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Phytochemical Screening and in-Vivo Anthelmintic Activity of *Allium sativum* Leaf Extract

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Abstract: The anthelmintic potency of ethanol extracts of Allium sativum was determined in-vivo against a round worm by infecting group of mice orally. Preliminary phytochemical screening revealed the presence of tannin, flavonoid, saponin, alkaloid and glycoside in the plant while anthraquinone was absent. The ethanol extract of garlic was observed to be potent against Ascaris lumbricoides with percentage fall egg count of 80.73% for 200mg/kg of garlic extract 84.26% for 400mg/kg of garlic and 91.78% for 800mg/kg of garlic extract respectively. The acute toxicity effect of the garlic extract recorded was zero (0%) mortality for all dose given to the mice. Allicin, the sulfur-containig compound in garlic acts not only by killing gastrointestinal parasites but also enhances natural immunity of the host.These findings therefore justify the traditional medicinal use of garlic.

Key Words: *Allium sativum, phytoconstituents, in-vivo assay, anthelmintic*

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1.0 Introduction

Medicinal plants have served through ages, as a constant source of medications for the treatment of several illnesses (Chopra *et al*.,1956). The history of herbal medicines is as old as human civilization. Plants are known to provide a rich source of botanical anthelmintic (Kala *et al*., 2004). The prevalence of animal diseases is one of the major livestock production constraints in Nigeria and all over the world with great impact in the livelihoods due to related economic loses affecting food security. A number of medicinal plants have been used to treat parasitic helminthics in both man and animals leading to stunted growth and other ailments. The invasion is caused by infections with several species of stomach and intestinal worms (Crompton et al 1985). Most synthetic drugs that are used for the treatment of various diseases have been reportedly to exhibit characteristics side effect or toxic symptoms because they are not green. Consequently, recent efforts in drugs design and development are directed green synthesis and applications (Karimi *et al* 2015). The use of plant extract for drug development or directly for the treatment of some medical conditions has received significant research attraction. Plant extracts that exhibit medicinal values have useful phytochemical constituents (Nagesh et al 2009). Based on these requirements; several plants have been investigated and tested for various biological activities (Negi *et al*., 2011). However, there is still research gap on pharmaceutical applications of *A. sativum*, hence the aim of the present study is to investigate anthelmintic activity of *A. sativum*. *A.sativum* belongs to the family Alliaceae and it's also known as garlic (Friesen *et al*., 2006). There are different species of Allium namely *Allium ursinum, A. vineale, A. oleraceum. A. canadense, A. ampeloprasum* (Gathuma *et al*., 2004)

However, *A. sativum* contains an active compound known as allicin (diallylthiosulfinate or diallyldisulfide) and alliin (S-allylcysteine sulfoxide) as the most abundant (Gebreselema and Mebrahtu, 2013). It is also rich in 17 amino acids, vitamins, minerals and 33 sulphur compounds

(Peter *et al*., 2008). Anti-parasitic effect include; ajoene (Ledezma and Apitz-Castro, 2006), allicin, allyl methyl thiosulfanate and ethyl allythiosulfanate (Josling, 2001), diallylmonosulfide, dially ldisulfide, and diallyltrisulfide (Tsao and Yin, 2001). Allicin, also known as 2-propene-1-sulfinothic acid S-2-propenyl ester; thio-2-propene-1-sulfinic acid S-allyl ester (Chester *et al*., 1944) has antimicrobial effects against many viruses, bacteria, fungi and parasites. Garlic oil and steam- distilled garlic do not contain significant amounts of allin and allicin, but instead contain various product of allicin transformation; none appears to have as much physiological activity as fresh garlic.

2.0 Materials and Methods

2.1 Sample collection and preparation

Fresh bulbs of *A.sativum* were collected from a local farm, located within Umuahia South Local Government Area of Abia State, Nigeria and was authenticated and identified by a botanist at the Department of Plant Science and Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike Abia State as Allium sativum

The samples were dried to constant weight and grounded to powder form. 100 g of the powder samples was soaked in ethanol for cold extraction, which was carried out at 700C.The extracted component (whose yield was 16.24%) was dried in an oven to a constant weight at 400C.

2.1.1 Collection of animals

A total of 50 adult albino mice obtained from the laboratory animal unit in Michael Okpara University of Agriculture, were used for this study. 25 animals were used for LD50 determination while the other 25 were used for the anthelmintic study. The animals were housed in ventilated caged, fed (grower guinea feed) ad libitum and clean water but starved for 24 hours prior to commencement of the experiment. All animal experiments were conducted in compliance with NIH guidelines for care and use of laboratory animals (PUB. No 85-23, Revised 1985) as expressed Akah *et al*, (2009)

These experiments were carried out in various laboratories including parasitology, physiology and pharmacology in the college of veterinary medicine, Michael Okpara University of Agriculture, Umudike, Abia state.

