during first 30 minutes after dosing, periodically during 24 hours with special attention given during first 4 hours and daily thereafter for total of 14 days. All observations were systemically recorded with individual records being maintained for each animal [22, 23].

Limit test: Literature search reveals that *A. sativum* is a commonly used food and nutracutical used in Indian traditional and alternative system of medicine and most likely to be nontoxic. As n-butanol fraction of ethanolic extract was the study drug it was decided to start the limit test from 500 mg/kg dose. One animal was administered the test dose and observed for 48 hours. The animal survives, so 2 additional animals were dosed, one at a time and observed for 48 hours. All animals survived, but the animals were observed for further 14 days for check if there are any late deaths. As all 3 animals survived the LD_{50} is greater than the test dose 500 mg/kg. The *in vivo* diuretic study was conducted at a dose level of 10 and 20 mg/kg, as higher doses are likely to provoke hypotension, bradycardia and T-wave inversion [15].

Diuretic Potential study:

Experimental Protocol: The rats were randomly divided into 4 groups of 5 animal each as Group I- vehicle control given 2 % tragacanth suspension in distilled water 1 ml/kg (p.o); Group II- standard drug frusemide (20 mg/kg, p.o); Group III- n-butanol extract (10 mg/kg, p.o); Group IV- n-butanol extract (20 mg/kg, p.o). Animal 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 vehicle, standard drug and different doses of extracts 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 $25\pm0.5°C$ [24]. Urine was collected in a graduated cylinder and its volume was measured for next 5 hr. During this period no food and water was made available to animals. Collection time for first drop of urine and total volume of urine collected from both control and treated groups were measured. Cumulative urine excretion was calculated in relation to body weight and expressed as ml/100 gm body weight. Electrolyte (Na^+ and K^+) concentration and pH of collected urine was estimated at the end of the experimental period and expressed as Meq/100 gm body weight.

Measurement of Urine output and Analysis of Electrolytes: Na^+ and K^+ concentrations were measured using a digital flame photometer (Elico, India). The instrument was calibrated with standard solutions containing different concentration of Na^+ and K^+. Chloride ion concentration was estimated by titration with silver nitrate solution (N/50) using 3 drops of potassium chromate solution as indicator [25]. A pH meter (Jyoti Scientific, India) was used to measure the pH of freshly collected urine sample.

Statistical Analysis: The results were expressed as mean values ± SEM (standard error of mean) of 5 rats. Experimental data were analyzed using one way ANOVA followed by Turkey-Kramer multiple comparison test. P value less than 0.05 were considered statistically significant. Graph Pad Prism Version 3.02 was used for statistical calculations.

RESULTS:

Phytochemical Screening: Phytochemical analysis of *A. sativum* n-butanol fraction showed presence of steroids, triterpenoidal saponins and carbohydrates as major phytochemical constituents, and alkaloid and cardiac glycoside in slight quantity. Tannins, flavonoids, anthraquinone and cyanogenic glycosides were found to be absent (**Table 1**).

Acute Toxicity study: The LD_{50} is greater than the test dose 500 mg/kg. The *in vivo* diuretic study was conducted at a dose level of 10 and 20 mg/kg, as higher doses are likely to provoke hypotension, bradycardia and T-wave inversion [15].

TABLE 1: PHYTOCHEMICAL SCREENING OF N-BUTANOL FRACTION OF *ALLIUM SATIVUM* EXTRACT

Test for phytoconstituents	Observation
Carbohydrates	++
Alkaloids	+
Tannins	–
Flavonoid	–
Cardiac glycosides	+
Anthraquinone glycosides	–
Cyanogenic glycosides	–
Triterpenoidal Saponins	+++
Steroids	++

– = Compound not detected; + = compound detected.

Diuretic Activity: All animals were fed with priming dose of normal saline (25 ml/kg, orally). The mean basal urine output for control animals was 5.5 ± 0.84 ml over 5 hrs. Frusemide at 20 mg/kg induced a brisk and significant diuresis within 12 min of administration. A. sativum n-butanol fraction produced dose dependent diuretic activity. At 20 mg/kg dose onset of diuresis and total volume of urine formed was significantly (*P*<0.01-0.05) higher. Fifth hour urine volume at 20 mg/kg dose was 9.3 ml as compared to 5.5 ml of control as shown in **Table 2**.

