

ANTI-*HELICOBACTER PYLORI* EFFECTS OF THE METHANOL EXTRACTS OF *ALLIUM ASCALONICUM* (LINN.) (LILIACEAE) BULB.

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Summary: *Allium ascalonicum* bulb of the family Liliaceae is an annual herbaceous plant of smaller size than *Allium cepa*. The bulb is of considerable importance in African cooking and in salads. Various species have been reported to have anti-diabetic, hypocholesterolaemic, fibrinolytic, anti-ulcer and diuretic potentials. Crude methanol extracts of *Allium ascalonicum* bulb was screened against three strains of *Helicobacter pylori* (UCH 97001, UCH 98026 and UCH 97009) for antibacterial activity by the agar diffusion method on Muller-Hinton agar supplemented with defibrinated horse blood and grown in a microaerophilic incubator. All the strains were inhibited by the extract. Further investigation on the effect of the extracts on the urease activity of the *Helicobacter pylori* strains showed that urease activity of all the strains decreased with increase in the concentration of the extracts. Phytochemical screening of the plant revealed the presence of alkaloids, saponins, cardiac glycosides and essential oils while tannins were not detected. *Allium ascalonicum* bulb has some therapeutic potential against *Helicobacter pylori*, which may be explored by pharmaceutical companies and patients with gastroduodenal disorders.

Key Words- *Allium ascalonicum*, anti - *Helicobacter pylori*, urease activity.

Introduction

Allium ascalonicum (Linn) belongs to the family Liliaceae and is an annual herbaceous plant of smaller size than *Allium cepa*. The bulb is of considerable importance in African cooking and in salads (Irvine, 1956). In Ghana the bulbs are often pickled in vinegar made from palm-wine while chills are added to give piquancy (Irvine, 1961). In Northern Nigeria, some traditional herbalists, pound the *Allium ascalonicum* dry skin into powder, mix it with honey and carrot juice for the treatment of cancer. It has also been combined with *Carica papaya* (old leave), *Eliaeis guineensis* (roots), *Citrullus vulgaris* (roots), *Anisphylla species* (seeds), *Capsicum frutescens* (unripe fruits) and *Securidaca longipedunculata* (roots) taken with pap made from *Zea mays* for the treatment of Malaria (Akinniyi *et al*, 1986).

Various species of *Allium* have been reported to have antidiabetic, hypocholesterolaemic, fibrinolytic, anti-ulcer and diuretic potentials (Gills, 1992 and Augusti, 1996). The protein and other nutrient content is low, but there is value in the vitamin B content (Irvine, 1961). Phytochemical studies on *Allium* species plant shows that it contains many sulphur containing active principles mainly in the form of cysteine derivatives, viz. S-alkylcysteine sulfoxides, which decompose into a variety of thiosulfinates and polysulfides by the action of an enzyme allinase on extraction (Augusti, 1996).

Helicobacter pylori – a Gram negative, spiral shaped flagellated, microaerophilic organism has been implicated as the etiologic agent of acute gastritis, peptic ulcer, duodenal ulcer, gastric adenocarcinoma in humans (Marshall, 1984; Buck, 1990; Blaser, 1992). Its niche is restricted to the gastric mucosa. Among its virulence factors is possession of high level of urease enzyme which converts urea to ammonia, creating a local alkaline environment enabling the organism to survive the rather acidic environment of the stomach as well as aiding its initial colonisation of the gastric mucosa (David, 1996).

Dual therapy using proton pump inhibitors and a single antibiotic gives suboptimal eradication rate of *H. pylori*. Triple therapy using at least two antibiotics and either bismuth or a proton pump inhibitor gives eradication rates of 90% (Huang *et al*, 1997). However, these regimens are complicated with significant side effects and compliance problems leading to relapse. Since incomplete cure was achieved with triple therapy and the possible side effects, an alternative therapeutic agent is necessary. The antibacterial activities of plant extracts abound in the literature. The aim of this study is to investigate the effects of *Allium ascalonicum* bulb on *Helicobacter pylori* with special interest on susceptibility and effect on urease activity and then suggests its possible use for eradication of this pathogen.

Material and Method

Plant collection and preparation of extracts:

Fresh *Allium ascalonicum* leaves were bought from Jos Market, Plateau State, Nigeria and authenticated at both the Department of Botany and Microbiology, University of Ibadan and Forestry Research Institute of Nigeria (FRIN). Voucher specimen are deposited at both herbarium. The plant bulb was chopped, sun-dried and pulverised. Coarsely powdered plant sample weighing 300.27g was successfully extracted using soxhlet extractor with hexane and methanol as solvents for 24 hours in succession.