2.2 Acute toxicity studies

25mice of both sexes weighing 30 to 40 g were divided into 5 groups, each consisting of 5 mice. They were assigned graded doses of the extract in the order 200, 400, 600, 800, 100mg/kg body weight respectively. The animals were kept in aluminum cages and were given free access to feed and water while the toxicity signs and deaths within a period of 24hours were monitored. The LD50 value for the extract was determined according to lorke's method, adopted by Ijioma and Nwosu (2015).

2.3Helminth material

A mouse adapted strain of *Ascaris lumbricoide* larva (3rd stage larva) supplied by Dr. Ngongeh of the Department of Parasitology, College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike. The droppings of mice artificially infected with *Ascaris lumbricoide* were collected and soaked in water for 4 hours. Excess water was decanted. The feces were crushed in a glass mortar, transferred to plastic containers (in lots of about 5.0 g) and shaken with glass beads. The concentrated materials were mixed with about 1 litre of water and strained through several layers of gauze. The filtrate was transferred to jars. The supernatant solution was discarded and the sediment was mixed with vermiculite in labeled plastic petri dishes and incubated at 4oC for 7 days. The infective 3rd stage larvae (L3) were recovered from 7 to 14 days old vermiculite faecal cultures using modified baermann apparatus. The third stage (L3) obtained were washed several times with distilled water and their number determined by dilution counting. The volume was adjusted to give 200 L3in 0.2 ml.

2.4 Parasite inoculations

The mice used for anthelmintic studies were dewormed using Albendazole in their drinking water (7 mg/ml) for three days, one month prior to the experiment. Mice body mass were measured prior to infection. The mice were infected orally with *Ascaris lumbricoide* larva, 100 L3per 0.1ml per mice using an oral gavage on day 0.

2.5 Recovery of eggs

After 13 days of infection, freshly passed out feaces of experimentally infected laboratory mice were collected using a tea spoon. One gram of the feaces was homogenized in a mortar using pestle with 60ml of saturated NaCl solution, this

mixture was filtered using a tea sieve and 150 micrlitre sieve. The filtrate was transferred into a test tube and filled until the formation of upper

meniscus. A cover slip was used to cover each of the tubes and allowed to stand for 3 minutes. This was to enable eggs of the parasites to move upward, float and attach to the cover slip. The larvae were removed and placed on a slide for observation using microscope to confirm the presence of *Ascaris lumbricoide*.

2.6Chemotherapeutic trials/administration

Heavily infected mice with *Ascaris lumbricoide* were randomly assigned into five groups of five mice each. They were also grouped according to similar body weight.

2.7 Egg count

In-vivo parasite egg output was counted from 1-3 fecal pellets that were collected every 3 days from each group of mice (starting from the 14th day post *Ascaris lumbricoide* inoculation). Helminth eggs were recovered and examined qualitatively by flotation using saturated NaCl solution and egg output per gram of faeces were calculated. For each mice sample, two grids of a McMaster slide were counted and the average was used as the eggs per gram of faeces for the parasite.

2.8 Phytochemical screening

Alkaloids was determined using the method described by Harborne (1973), flavonoids and tannins were described by Odebiyi and Sofowora (1978) while cardiac glycoside and saponins were determined by the methods of Sofowora (1993) and Wall et al (1954) respectively.

Alkaloids and cardiac glycoside were carried out with the method by Harborne (1980). Saponin by Obadoni and Ochuko (2002), Flavonoids by Allen *et al* (1973) and tannins by Schanderl (1970

Group 1 – Served as normal, no treatment was given except normal animal feed and distilled water.

Group 2 - Served as positive control, mice was treated with standard drug albandazole (40mg/kg).

Group 3 – Were administered and treated with Allium sativum ethanolic extract at doses 200mg/kg.

Group 4 - and 5 – Were administered and treated with Allium sativum ethanolic extract at doses 400 and 800mg/kg body weight respectively.

3.0 Results and Discussions.

Results obtained from phytochemical screening of the plant extract are recorded in Table 1 while Table 2 presents data obtained for acute toxicity test conducted with the ethanol extract of the plant In-vivo effect of *Allium sativum* ethanolic leaf extract on fecal egg count of *Ascaris lumbricoide* in mice.

