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RESEARCH ARTICLE

Determain Kind and Concentration of *Allium sativum* L.Plant Ingredients and its Effect on Isolated Bacteria Causing Urinary Tract Infections

Ayyub J. Abdl-Rahmaan Al-Baytay, Abdul Kareem Fattah Omar, Luma Safe Al Ghanam

College of Women Education/Tikrit University/Iraq.

Abstract

Atotal of (190) samples urinary were collected patients with Urinary Tract Infections(UTIs) from the period 2017/11/18 to 2018/2/17 ,for determain kind and concentration of Allium sativum L.ingredients and its effect on growth of the Gram-negative bacteria isolated from urinary tract infections in Al-Alam city/Tikrit Governoratr/Iraq,The study was carried out in the laboratories of Al-Alam Hospital and the laboratories of the College of Education for Women , Department of life Science,chemical analysis by HPLC apparatua showed the plant contain severel active ingrdients: S-ally cysteine,Y-glutomycysteine, Vinyl-{4H}1,2dithlin(agoene),di-allyldisulfide and Diallytrisulfide with concentrate,5.72% 9.75%, 53.09%,9.72%,12..85% and 10.60% respectively,also appeared many bacteria genus on In the samples taken from patients patients: E.coli, Klebsiella, Pseudomonus,Proteus,Serratia and Chromobacterium with isolates concentration reached for each genus reached, 44.2%, 23.5%, 17.6%, 5.5%, 5.8% and 2.9% respectively,and the current study for aquoues and alcoholic for extracts appeared a clear contrast on inhibition of studied bacteria kinds, as it was alcoholic extract of Allium sativum more inhibiton than aquoues extract on the concentration 100% throught observation inhibition diameter on the concentration 25,50 and 100%.

Keywords: *Allium sativum+plant extract+bacteria.*

Introduction

Urinary Tract Infections (UTIs) the most common diseases, and comes after it respiratory system diseases, which leads to the death of many people infected with them [1]. (UTIs) infect any part or place of urinary tract and it caused by many kind of gram negative and possative stain which be on causes of the diseases [2] And the number of people whom infected by it reaches millions, including females and males on different ages,and women are more likely to get the diseases than men [3]. The increased important on study of bacteria negative gram stain not only because its ability on configuration diseases for peopils who sleep in hospitals but also because of increasing the ability to resist antibiotics, which became healthy problems scattered in the world [4].

Verily negative bacteria is more common to produce the infection for example kinds do not fermenting glucose such as E Pseudomonas aeruginosa, which are considered most prevalent and most infection after E. coli Because of because it has a ferocity factor Characterized by it and giving effective resistance for antibiotic[5]. It has been found 50-60% from this diseses infections in Iran,also it had seen increasing resistance this kind for antibiotics during afew past[6],the negative gram stain bacteria are more kinds finding on urinary tract infection diseses ,may be rody shaped, some of them moved and grow on temperature 37^{0}C and some of 30-35^{0}C and its aerobic bacteria negative for oxidase enzyme produce and positive for catalase test except Shigella dysenteriae type which exist in water,soil, plant surface and animal body [7].

They found that the largest living organisms that cause UTIs disease are bacteria, including the negative, Enterobacteraceae and Speudomonaceae and the positive bacteria, including the Staphylococcus and Spongy [8, 9], this disease considered from medicinal problems which exists on more

countries of the world, either in Iraq it ranks first in between of others bacterial diseases up to 23% [10**]** .Discovery of antibiotics had important effect on putting ends and outbreaks of diseases and this lead to formation unlimited mounts from it which had role on eradicate the on severel type of bacteria [11],but its effect on bacteria began to decline because it had became resistance on this antibiotics and thgus become with weak effect on the bacteria,and ait has defensive means, and this means be on top of it when this antibiotic used randomly and irregular and and in large because quantities bacteria contain plasmid has ability to confrontation the antibiotic and also it has ability to on access to wide distances amoung the bacteria [12, 13].

The more and bad use of antibiotic be related with fast ability to formation bacterial Strain capable of resistance antibiotic and the relationship will be trivial between bacteria and antibiotic [14],and because of this problem and its side effect the scientists turned to use natural plants to make it treatment for more diseass [15, 16], which sweeps the body Including UTIs disease because medicinal plant has non or limited side effect [17], and with excellent ability on make a physiology change more than industrial and chemical material [18, 19] Treatment with plants and medicinal herbs is one of the method, used throughout the ages in treating various diseases. Many plants have been used for treatment by civilizations, including Indian and Chinese.