Each extract was filtered, concentrated *in-vacuo* and stored at 40°C until needed for analysis. 100mg/ml and 50mg/ml of the concentrated extract were prepared by dissolving 0.4g and 0.2g of the concentrated extract in 50% methanol respectively. Few drops of Tween 80 was added to enhance proper dissolution of the extracts. They were then used for antibacterial assays.

Microorganisms

The 3 strains of *Helicobacter pylori* used were cultured from gastric biopsy specimens of patients attending the endoscopy unit of University College Hospital (UCH) Ibadan, Nigeria. *Helicobacter pylori* bacterial cells were identified according to colony morphology, Gram staining, microaerophilic growth (at 37°C), oxidase positive (+ve), catalase(+ve), urease(+ve), nitrate(-ve), Hydrogen sulphide (-ve), hippurate hydrolysis(-ve) and nalidixic acid. The strains are UCH 97001, UCH 98026 and UCH 97009. They were subcultured in Mueller-Hinton broth supplemented with 3% sterile fetal calf serum, incubated under microaerophilic conditions at 37°C for 3 days and stored in a refrigerator for subsequent use.

Media

The media used were Mueller-Hinton agar and Mueller Hinton Broth (pH 7.3±0.1) made by DIFCO Laboratories, Michigan, USA. Horse-blood and sterile fetal calf serum were also employed as enrichment substances for the agar media and broth used respectively.

Antimicrobial agents

Pylorid® (25 mg/ml) and Bismuth citrate (25mg/mL) were included as positive controls while 50% methanol was used as negative control.

Phytochemical screening:

A quantitative chemical analysis of the powdered sample was carried out to detect the presence of various secondary metabolites such as alkaloids, anthraquinones, saponins, cardiac

glycosides, tannins, cyanogenetic glycosides, steroidal nucleus essential oil and flavonoids using the method described by Harbone, 1991.

Antimicrobial screening of crude extracts

Antimicrobial screening of the extracts was carried out as described by Diker *et al*, 1994.

A 0.6ml of 1:100 dilution fresh overnight culture of the *Helicobacter pylori* strains grown in Mueller-Hinton broth supplemented with sterile fetal calf serum was dispensed into sterile petri dishes aseptically.

Thereafter, molten Mueller-Hinton agar enriched with defibrinated horse blood was aseptically poured into the petri - dishes, mixed properly and allowed to solidify. A sterile cork borer of 8mm diameter was used to make equidistant and uniform wells on the surface of the agar medium. About 60µl of the resuspended extracts were placed inside the wells. The positive and negative controls were introduced into their own wells separately.

The plates were allowed to stay for 1 hour for proper diffusion of the extracts. Incubation of the plates was done under microaerophilic conditions at 37°C for 3 days. The diameters of clear zones of inhibition were measured to the nearest mm using a standard transparent meter ruler. Result are average of triplicate experiment.

Urease activity assay

The effect of the methanol extract of *Allium ascalonicum* bulb on the urease activity of the *H. pylori* strains was investigated using the alkalimetric method (Hamilton-Miller and Gargan, 1979; Mobley *et al*, 1988).

Fresh overnight cultures of *H. pylori* strains grown in Mueller-Hinton broth supplemented with sterile fetal calf serum was centrifuged and the sediment washed twice with 0.02M phosphate buffer solution (PBS-pH 6.8); resuspended again in the same PBS (0.8-1.0 at OD560nm) and used for urease activity assay.

Urease activity assay(control):

In the control experiment, a 0.1ml of the bacterial suspension was added to sterile test tubes containing 2.5ml of 0.03M PBS (pH 6.8) 0.1ml of phenol red (7µg ml⁻¹) and 0.4ml of urea (330µg/L). The tubes were properly shaken and the optical density OD-560nm and % Transmission (%T) was recorded for a period of 1 hour using a colorimeter.

Effect of Extract on Urease Activity:

To a sterile test tube containing similar reagents as in the control experiment, 60µl of increasing concentrations of the extract (25mg/ml, 50mg/ml, 100 mg/ml and 300mg/ml) was added and

shaken properly. The OD at 560nm and % Transmission (%T) were determined and recorded at 23°C for a period of 1 hour as in the control experiment. The OD (560nm) and %T values are the urease activity values.

Results

The percentage yield of methanol extract was greater (17.2%) than that of hexane extract (2.5%). Phytochemical screening revealed the presence of alkaloids, cardiac glycosides, essential oils and saponins while anthraquinones and tannins were absent. Alkaloids and cardiac glycosides and essential oils were present at a high concentration

while saponins were present at a low concentration (Table 1). All the *H. pylori* strains tested were susceptible

to the extract at the used concentrations with strain UCH 97009 showing the greatest susceptibility pattern (Table 2). Urease activity of all the strains decreased with increase in concentration of the extracts. The effect of the extract on strain UCH 97009 is presented graphically in figure 1. The other two strains has similar effect.