Table 3 showed that after 13 days of infection, all mice in the groups had a significant ($p<0.05$) fecal *Ascaris lumbricoide* counts. In day zero (0), the fecal egg load in group 2 (negative control) was 254.60±28.22 eggs/g while in group 3 (abendazole 30 mg/kg) was 215.40±17.06 egg/g. Facal loads for groups 4 and 5 (Allium sativum ethanolic leaf extract 400 mg/kg and 800mg/kg) were 184.80±2.96 eggs/g and 245.40±33.93 eggs/g respectively. Group 1 (normal control) consisted of normal mice that were not infected and hence zero fecal egg counts.

Table 1: Phytochemical screening (Qualitative and Quantitative)

Phytochemical	Status	Concentration (g/100g)
Tannin	+	2.52 ± 0.12
Saponin	+	0.24 ± 0.04
Cardiac glycoside	+	1.88 ± 0.26
Alkaloid	+	0.12 ± 0.02
Flavonoid	+	0.05 ± 0.03

Key= +present; - absent, Values are expressed as Mean ±SEM (n= 3)

Table 2: Acute toxicity (LD50) effect of Allium sativum ethanolic extract (in-vivo)

Group	Dose (mg/kg)	No of Deaths	Percentage mortality
1	200	0	0.00
2	400	0	0.00
3	600	0	0.00
4	800	0	0.00
5	1000	0	0.00

Treatment with both the standard drug (abendazole) and Allium sativum ethanolic leaf extract significantly lowered fecal *Ascaris lumbricoide* egg counts in all test groups when compared with those observed in group 1 (control) and completely eliminated the ova at various stages of treatment. At the end of the 3rd day of treatment, fecal egg count in the negative control was 250.20±19.56 eggs/g while group 3 (which represented those treated with 30mg/kg of abendazole) indicated significant reduction in fecal load had up to 86.20±5.99 eggs/g. Group 4 and 5 treated with 400 and 800mg/kg *Allium sativum* ethanolic leaf extract at this stage of treatment had fecal egg count values of 100.60±14.93 eggs/g and 90.80± 19.83 eggs/g respectively. In the 6th day of treatment, fecal egg count in the Abendazole treated group (group 3)

reduced to 0.600±0.40 eggs/g. Groups treated with 400 and 800 mg/kg of the ethanolic leaf extract of the tested plant had fecal egg counts value of 0.80±0.56 eggs/g and 0.20±0.20 eggs/g but those in group 2 (negative control) displayed increase in fecal egg load to a maximum value of 284.00±7.07 eggs/g. In the 9th day of treatment *Ascaris lumbricoide* eggs were absence in the collected fecal samples from all the infected mice (that were treated with both abendazole and Allium sativum leaf extract. However, in group 2 (negative control) fecal egg counts were observed to increase to a maximum value of 306.60±4.18 eggs/g in the 12th day.

Table 3: Percentage fall in fecal egg count of *Ascaris lumbricoide* following treatment of Abendazole (40 mg/kg) and Allium sativum ethanolic leaf extract (200,400 and 800 mg/kg)

Groups	Treatment	Post infection egg count	4days post treatment	8days post treatment		% fall in egg count
1	Control	233.20±14.89	308.00±18.93	328.20 ±9.75		-42.29 ± 6.73
2	40mg/kg Albenzadole	203.00±11.88	87.40±11.73*	0.80 ± 0.37*		99.61±0.18*
3	200mg/kg garlic extract	235.40±19.10	163.20±12.62*	45.60 ±6.98*		80.73 ±2.47*
4	400mg/kg garlic extract	266.20±17.53	121.00±5.20*	41.00 ±7.70*		84.26 ±3.17*
5	800mg/kg garlic extract	250.20±8.10	117.40±5.78*	20.60 ±1.40*		91.78 ±0.39*