The electrolyte changes induced by the standard drug, vehicle and different doses of extract are shown in **Table 3**. Frusemide induces diuresis on 12.3 min compared to normal urination at 36.4 min where as A. sativum at 20 mg/kg dose induces diuresis at 17.6 mins. The delay in onset of diuresis induced by the extract may be attributed to poor absorption of the active principles present in the crude preparations.

The *A. sativum* n-butanol extract induced a significant (P<0.05) increase in urinary Na^+ loss at 20 mg/kg dose with a Na^+/K^+ ratio of 1.590. Frusemide caused the expected increase in the renal excretion of Na^+, Cl^- and K^+ as a result urine Na^+/K^+ ratio was decreased to 1.440 as compared to 1.674 of control. The effect of *A. sativum* on sodium excretion was comparatively more on sodium than that of potassium. Extract at 20 mg/kg dose produced 24.57% increase in Na^+ excretion against 132.65% increase by frusemide when compared to control. *A. sativum* n-butanol extract had significant natriuretic and aquaretic responses in experimental animals.

TABLE 2: EFFECT OF *A. SATIVUM* N-BUTANOL FRACTION ON URINE OUTPUT IN RATS

Treatment (mg/kg, p.o)	Collection time for 1st drop of urine in min. (M ± SEM)	Total volume of urine on the 5th hr. (M ± SEM)
Vehicle control	36.4 ± 2.03	5.5 ± 0.84
Frusemide (20)	12.3 ± 0.73***	11.3± 1.04**
A. sativum n-butanol fraction (10)	23.5 ± 1.13*	7.5 ± 0.97^{ns}
A. sativum n-butanol fraction (20)	17.6 ± 0.97**	9.3 ± 0.84*

n = 6. *P<0.05, **P<0.01, ***P<0.001 and ns = not significant when compared to control group.

TABLE 3: EFFECT OF *A. SATIVUM* n-BUTANOL FRACTION ON URINARY ELECTROLYTE EXCRETION ON RAT

Treatment (mg/kg, p.o)	Electrolyte excretion				
	Na^+ in mEq/L (M ± SEM)	K^+ in mEq/L (M ± SEM)	Cl^- in mEq/L (M ± SEM)	Na^+/K^+ ratio	% increase in Na^+ excretion
Vehicle control	163.56 ± 8.32	97.69 ± 4.66	73.50 ± 4.07	1.674	---
Frusemide (20)	380.53 ± 11.14***	264.15 ± 10.71***	226.05 ± 10.75***	1.440	132.65
A. sativum n-butanol fraction (10)	183.25 ± 9.45^{ns}	122.30 ± 8.90^{ns}	75.80 ± 4.46^{ns}	1.498	12.03
A. sativum n-butanol fraction (20)	203.81 ± 8.12*	128.11 ± 10.08^{ns}	98.57 ± 4.02^{ns}	1.590	24.57

n = 6. *P<0.01, ***P<0.001 and ns = not significant when compared to control group.

DISCUSSION: The composition of the garlic bulbs is approximately 84.09% water, 13.38% organic matter and 1.53% inorganic matter. The phytochemicals responsible for the sharp flavor of garlic are produced when the plant's cells are damaged. When a cell is broken by chopping, chewing, or crushing, enzymes stored in cell vacuoles trigger the breakdown of several sulfur-containing compounds stored in the cell fluids. The resultant compounds are responsible for the sharp or hot taste and strong smell of garlic. Some of the compounds are unstable and continue to evolve over time.

Among the members of the onion family, garlic has by far the highest concentrations of initial reaction products, making garlic much more potent than onions, shallots, or leeks.

Diuretic herbs promote the formation of urine by the kidney. They help the body to get rid of excess of fluids, salts and toxic substances by increasing the rate of urine production. Diuretics work on two different ways: first way they reduce the water reabsorption in the nephrons of the kidney and second way they change the osmotic balance causing more water to be lost. Various herbs in diuretic category are *Achillea millefolium*, *Achyranthes aspera*, *Withania somnifera*, *Bacopa monniera*, *Boswellia serrate*, *Emblica officinalis*, *Lepidium sativum*, *Pinus roxburghii* and *Ziziphus jujube* [26]. Ursolic acid (a triterpene derivative) contributes to the diuretic action of the Bearberry Herb (Ericaceae) extract. Dandelion (*Taraxacum officinale*) root and leaves containing triterpenes extract exhibit pronounced diuretic effect.

