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Full Length Research Paper

Phytochemical screening, elemental analysis and acute toxicity of aqueous extract of *Allium sativum* L. bulbs in experimental rabbits

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There seems to be little literature about the toxicity of the widely used garlic, which makes it important to investigate chemical elements as well as chemical compounds that could be present in garlic bulbs and their safety for human handlers and consumers. Phytochemical screening and elemental analysis of powdered bulb of *Allium sativum* L. and toxicological effects of its aqueous extract were investigated in experimental rabbits. Acute toxicity study was conducted following subcutaneous administration of graded doses of the plant extract in experimental rabbits. LD_{50} was found to be 3034 mg/kg and maximum tolerated dose was 2200 mg/kg. Mortality occurred in rabbits given the extract at 3200 and 4200 mg/kg with other behavioural signs like loss of appetite and partial paralysis. The percentage yield of the extract was 75.8%. Elemental analysis indicated that the powdered plant material contained mainly potassium, phosphorus and iron among other elements. While the phytochemical screening revealed presence of the following chemical compounds: saponins, steroids, tannins, carbohydrates and cardiac glycosides, whereas, alkaloids, cadenolide, flavonoid, anthraquinone and cyanogenic glycosides were found absent.

Key words: Garlic, chemical elements, chemical compounds, safety margin, rabbits, Nigeria.

INTRODUCTION

Allium sativum L. (Liliacea) is a perennial bulb with a tall, erect flowering stem that grows to between 2 and 3 feets. The plant produces pink to purple flowers that bloom from July to September and the bulb is odifereous (McMahon and Vargas, 1993). The name *A. sativum* L. comes from the Celtic word “all” meaning burning or smarting. 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, and the American Indian used garlic as a remedy for ear aches, flatulence and scurvy. The bulb of the plant has been used in many parts of the world as a stimulant, carminative, antiseptic, anthelmintic, expectorant and diuretic (Mikail, 2003).

The bulbs of the plant has been used in many parts of the world as a stimulant, carminative, antiseptic, anthel-mintic (ascaris and oxyuris), diaphoretic, expectorant, diuretic, antisorbutic aphrodisiac and antiasthmatic, in pulmonary diseases such as croup, whooping cough, tuberculosis, bronchoectasis and gangrene. Furthermore, the herb has been used externally as a ruberfacient and vesicant and as an antirheumatic agent. The plant has also been used as a febrifuge and has been taken mashed with honey, for the relief of rheumatic pains (Mahon and Vargas, 1993).

As the demand for garlic increased, researches had been made as to the economic value and medical use-fulness of this crop. It was found out that garlic is not only beneficial as medicinal plant, but it can be used as repellant to some plant pests and diseases. Thus garlic has been used as a very effective insecticide (Ramasasa,

2009).

Fresh garlic is a source of numerous vitamins, minerals and trace elements, although most are found in only minute quantities. Garlic contains the highest sulfur content of any member of the *Allium genus*. Two trace elements, germanium and selenium are found in detectable quantities and have been postulated to play a role in the herb's antitumor effect (Ariga et al., 1980).

Garlic contains about 0.5% of a volatile oil which is composed of sulfur containing compounds {diallyl disulphide, diallyl trisulphide and methylallyl trisulphate (Ariga et al., 1980)}. The bulbs contain an odourless sulfur containing amino acid called alliin (s-allyl-1-cysteine sulfoxide), which has no pharmacologic activity (Castleman, 1991). When the bulb is grinded, the enzyme alliinase is released which results in the conversion of alliin to 2 - propenesulforic 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 (McCaleb, 1993).

There seems to be little literature about the toxicity of the widely used garlic, which makes it important to investigate chemical elements as well as chemical compounds that could be present in garlic bulbs and their safety for human handlers and consumers.

The objective of the present study is to carryout phytochemical screening and elemental analysis of the powdered garlic bulbs as well as evaluating its aqueous extract safety margin in experimental rabbits.