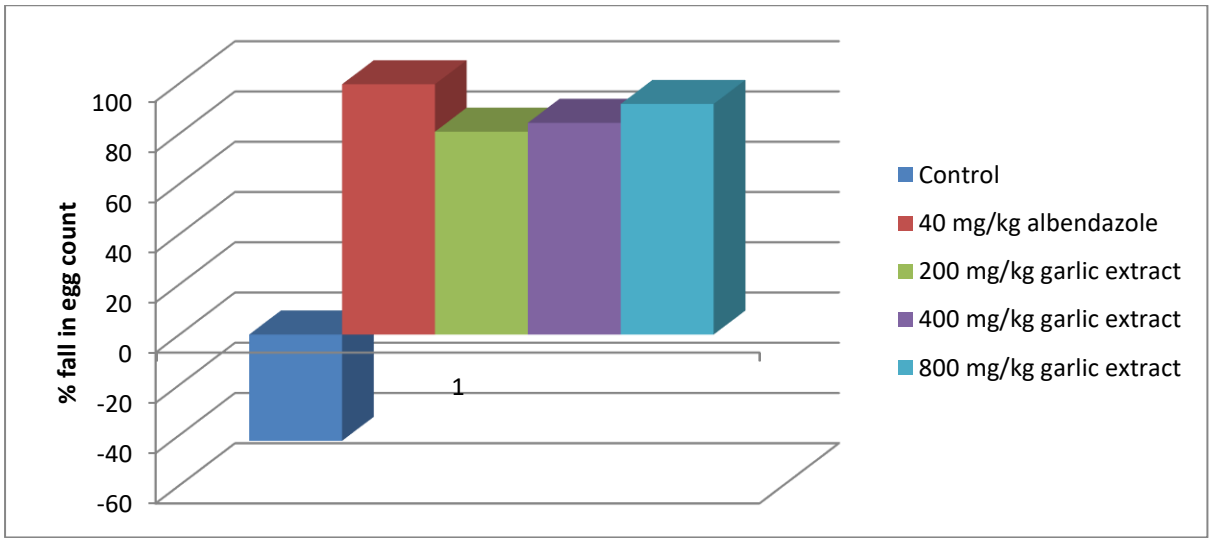

Fig 1: Percentage fall in fecal egg count of *Ascaris Lumbricoide*

The administration of Allium sativum ethanolic extract to the infected mice significantly ($P<0.05$) lowered the parasites in all treatment groups (2,3,4 and 5) compared to the control (group 1). The percentage reduction in fecal egg count, at day 3 after treatment with abendazole (group 2) was 99.61% while in groups 3,4 and 5, treatment with 200, 400, and 800mg/kg of Allium sativum extracted to percentage reduction of 80.73%,84.62% and 91.76% respectively. Treatment of mice in group 2 with abendazole (40mg/kg),led to the extermination of *Ascaris lumbricoide* ova in the 9th day.. This represent 100% effectiveness. Similar findings were observed for mice in groups 3,4 and 5treated with 200,400 and 800mg/kg of the plant extract. Calculated toxicity index (LD_{50}) for the studied plant extract suggest that the extract may not be toxic to living systems, which also confirm why the plant is useful to man as food and as curative agent. The safety of this extract is in agreement with the recommendation of the Organization for Economic Cooperation and Development (OECD) guideline for acute toxicity studies. The

guideline stipulates that mortality is the expected end point of acute toxicity and that where no mortality occurred within the acute toxicity study period in a population treated with a dose range at which mortality is expected, hence, the administered agent may be adjusted to be well tolerated and free of acute toxicity (OECD, 2001). Similar conclusions were made in other acute toxicity investigations involving Allium sativum (Akah *et al*., 2009). The observed effect of *Allium sativum* suggests that the extract may contain ingredients with strong anthelmintic activity. Allium sativum have been reported to contain phenolic compounds and phenolic compounds have been said to possess strong anthelmintic activity (Udoha *et al*., 2015)

The mechanism for the inhibition of eggs hatching larval development of Allium sativum may be related to the inhibition of cell in the organisms and consequently, inhibiting the formation and development of vital structures of the parasites. Allium sativum extract may also have acted like albedazole, a standard anthelmintic agent whose primary mode of action is to inhibit the polymerization of the parasitic tubulin into microtubules. The high affinity of abendazole to the tubulin causes the loss of the cytoplasmic microtubules which leads to impaired uptake of glucose by the larval and adult stages of the parasites and making the parasite to be unable to maintain energy production. This chain of events leads to immobilization and eventual death of the parasites (Tsao et al 2001). Allium sativum extract may have acted in addition to the above mechanism by inhibition of enzyme fumarate reductase- a secondary mechanism compared favorably with that of the standard drug abendazole.

4.0 Conclusion

The results and findings of the study reveal that Allium sativum has the potential of curing and preventing tumor,cancer of the stomach,colon,lungs and rectum, fights intestinal and stomach parasites,strongly reduces cholesterol, sore throat, tonsillitis mouth ulcers and sinusitis. The curative efficacy can be attributed to the presence of allicin, which aid the enzyme, allinase to act on the stable precursor, allin and exhibit anthelminthic activity, whose efficacy is comparable to that of albendazole. The present study has produced baseline information on the possibility of extrapolating the identity of the study plant extract in the treatment of some infection in man after trial test.

5.0 References

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Conflict of Interest

The authors declared no conflict of interest.