Phytochemical screening of the n-butanol fraction of hydroalcoholic extract revealed the presence of steroid, triterpene and glycosides. It was quiet clear that the oral administration of the extract of *A. sativum* in a dose of 20 mg/kg significantly increased the urine volume with regards to control. The marked elevation in the urine volume within the treated group proved the diuretic effect. Diuresis is achieved by increased urinary electrolyte concentration with significant increase in the urinary output [27]. These two processes are involved in the suppression of renal tubular reabsorption of electrolytes, water and low molecular weight organic compounds into blood stream and as a consequence, promoting the formation of urine [28].

The mechanism of the diuretic action of the n-butanol extract of *A. sativum* was investigated by its comparison with the effects of furosemide, the reference drug. Furosemide, a loop diuretic known for its diuretic and saluretic effect [29], was selected as the reference drug, since it is used clinically as a diuretic in edematous states (congestive heart failure, hepatic cirrhosis and nephritic syndrome) and hypertension. Furosemide increased the excretion of Na^+, K^+ and Cl^-. Furosemide acts by inhibiting electrolytes re-absorption in the thick, ascending limp of the loop of Henle by inhibiting $Na^+/K^+/2Cl^-$ symporter (co-transporter system) in the thick ascending limb of the Loop of Henley [30, 31]. It was noted that n-butanol extract of *A. sativum* caused increase in both urine and Na^+ excretion qualitatively in similar manner to furosemide which is known by its potential saluretic and diuretic effects. The administration of *A. sativum* n-butanol showed little effect on potassium excretion which is essential quality of a good diuretic. The increase in the ratio of concentration of extracted sodium ion for the tested extract, compared to control, indicates that the extract increases sodium ion excretion to a greater extent than potassium, which is a very essential for good diuretic with lesser hyperkalaemic effect [32].

A test for potassium in the urine checks how much potassium is excreted in the urine. Potassium is both an electrolyte and a mineral. It helps to keep the water (the amount of fluid inside and outside the body's cells) and electrolyte balance of the body. Potassium is also important for the working of nerves and muscles. A low sodium/potassium ratio indicates some kidney weakness. *A. Sativum* do not have similar diuretic and saluretic activity like furosemide, it is likely that the active component(s) of the garlic bulb does not have a furosemide-like action.

However, based upon the sodium/potassium excretion ratios of *A. Sativum* extract at 20 mg/kg dose and furosemide, respectively, it appears that the plant extract is more potassium sparing than furosemide. As reported by Pantoja (2000) the intravenous administration of purified fractions of *A. sativum*, exhibits a significant biphasic and natriuretic response. Chloride ions follow the natriuretic profile but potassium ions do not. No changes were observed in arterial blood pressure or in the electrocardiogram. The purified garlic fractions also bring about a suppressive dose dependent effect on Na-K-ATPase. Therefore it may cause diuresis by increasing the volume of urine [17].

Diuretic relieves pulmonary congestion and peripheral edema. Diuretic agents are useful in reducing the syndromes of volume overload, orthopnea and paroxysmal nocturnal dyspnoea. They decrease plasma volume and subsequently venous return to the heart (preload). This decreases cardiac workload, oxygen

demand and plasma volume, thus decreasing blood pressure. Thus diuretics play an important role in hypertensive patients [33].

A. sativum contains other active principles which explain its hypotensive properties such as: adenosine, arginine, ascorbic-acid, calcium, magnesium, potassium, quercetin, tryptophan and tyrosinase [34, 35].

A furostanol saponin named proto-eruboside-B was isoloated from a crude glycoside fraction prepared from a methanolic extract of frozen garlic bulbs by a reversed-phase porous polymer [36]. Peng *et al*., (1994) reported the isolation and structure determination of new steroid saponins named proto-isoeruboside-B and isoeruboside-B [37]. Steroid saponins in the crude glycoside fraction, prepared from a methanolic extract of crushed raw garlic at room temperature yielded new spirostanol saponins, named sativoside-B2, -B3, -B4, and -B5, along with eruboside-B [38].

Koch (1993) indicated that the cholesterol-lowering effect of garlic was probably due to the saponin content [39]. Other studies report that the crude glycoside fraction from methanolic raw-garlic extracts, which mainly contains spirostanol saponins produced by the conversion of furostanol saponins via ß-glucosidase, lowered total plasma cholesterol and LDL cholesterol without changing HDL cholesterol levels in hypercholesterolemic animal models [40, 41].