MATERIALS AND METHODS

Plant material

The bulbs of *A. sativum* L. (Garlic) were purchased at Sokoto Central Market in May, 2002 and identified by traditional herbalist (Ardo, personal communication, 2002) and a botanist at Usman Danfodiyo University, Sokoto. A voucher specimen numbered 250 was deposited at the Botany Departmental herbarium of the University.

Extract preparation

500g of air-dried bulbs of the garlic were cut into small pieces and pulverized. The powdered bulbs were then soaked in distilled water and heated at 100°C; this was allowed to cool down and then filtered into another clean container. The recovered filtrate was oven dried to concentrate the sample. The resultant yellowish, crystalline crude extract was used for the toxicity study.

Acute toxicity

Twenty one New Zealand rabbits of both sexes were divided into 7 groups of 3 rabbits each. They were fed with standard rabbits feed and supplied with water *ad libitum*. The groups were treated with the graded doses of the bulbs aqueous extract (300, 600, 1200, 2200, 3200 and 4200 mg/kg) subcutaneously and distilled water was given to the seventh group (control group).

The rabbits were observed for signs of acute toxicity like behavioural changes and death over 72 h and LD_{50} (Median lethal dose) was determined using the Arithmetic method of Karber modified by Aliyu and Nwude (1982). Post mortem examinations were performed to determine any gross pathological changes. The maximum tolerated dose of the extract was also determined.
The Acute toxicity (LD_{50}) was calculated using the formula:

$$LD_{50} = Ld_y - {}^{l\text{-}r}\sum (Dd \times Md)/n \quad (1)$$

Where; LD_y = highest dose and n = number of animals per group (n = 3).

Elemental analysis

Powdered bulbs of *A. sativum* were used for this analysis and this was done using atomic spectrophotometer (210 VGP BUCK, scientific, U.K) at the Faculty of Agriculture multipurpose Laboratory, Usman Danfodiyo University, Sokoto, Nigeria. The result obtained was analysed using SPSS 17.0 statistical soft ware.

Phytochemical screening

The powdered bulbs of *A. sativum* was screened for the presence of chemical compounds as described by Evans (1996), Parekh and Chanda (2007). This was done at the Faculty of Pharmaceutical Sciences, Pharmacognosy Laboratory of Ahmadu Bello University, Zaria, Nigeria.

Preliminary phytochemical screening

The powdered plant material was subjected to preliminary phytochemical analysis to test for the presence or absence of phytochemical constituents using the following methods: carbohydrates [(500 mg plant material boiled in 30 ml distilled water, filtered); 1 ml filtrate + 1 ml of Molisch's reagent +1 ml conc. H_2SO_4. A reddish ring indicates the presence of carbohydrate; 1 ml filtrate + 2 ml of Fehling's solution + boiled for 5 min. A brick red precipitate indicates the presence of reducing sugars; 1 ml filtrate + 1 ml Barfoed's reagent + heat. A red precipitate indicates the presence of monosaccharide (Evans, 1996)]. Tannins [2 ml filtrate + 1 ml $FeCl_3$, blue-black or greenish-black precipitate indicates tannins; 1 ml filtrate, + 3 drops of lead sub acetate, a colored precipitate indicates the presence of tannins (Evans, 1996)]. Saponnins [frothing test: 0.5 ml filtrate + 5 ml distilled water, shaken for 30 s, persistence frothing indicates saponnins (Evans, 1996)].

Flavonoids [Shinoda's Test: (200 mg plant material in 5 ml ethanol, filtered) 1 ml filtrate, + magnesium ribbon + conc. HCl a pink or red color indicates the presence of flavonoids]. Terpenes/steroids [Liebermann - Burchard's Test: (200 mg plant material in 10 ml chloroform, filtered; 2 ml filtrate + 2 ml acetic anhydride + 1 ml of conc. H_2SO_4. A blue – green ring indicates the presence of terpenes/steroids (Parekh and Chanda, 2007)].