Table 1: Phytochemical screening of *Allium ascalonicum* bulb

PHYTOCHEMICAL GROUPINGS	OBSERVATION
Alkaloids	+++
Anthraquinones	-
Cardiac glycosides	
(i) Sugar Test	+++
(ii) Glycoside test	+++
Saponins	+
Tannins	-
Essential oils	++
Flavonoids	+

Constituents—Not detectable:

+++ = High concentration;

+ = Low concentration; ++ = Medium concentration

NT = Not Tested

Table 2: Antimicrobial Susceptibility of *Helicobacter pylori* strains to Methanol Extracts of *Allium ascalonicum* bulb.

<i>Helicobacter</i> <i>Pylori</i> strains	*Diameter Zones of inhibition (mm)				
	Methanol Extract (mg/ml)		Pylorid®	Bismuth citrate	Methanol
	100	50	25mg/ml.	25 mg/ml.	50%
UCH 97001	13 ± 0.3	13 ± 0.2	31 ± 0.4	30 ± 0.3	0
UCH 98026	14 ± 0.1	14 ± 0.3	18 ± 0.2	22 ± 0.2	0
UCH 97009	18 ± 0.2	17 ± 0.3	18 ± 0.3	22 ± 0.2	0

* Result is average of triplicate experiment.

Fig.1a: Urease Activity of *Helicobacter pylori* (UCH 97007) strain in different concentrations of methanol extract of *Allium association bulb*

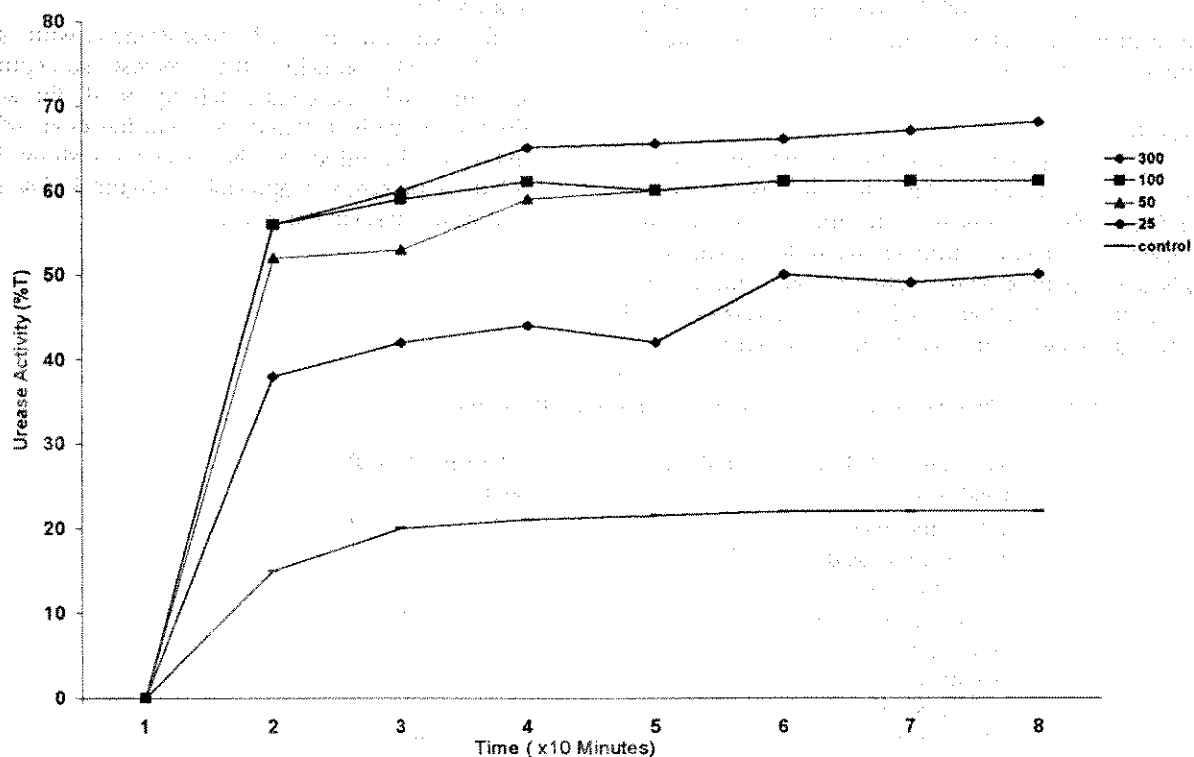
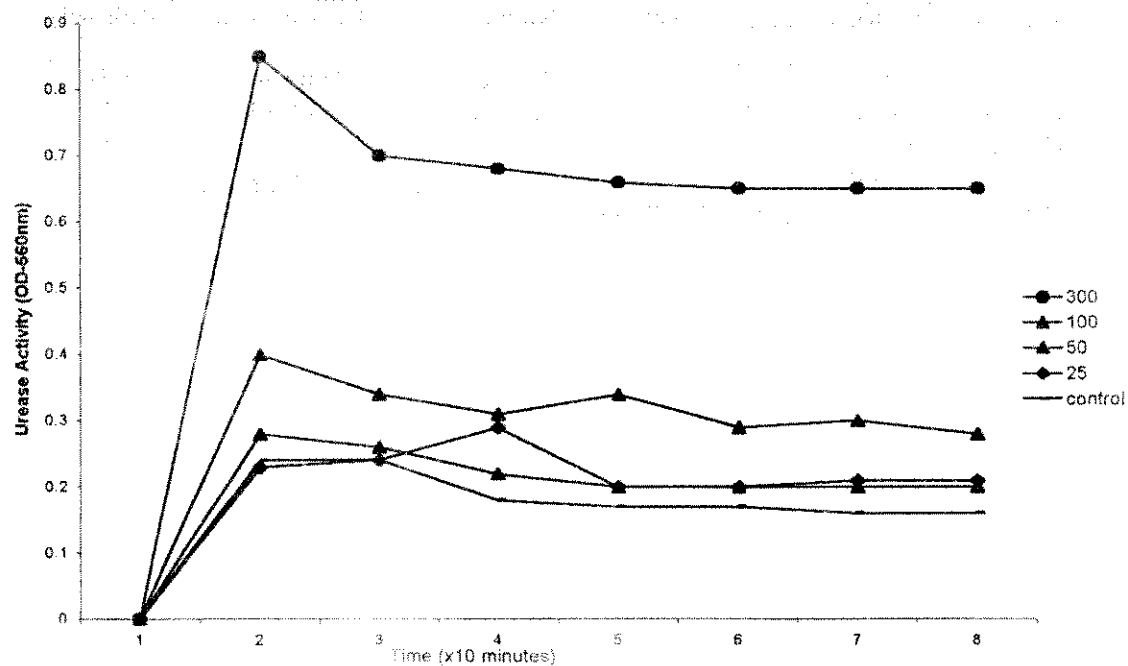