Plasma and urine creatinine clearance parameters were not determined, though volume of urine has a direct correlation with Glomerular filtration rate (GFR). The *A. sativum* extract at 20 mg/kg dose significantly increased urine volume as like furosemide which is reported to increase urine volume as well as GFR significantly [42]. The increase in urine filtration rate inturn GFR by *A. sativum* extract is possibly may be due to:

(i) Detergent like interaction of triterpenoidal saponins with structural components of glomerular membranes affecting fluid filtration [43].

(ii) A decrease in renal perfusion pressure, attributable to decrease in the resistance of the afferent arteriole, and/or increase in the resistance of the efferent arteriole [44].

(iii) Direct effect on arterial pressure affecting glomerular blood flow [44] as garlic reported to relax vascular smooth muscles inducing vasodilation [45]. The known vasodilative effect of garlic is possibly caused by catabolism of garlic-derived polysulfides to hydrogen sulfide in red blood cells, a reaction that is dependent on reduced thiols in or on the RBC membrane [46].

(iv) Decrease in glomerular active transport of Na^+ ion by inhibiting Na^+, K^+-ATPase activity. The tubular reabsorption of sodium generally requires active transport via Na^+, K^+-ATPase. Aqueous garlic extract decreases active Na^+ transport in the toad skin and induces in vitro inhibition of Na^+, K^+-ATPase activity [47]. A highly purified fraction of aqueous garlic extract inhibited in a dose-related manner kidney Na^+, K^+-ATPase activity. Pantoja et al., (2000) concludes the diuretic and natriuretic responses induced by garlic purified fraction are probably mediated by a sodium pump inhibition at the sodium tubular reabsorption level of the kidney [17].

As it is not known as to which component(s) of the n-butanol extract of *A. sativum* is responsible for the diuretic effect. The activity could be due to any one or more of phyto compounds present in garlic like furostanol saponin named eruboside, proto-eruboside, proto-isoeruboside and isoeruboside, and steroidal like sativosides. Sesquiterpene lactones and triterpenes have been shown to have a diuretic and saluretic effect [48, 49].

However, the exact components of garlic responsible for the diuretic activity are unknown. Crude n-butanol extract of *A. sativum* induced a significant increase in diuresis and natriuresis. Air-dried whole plants extract of *Hygrophila auriculata* in n-butanol containing triterpene increased the urinary output (by 316%) and electrolytic excretion of Na^+ (by 140%) and K^+ (by 177%), without significant renal excretion of Cl^- as compared to control. This study results raise the possibility of existence of better diuretic activity in n-butanol fraction of *H. auriculata* by inhibiting tubular reabsorption of water and sodium ion compared to chloroform and alcoholic extract [50].

The present study clearly demonstrated the diuretic effect of the garlic bulb n-butanol extract. The study confirmed the ethnopharmacological and Ayurvedic use of *A. sativum* as a diuretic agent, but further studies are necessary to evaluate the mechanisms involved in its biological activity and safety following repeated exposure.

REFERENCES:

1. Mukherjee PK: Quality Control of Herbal Drugs, Business Horizons Publishers, First Edition 2002.
2. Nadkarni KM: Indian Materia Medica, Popular Prakashan, Bombay, Edition, Vol. 1, 2007:65-71: 1296.
3. Augusti KT: Therapeutic values of onion (*Allium cepa*L.) and garlic (*Allium sativum* L.). Indian Journal of Experimental Biology 1996; 34: 634-640.
4. Ali M and Thomson M: Consumption of a garlic clove a day could be beneficial in preventing thrombosis. Prostaglandins Leukot Essent Fatty Acids 1995; 53: 211-212.
5. Bordia T, Mohammed N, Thomson M and Ali M: An evaluation of garlic and onion as antithrombotic agents. Prostaglandins Leukot Essent Fatty Acids 1996; 54: 183-186.
6. Thomson M, Mustafa T and Ali M: Thromboxane-B2 levels in serum of rabbits receiving a single intravenousdose of aqueous extract of garlic and onion. Prostaglandins Leukot Essent Fatty Acids 2000; 63: 217-221.
7. Augusti KT and Sheela CG: Antiperoxide effect of S-allyl cysteine sulfoxide, a insulin secretagogue, in diabetic rats. Experientia 1996; 52: 115-120.
8. Anwar MM, Meki AR: Oxidative stress in streptozotocin-induced diabetic rats: effects of garlic oiland melatonin. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 2003; 135: 539-547.
9. Bakri IM and Douglas CW: Inhibitory effect of garlic extract on oral bacteria. Archives of Oral Biology 2005; 50: 645- 651.
10. Rees LP, Minney SF, Plummer NT, Slater JH and Skyrme DA: A quantitative assessment of the antimicrobialactivity of garlic (*Allium sativum*.). World Journal of Microbiology and Biotechnology 1993; 9: 303-307.
11. Yoshida H, Iwata N and Karsuzaki H: Antimicrobial activity of a compound isolated from an oil-maceratedgarlic extract. Bioscience Biotechnology and Biochemistry 1998; 62: 1014-1017.
12. Ali M, Al-Qattan KK, Al-Enezi F, Khanafer RM and Mustafa T: Effect of allicin from garlic powder on serum lipids and blood pressure in rats fed with a high cholesterol diet. Prostaglandins Leukot Essent Fatty Acids 2000; 62: 253-259.
13. Banerjee SK and Maulik SK: Effect of garlic on cardiovascular disorders: a review. Nutrition Journal 2002; 1: 4.
14. Amber Keefer. 2011. How Does Garlic Speed Up Metabolism? Available at: http://www.livestrong.com/article/51075-garlic-speed-up-metabolism/. Accessed on: 20th Jan 2012.
15. Pantoja CV, Chiang LC, Norris BC and Concha JB: Diuretic, natriuretic and hypotensive effects produced by *Allium sativum* (garlic) in anaesthetized dogs. Journal of Ethnopharmacology 1991; 31:325-31.
16. Pantoja CV, Norris BC, and Contreras CM: Diuretic and natriuretic effects of chromatographically purified fraction of garlic (*Allium sativum*). Journal of Ethnopharmacology 1996; 52: 101–105.
17. Pantoja CV, Martin NT, Norris BC and Contreras CM: Purification and bioassays of a diuretic and natriuretic fraction from garlic (*Allium sativum*). Journal of Ethnopharmacology 2000; 70: 35–40.
18. Smoczkiewicz MA, Nitschke D and Wieladek H: Microdetermination of steroid and triterpene saponin glycosides in various plant materials I. Allium species. Microchimica Acta 2004; 78:43-53.
19. Kambou G and Guissou IP: Phytochemical Composition and Insecticidal Effects of Aqueous Spice Extracts on Insect Pests Found on Green Beans (*Phaseolus vulgaris*) in Burkina Faso. Tropicultura 2011; 29:212-217.
20. Trease E G and Evans W C: Pharmacognosy, Balliere, Tindall, London, Eleventh Edition 1978.
21. Kokate C K, Purohit A P and Gokhale S B: Pharmacognosy, Nirali Prakashan, Pune, 34th Edition 2006.
22. Lipniek RL, Cotruvo JA, Hill RN, Bruce RD, Stitzel KA and Walker AP: Comparison of the Up and Down, Convensional LD_{50} and Fixed Dose acute toxicity testing procedures. Food and Chemical Toxicology 1995; 33: 223-31.
23. Organization for Economic Cooperation and Development (OECD). Guidelines for testing of chemicals. Section 4: Health Effects. Addendum to test guideline 401, Fixed dose Procedure, Paris, France. 1992.
24. Leander JD: A kappa opioid effect: increased urination in the rat. Journal of Pharmacology and Experimental Therapeutics 1983; 224:89-94.
25. Jeffery GH, Bassett J, Mendham J and Denney RC. Vogel's Textbook of Quantities Chemical Analysis. Addison Wesley Longman Inc. London, Edition 5, 1989: 582-83.
26. Diniz LR, Santana PC, Ribeiro AP, Portella VG, Pacheco LF, Meyer NB, César IC, Cosenza GP, Brandao MG and Vieira MA: Effect of triterpene saponins from roots of Ampelozizyphus amazonicus Ducke on diuresis in rats. Journal of Ethnopharmacology 2009; 123:275-9.
27. De Stevens G. Diuretics: Chemistry and pharmacology. 1st Ed., Academic Press, New York 1963.
28. Milton JC, Sheldon M and Brainerd H. Handbook of Medical Treatment. 12th Ed. Lange Medical Publication, Oxford, Blackwell Scientific Publication , 1970.
29. Leuschner J: Anti-inflammatory, spasmolytic and diuretic effects of a commonly available Solidago gigantean herb extract. Arzneimittel Forschung (Drug Research) 1995; 45:168–195.
30. William BS, Paul JC and Henry OH: Furosemide: A Clinical Evaluation of Its Diuretic Action. Circulation 1966; 34:910-920.
31. Shinkawa T, Yamaski F, Notsu T, Nakakuki M, Nishijima K, Yoshitomi K and Imai M: Loop and distal actions of a novel diuretics M 17055. European Journal of Pharmacology 1993; 238: 317-325.
32. Bose A, Gupta JK, Dash GK, Ghosh T and Panda DS: Diuretic and antibacterial activity of aqueous extract of *Cleome rutidosperma* D.C. Indian Journal of pharmaceutical Science 2007; 692:292-294.
33. Mute V, Awari D, Vawhal P, Kulkarni A, Bartakke U and Shetty R: Evaluation of diuretic activity of aqueous extract of *Raphanus sativus*. European Journal of Biological Sciences 2011; 3:13-15.
34. Harunobu A: Clarifying the Real Bioactive Constituents of Garlic. Journal of Nutrition 2006; 136:716S-725S.
35. Rangel JAO., Synergistic anti-hypertensive phyto-nutraceutical composition. Available online http://www.faqs.org/patents/app/20080267939. Publication