Alkaloids [200 mg plant material boiled in 20 ml of 1% H_2SO_4 in 50% ethanol, filtered; filtrate + 5 drops conc. NH_4OH + 20 ml chloroform and the two layers separated. Chloroform layer was extracted with 20 ml dilute H_2SO_4. Extract + 5 drops of Mayer's/ Wagner's/Dragendorff's reagents, a creamy/brownish-red/orange-red precipitate indicates the presence of alkaloids (Evans, 1996)].

Anthraquinones [Borntrager's test: 100 mg of powdered plant in 5 ml of chloroform, filtered. 2 ml filtrate + 2 ml 10% NH_4OH. A bright pink colour indicates the presence of anthraquinones; Modified Borntrager's test: 200 mg plant material boiled in 5 ml 10% HCl, filtered. Filtrate extracted with 5 ml benzene and benzene layer shaken with 5 ml 10% NH_4OH. A rose pink or cherry red colour indicates the presence of anthraquinone derivatives (Evans, 1996).

Table 1. Elemental analysis of the extract of *A. sativum*.

Elements	mg/gwt of *A. sativum*
K	1566.5
Na	10.3
Ca	46.8
Mg	7.2
P	212.5
Fe	375.0

Descriptive variables = K, Na, Ca, Mg, P and Fe; Statistics = Mean, STDDEV, Min, Max.

Table 2. Descriptives statistics.

Elements	N	Minimum	Maximum	Mean	Std. deviation
K	3	1566,30	1566.70	1566.5000	0.20000
Na	3	10.20	10.40	10.3000	0.10000
Ca	3	46.50	47.10	46.8000	0.30000
Mg	3	7.10	7.30	7.2000	0.10000
P	3	212.20	212.80	212.5000	0.30000
Fe	3	374.80	375.20	375.0000	0.20000
Valid N (list wise)	3				

Other chemical compounds were screened following standard procedures descried by Evans (1996), Parekh and Chanda (2007).

RESULTS

Elemental analysis

The plant extract yielded 75.8% relative to the starting material. Elemental analysis of the extract presented in Tables 1 and 2, showed that the plant contained major elements like Na, K, Ca, Mg, P and Fe.

Toxicity

The extract induced behavioural signs like loss of appetite, depression, partial paralysis and death at the higher doses (3200 and 4200 mg/kg). In contrast, there was no death recorded in animals given 300 - 2,200 mg/kg of the extract (Table 3).

Phytochemical screening

The phytochemical screening showed that the powdered plant material contains the following chemical compounds (Table 5).

DISCUSSION

The percentage yield of the extract of *A. sativum* was found to be 75.8%, while it is powdered bulbs elemental analysis revealed the presence of major elements like Na, K, Ca, Mg, P and Fe. The concentration of Fe is high compared to species like wild garlic which posses 130 mg of Fe/kg of the plant material. The detected elements could be useful, dietary, physiologically and pharmacologically to any living system. However, presence of chemical compounds such as saponins, steroids, tannins, carbohydrates and cardiac glycosides as well as absence of alkaloids, cadenolide, flavonoid, anthraquinone and cyanogenic glycosides call for further research about the pharmacological implications of the presence and or absence of these chemical compounds in the powdered plant material of *A. sativum.*

There was neither death recorded nor discernible gross pathological lesion seen in animals dosed with 300, 600, 1200 and 220 mg/kg of the aqueous extract, thus 2200 mg/kg was considered as maximum tolerated dose. While those given 3200 – 4200 mg/kg had slightly congested liver with recorded numbers of death. The median lethal dose (LD_{50}) was calculated to be 3034 mg/kg (Table 4). According to the (Clarke and Clarke, 1979), any substance whose LD_{50} is above 1000 mg/kg is regarded relatively safe. Similarly, WHO (1991) considers extract or agents with LD_{50} above 3000 mg/kg as essentially safe. The fact that in this study, high LD_{50} was obtained is an indication that this result is useful for the safety of persons, such as people who consume enough amounts of garlic or who handle or apply insecticide of garlic origin in agricultural crops or plantations. The elemental analysis and phytochemcial screening of the plant material revealed the presence of certain elements