This plants has active compounds help human a lot in disposal the diseases [15, 20], compounds as alkaloides, essential oils, glycosides and commarins [21, 22]. Allium sativum plant used around the world because its spicy flavor as one type of spices and this clovers are more uses [23], and it has volatail oil and sulfide compounds such as allicin, minerals, protein and amino acid [24], in addition of its containing from elements Ca, P, Fe, C and Vitamins which give it an important to Eliminatation microorganisms and give the body ammunity to counter a lot diseases [25] studies confirmed that

A.sativum composed of many components of which glucoside sulfure, essential oil, homogeneous mixture of allyle oxide, sulfure, iode and silice and material similar to antibiotics as allicine and garlicine which they has effective effect against Streptococcus bacteria [26], the cellulosic fiber is the main components of A. sativum clovers which uses medicinaly with distinctive aroma which is attribute its effect as awid field antibiotic of microorganisms [27]. In iraq many researchers they did study about effect of the plant and effect its extracts on the bacteria, [28], showed effective role of aqueous and alcoholic plant extracts on growth of bacteria isolated from tonsils, and there in an important for plant extracts on resistance of lung system bacteria [29], Extracts of Apium graveolens and Trigonella foenum Working on prevent growth UTIs bacteria a nd the incidence of the disease [30]. The A.sativum plant has been studied and its effect on the growth of negative bacteria has been used in its seeds. The aim of the research to determain kind and concentration of A. sativum ingrdients and isolation also diagnosis of negative bacteria with Test the effect of plant extracts on the growth of bacteria.

Material and Methods

About 190 samples of negative gram stain bacteria from patients of UTIs were collected on 18\11\2017 to 17\2\2018,then diagnosed bacteria after their development at the medium blood agar that contain the 5% of the blood of the human and medium macConkey and EMB sterile autoclave degree 121 degree Celsius and for 15 minutes. As a person depending on the qualities of formal and tests biochemical [31].Also use the system API 20E make sure of the diagnosis [32].

Preparing Plants Extracts

A. sativum cloves were washed by tab water then with sterilized water and air dried at room temperature,100gm of cloves milled and used for extraction in 100ml of both hot water and ether alcohol is well known size more for a period of 24 hours in the sitter vibratory and are nominated placed streaming in a centrifugal quickly 3000/5000 cycle/min, and than nominate of new and take streaming and placed in evaporator rotor for getting dry powder then different concentration 25,50 and100% prepared from the dried extraction [33].

Active Ingredients Appreciation

About 10 gm from *A. sativum* put in 50 ml boiled water (90-100C0) for 3 hours then

extracted whattman papers no.1 the extraction collected and put in closed glass tube in order measuring the concentration of active ingredients by High Performance Liquid Chromotography apparatus(HPLC) which supplied by Shimadzu company (Japan) type,LC-10A 2000 supplied with spectrum scale (Spectro photo meter – spd – 10A – UV),Asample size 20µl injected on Fast liquid chramotographic column (LC) with diamention(50×4.6mm I.D) by the injector type (Rheodyn-712) at condition show in (table 1)and the data recorded by calculator which drawed the pick area and retention time.Astandard solution of *Allium sativum* plant used and sperated by HPLC apparatus and identification the pick area and retention time of standard solution (Table 2 Figure 1)and comparing it with the pick area and retention time of studed plant sample at the same condition [34]. Concentration of compounds in the sample calculated by the aquation:

$$\text{Conc. Compound in the plant} = \frac{\text{Pick area of compounds}}{\text{Pick area of standard pattern}} \times \text{standard pattern conc.} \times \text{delution factor}$$

Table 1: chromatographics separate condition

Colum	Mobile Phase	Following rate	Type of detector	Temperature	Fast of recorder paper	Sample Size
Reverse	20mm	1.0 ml/min	Ultra	38C^0	8 mm/min	3µl
Phase	sodium hydrogen		violate ray			
Column	phosphate: 10 mm		at210 nm			
(50×2.0	octan sulphonate					
Mm I.D)						50 (v/v)

Bacteria Isolation and Identification

Bacteria after their development at the medium blood agar that contain the 5% of the blood of the human and medium macConkey and EMB sterile autoclave degree 121 degree Celsius and for 15 minutes. As a person depending on the qualities of formal and tests biochemical [31].Also use the system API 20E make sure of the diagnosis [32].