date: 10/30/2008. Patent application number: 20080267939 accessed on 3 March 2012.
36. Matsuura H, Ushiroguchi T, Itakura Y, Hayasi N and Fuwa T: A furostanol glycoside from garlic bulbs of *Allium sativum*. Chemical and Pharmaceutical Bulletin (Tokyo) 1988; 36:3659–3663.
37. Peng J, Yao X, Okada Y, Okuyama T: Further studies on new furostanol saponins from the bulbs of *Allium macrostemon*. Chemical and Pharmaceutical Bulletin (Tokyo) 1994; 42:2180–2182.
38. Matsuura H, Graham J, Farnsworth NR and Beecher CWW: New spirostanol glycosides from garlic.The 38th annual meeting of the American society of pharmacognosy, Iowa, July 1997.
39. Koch HP: Saponine in knoblauch und küchenzwiebel. Dtsch Apoth Ztg 1993; 133:3733–3743.
40. Matsuura H: Saponins in garlic as modifiers of the risk of cardiovascular disease. Journal of Nutrition 2001; 131:1000S–1005S.
41. Slowing K, Ganado P, Sanz M, Ruiz E, Beecher C and Tejerina T. Effect of garlic in cholesterol-fed rats. Journal of Nutrition 2001; 131:994S–999S.
42. Aissaouia A, El-Hilalya J, Israilib ZH and Lyoussi B: Acute diuretic effect of continuous intravenous infusion of an aqueous extract of *Coriandrum sativum* L. in anesthetized rats. Journal of Ethnopharmacology 2008; 115: 89–95.
43. Natochin, Yu. V, Shakhmatova EI and Bakos P. Reversible inactivation of water and sodium transport systems in frog urinary bladder and skin in the presence of detergents. Physiologia Bohemoslovaca 1990; 39:157–170.
44. Bevevino LH and Mello Aires M: Effect of crude extract of roots of Bredemeyera floribunda Willd. I. Effect on arterial blood pressure and renal excretion in the rats. Journal of Ethnopharmacology 1994; 43:203–207.
45. Ginter E and Simko V: Garlic (*Allium sativum* L.) and cardiovascular diseases. Bratisl LekListy 2010; 111: 452-56.
46. Benavides GA, Squadrito GL and Mills RW: Hydrogen sulfide mediates the vasoactivity of garlic. Proceeding of National Academy of Science USA 2007; 104: 17977-17982.
47. Norris BC, Pantoja CV, Chiang L Ch, Concha JB, Neuman VR and Ferrada OV: Inhibitory effect of garlic (*Allium sativum*) on sodium transport in isolated toad skin. Journal of Ethnopharmacology 1991; 31: 309–318.
48. Ahmed M, Sadhu SK, Datta BK, Kunu JK, and Bachar SC: Preliminary studies on the antiinflammatory, analgesic and diuretic activity of stagninol, a sesquiterpene isolated from *Persicaria stagnina*. Die Pharmazie 1997; 52:472–475.
49. Somova LI, Shode FO, Ramnanan P and Nadar A, Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from *Olea europaea*, subspecies africana leaves. Journal of Ethnopharmacology 84 (2003), pp. 299–305.
50. Hussain S, Ahmed N and Ansari ZH: Preliminary Studies on Diuretic Effect of *Hygrophila auriculata* (Schum) Heine in Rats. International Journal of Health Research 2009; 2: 59-64.

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