Figure 1b: Urease Activity of *Helicobacter pylori* (UCH 97009) strain in different concentrations of methanol extract of *Allium ascalonium bulb*



Anti-*Helicobacter pylori* effects of *Allium ascalonicum* bulb.

Discussion:

Over the years medicinal plant extracts have been shown to contain substances of therapeutic significance (Valnet, 1994). Antimicrobial chemotherapy does not achieve the much expected success in the eradication of microbial infections – *Helicobacter pylori* inclusive. Therefore the need for novel antimicrobial agents against *H. pylori* needs not to be overemphasized. The result of this work showed that *Allium ascalonicum* bulb inhibited the growth of *H. pylori* in a comparable level with the positive controls. Inhibition may be due to the presence of secondary metabolites in the plant as revealed by the phytochemical screening result.

Urease activity was decreased by the plant extract in all the strains as the concentration increases. It followed the pattern 300<100<50<25<control. For example in strain UCH 97001, at 20 minutes, urease activity was decreased from 73% to 1.6%, 54%, 56% and 72% for the control 300mg/ml, 100mg/ml, 50mg/ml and 25mg/ml respectively. The decrease of urease activity of all *H. pylori* strain by the *Allium ascalonicum* bulb methanol extracts is a big plus therapeutically. This is because urease enzyme present in both the cytoplasm and on the surface layer of *H. pylori* cells helps the organism to hydrolyze urea releasing ammonia, which neutralizes acid in the gastric mucosa, allowing the survival of the bacterium and its initial colonisation in the gastric mucosa (Hu *et al* 1990).

This decrease therefore means that the extracts will affect the survival of the organism in the gastric mucosa, which may cause them to be eradicated. Infact the use of proton-pump inhibitors like omeprazole and lansoprazole which are potent inhibitors of urease of *H. pylori* in the current treatment of *H. pylori* infections lends credence to this (Nagata *et al*, 1995).

However, inhibition of *H. pylori* strains may not be associated with a decrease in urease activity. Nagata *et al*, 1995 showed that the inhibitory action of lansoprazole and its analogs against *H. pylori* was not related to inhibition of urease. Therefore more work should be done to investigate the mechanism of inhibition of *H. pylori* strains by the plant extract.

In conclusion, *Allium ascalonicum* bulb has some anti-*Helicobacter pylori* effects as shown from this work. The use of this plant for the treatment of gastroduodenal disorders by some traditional herbalists in Northern parts of Nigeria is endorsed by this work. Also patients with gastroduodenal

disorders should make *Allium ascalonicum* bulb a dominant part of their daily diet.

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EDITOR