Table 2: Compounds, Retention time, Pick area and the concentration of *H. suaveolens*

Compounds	Retention time	Pick area	Concentration (µg/ml)
S-ally cysteine	2.21	157472	25
Y-glutomy cysteine	3.15	270205	25
Allicin	4.43	178054	25
Vinyl-{4H} 1,2dithlin (agoene)	5.26	287693	25
Di-allyldisulfide	6.31	292239	25
Diallyltrisulfide	7.46	336037	25

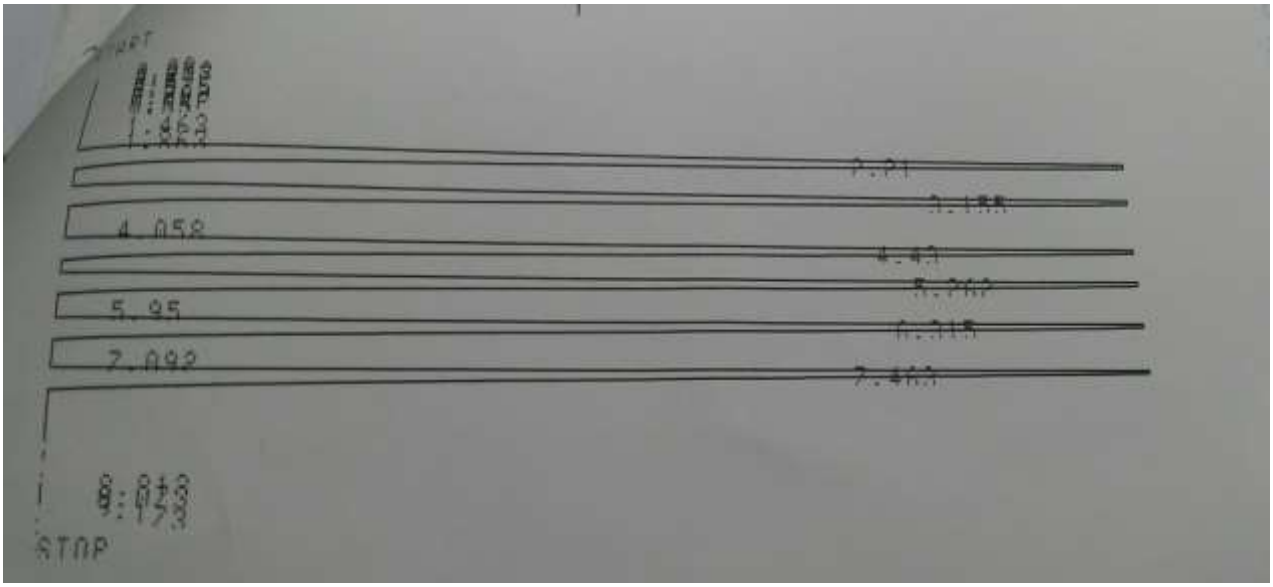

Fig.1: Chromatogram HPLC analysis of standard solution of *A. sativum*

Results and Discution

Verily Analysis by HPLC apparatus showed existence many compounds in the A.sativum cloves as S-ally cysteine, Y- glutomy cysteine, Allicin, Vinyl-{4H} 1,2dithlin(agoene), di-allyldisulfide, Diallyltrisulfide with concentrations were 5.72%, 9.57%, 53.09%, 9.72%, 12.85% and 10,60% respectively (table 3 and Figure 2). Material and active compounds analaysis by HPLC apparatus proved its activity in fast on diagnosis this compounds through its ability on calculating the curve with its hight and determine active ingredients in one operation [35].

It is also has the advantage in compare with other methods such as GC by ability on the dealing with non volatail materials including inorganic ions and thermally stable materials [36], results of study agree with [24, 37] whom refers To contain A. sativum to Allicin compound and agree with[38] whom confirmed contain this plant to sulfide compounds. After diagnostic tests,number and concentration of isolated bacteria reached 15 ,8,6,2 and 1 isolation by percentage reached 44.2%,23.5%,17.6%,5.8% and 2.9% for each of *E.coli*, *Klebsiella* , *Pseudomonas, Proteus, Serratia* and C*hromobacterium* respectively (table 4),the high percentage of *E.coli* due to its own a lot infection factors such as adhesion on Epithelial cells which lining urinary tracts and its own cilias which assist them adhesion and resistance antibiotics by proudcing the Hymolycin and its ability to growing fastly as it has short generation time [39] *Klebsiella* has factors assist them to occurrence the disease as portfolio protect them from unsuitable conditions and resist phagocytosis process besides of cilias ,and which givt them another chance to cause disease [40].

While appearance Serratia in this percentage agree with [41] pseudomonas were and pseudomonas is coliform shaped it exists in the form single or pairs or as chains [42] while proteus spherical shape and moving bacteria [43] its percentage disagree with [44], the last bacteria species *Chromobacterium* appeared by 2.9% percentage is the most kind cause the disease and which are considered natural flora existing in human and animal intestines [3].