Table 3. Mortality rate and behavioural signs in rabbits given *A. sativum.*

Group	Dose (mg/kg)	Route	Toxicity sign	Mortality
1	300	S.C	No toxic signs noticed	No death recorded
2	600	S.C	No toxic signs noticed	No death recorded
3	1200	S.C	No toxic signs noticed	No death recorded
4	2200	S.C	Became dull after 4 h, loss of appetite, became active and regain appetite in 12 h time	No death recorded
5	3200	S.C	Became dull in 3 h time with glossy eyes, eyes closed, layed down for about 3 h, loss of appetite, partial paralysis of the fore limbs.	Two died after 48 and 72 h
6	4200	S.C	Became dull within 3 h time with glossy eyes, eyes closed, layed down for about 3 h, loss of appetite, partial paralysis of the fore limbs.	All died between 48 to 72 h.
7	Distilled water	S.C	No toxicity	No death

Table 4. Determination of acute toxicity (LD_{50}) of *A. sativum* (garlic) in rabbits using Karber method.

Dose (mg/kg)	Dose difference	No of dead(n)	Mean dead	Dose difference × mean dead
30	0	0	0	0
600	300	0	0	0
1,200	600	0	0	0
2,200	1000	0	0	0
3,200	1000	2	1	1000
4,200	1000	3	2.5	2500
Control	0	0	0	0
				3500

LD_{50} = 4,200 – 3500/3 = 3034 mg/kg.

Table 5. Phytochemical screening of the powdered bulbs of *A. sativum* L.

Chemical compounds	Result
Saponins	+
Steroids	+
Alkaloids	-
Tannins	+
Cadenolide	-
Carbohydrates	+
Cardiac glycosides	+
Flavonoid	-
Anthraquinone	-
Cyanogenic glycosides	-

- = Compound not detected; + = compound detected.

and chemical compounds which may alone or in combination account for the diverse folkloric use of this bulb in traditional medicine.

Garlic and its extract have a long history of folk use. In many parts of the world, the strong-smelling, pungent tasting bulb of *A. sativum* is used as a flavouring agent in cookery. In addition to its use as a condiment and food, high LD_{50} and absence of cyanogenic glycosides as

recorded in this study, is an indication of relatively safe and reduced risk of cyanide poisoning other toxicities to consumers and handlers of this plant. However, garlic has been used in traditional medicine since ancient times and it still enjoys a considerable vogue in folklore medicine (Ojewole and Adewunmi, 2001). An earlier study had shown that with garlic ingestion, a population is protected from coronary hearty disease (Malik and Siddiqui, 1981). This could be due to the presence of cardiac glycosides as revealed by this study. Cardiac glycosides are used in the treatment of congestive heart failure and cardiac arrhythmias. These glycosides are found as secondary metabolites in several plants and in some animals.

Carbohydrates are the major source of fuel for metabolism, steroids were also recorded. Anabolic steroids interact with androgen receptors to increase muscles and bones synthesis. Saponins were also found positive, soap nuts (Sapindus), especially *Sapindus mukorossi* are used medically as expectorant, emetic and for treatment of excessive salivation, epilepsy, chlorosis and migraines. Soap nuts are used in Ayurvedic medicine as treatment of eczema, psoriasis and for removing freckles (National Geographic, 1995). Tannins recorded in this study has a wide variety range of usage, ranging from antiviral (Bruce et al., 1989), antibacterial (Akiyama et al., 2001), antiparasitic (Herbert and Albrecht, 2005) as well as inhibition of HIV replication in infected H9 lymphocytes with little toxicity as in epigallitannins. Garlic contains the trace elements germanium and selenium, which have been thought to play a role in improving host immunity.

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