Table 3: Ingrdients, Retention time, pick area, Concentration of *A.sativum*

S	Ingredients	Retention time	Pick area	Concentration µg/ml	Percentage (%)	Dilution
1	S-allyl cysteine	2.17	46894	314	%5.72	50
2	Y-glutomyl cysteine	3.12	64462	438,32	%9.57	50
3	Allicin	4.39	429028	2909,7	%53.09	50
4	Vinyl-{4H}1.2dithlin (agoene)	5.18	80085	533,2	%9.72	50
5	di-allyldisulfide	6.26	102374	704,26	%12.85	50
6	Diallyltrisulfide	7.38	80935	581,09	%10.60	50

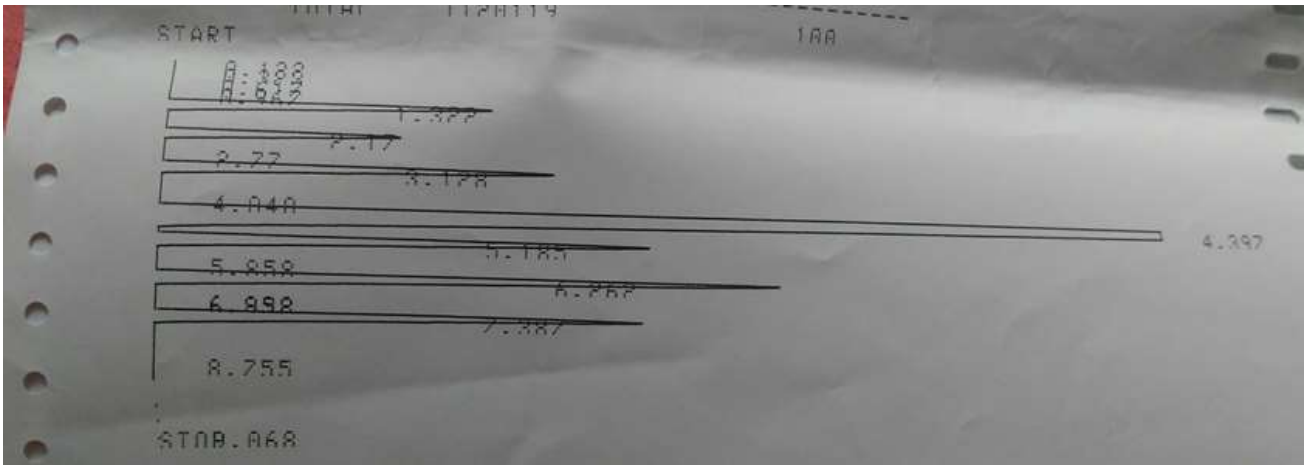

Fig. 2: Retention time and Pick area of *A.sativum* plant ingredients

Table 4.kinds of isolated bacteria

S	Bacteria genus	No.Isolation	Percentage (%)
1	*E.coli*	15	44.2
2	*Klebsiella*	8	23.5
3	*Pseudomonas*	6	17.6
4	*Proteus*	2	5.8
5	*Serratia*	2	5.8
6	*Chromobacterium*	1	2.9

The present study of aqueous and alcoholic extracts of *A.sativum* appeared aclear contrast on bacteria inhibition table 5 and *Pseudomonas* bacteria were most affected than other isolations, and alcoholic extract were more ihibtion than aqueous extract by observation inhibition diameter ,highest inhibition diameter by concentration 25% ,50% and 100% reached (13,16 and 20 mm) respectively. The same study showed that *Pseudomonas* more affected by different concentration of aqueous extract than other bacteria 25%, 50% and 100% concentration gaved high inhibition diameter reached (12, 14 and 17 mm) respectively, while *E.coli* bacteria were less affected at the same concentration of both alcoholic and aqueous extraction by inhibition diameter reached (6, 8 and 10 mm) and (5, 6 and 8 mm) respectively. The wide-ranging antagonism of *A. sativum* due to its containing sulfide compounds such as Allicin, Thiosulfinates [45, 46] whereas allicin works to Partial inhibition of formation the DNA and protein and total inhibition of RNA [47] as that presence of allicin substance has effect on enzymes oxidate [48] In addition to existence sulfide compounds such as Dimethyl disulfide, Methyl methey ethiosulfphonate, diallylsulfide, this compounds has effectiveness against for microorganism growth in addition to non sulfide compounds such as Vitamin B, proteins, Fe, Soaponins and Flavonoids [49] the resultas agree with [50] whom refer to effectiveness of aqueous and alcoholic extracts against isolates negative and positive gram stain bacteria, also agree with [51] whom showed effectiveness aqueous and alcoholic extracts of *A.sativum* against some kind of *Proteus, E. coli, Pseudomonas* bacteria.

Table 5.Effect aqueous and alcoholic of *A.sativum* extracts on bacteria isolates

Extracts / Isolates bacteria	**Aqueous**			**Alcoholic**		
	Concentrations (%)			Concentrations (%)		
	25	50	100	25	50	100
E.coli	5	6	8	6	8	10
Klebsiella	6	10	12	8	10	14
Proteus	6	8	10	10	12	15
Serratia	6	7	8	6	8	11
Pseudomonas	12	14	17	13	16	20
Chromobacterium	8	12	14	10	14	16

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