Journal of Medicinal Plants Studies
www.PlantsJournal.com

**ISSN (E): 2320-3862**
**ISSN (P): 2394-0530**
**NAAS Rating: 3.53**
JMPS 2020; 8(1): 26-30

Received: 16-11-2019
Accepted: 18-12-2019

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# GC-MS analysis and antibacterial activity of garlic extract with antibiotic

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**Abstract**
**Background and Objective:** Garlic (*Allium sativum*) is one of the herbs that used by traditional practitioners for preparation of herbals medicine. In the present study the antibacterial activity of aqueous and methanolic extracts of each local and imported garlic had been assayed separately against drug resistant clinical bacterial isolates include: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Salmonella typhi,* in addition compare it's effect with conventional antibiotics.
**Materials and Methods:** The antibacterial activity was determined by disc and a gar well diffusion method. The analysis of local and imported garlic constituents were done by gas chromatography-mass spectrometry (GC-MS).
**Results:** The results showed that there were differences in the antibacterial effect of garlic types and each extract. The aqueous extracts were more potent especially local garlic than the methanolic extracts, and all combinations were inferior in activity, when compared to the standard Ampicillin and the two types of garlic individually. The Inhibition zone of garlic varied ranging diameters (9.6 – 23.7 mm). The minimum inhibitory concentration of different bacterial species varied from 0.048 g/ml to 0.768 g/ml. The GC-MS analysis indicated that GC-MS analysis of local aqueous garlic showed the presence of: 3-chlorothiophene [7.35%]; diallyldisulphide [13.93%];-3-vinyl-1,2-dithiacyclohex-4-ene[18.40%]; 3-vinyl1, 2 dithiacyclohex-5ene [4.12%]; ethyl trifluoro methyl trisulphide [1.78%]; amidino thiourea[1.04]. 3,3 ‟thiobis1-propene [3.34%]; 1,4-diathiane [2.75%]; N, N‟-dimethyl-thiourea [0.72%]; 3-hydroxy, methyl-esteroctadecanoic acid [1.34%]; 2-chlorobutoxyethylester acetic acid [2.73%]; and 2-methoxy-n-tetrahydro furfuryl acetamide [1.54%].
**Conclusion:** Garlic extracts showed a powerful inhibitory effect against *pathogens* compared with Ampicillin.

**Keywords:** Garlic extract, antibacterial activity, clinical bacteria, antibiotics, GC-MS analysis

## Introduction
Garlic is well known to an array of photochemical, these bioactive molecules play a pivotal role in maintaining human physiology and having potential to reduce various ailments, so the composition of garlic that mentioned before varied according to geographical location, harvesting time and agronomic practices [1].
The consumption of traditional plants, particularly garlic, has gradually increased in worldwide due to their greater efficiency, lower side effects and relatively lower cost [2]. Scientific investigations have depicted that it contains 65% water, 30% carbohydrates, along with 5% of other bioactive moieties mainly sulphur- containing and nonsulphur - containing compounds. Among these organosulphur compounds, particularly cysteine sulphoxides and thisulphinates have greater importance [3].
The secondary plant extraction sources are usually prepared by using fresh and dried materials. Many authors has stated that the fresh plant tissues are used for plant extraction preparation, as they assumed that the ethno medicinal use fresh materials among the tribal people, in the same time, the others dry the plant in the oven at about 40 °C for 72 hrs. to prepare the aqueous extractions [4].
The crucial factor for the ultimate success of an investigation in to bioactive plant constituents is thus the selection of plant materials the most common extra intestinal bacterial infections and the second most common infectious disease encountered in community practice are urinary tract infection (UTI). It is alone poses a serious health problem affecting millions of people each year with total cost for treatment being in billions of dollars, that is worldwide, each year about 150 million people are diagnosed with UTI [5].

Antibiotics have been commonly using to treat bacterial infections such as Ampicillin and Ciprofloxacin. There is an increasing problem of antibiotic resistance to conventional drugs and a requirement for new solutions. Antibiotics have revolutionized medicine in many respects; their discovery was a turning point in medicinal history [6].

Regrettably, the use of these wonder drugs has been accompanied by the rapid appearance of resistant strains[7].The increasing reliance on drugs from natural sources has lead to the extraction and development of several drugs and chemotherapeutic agents from traditional herbs, which are present in abundance in the tropics [8]. Reviewing the effectiveness of plant based antimicrobial compounds, noted that a good proportion of such compounds are agents with weak or narrow spectrum of activities that act in synergy with intrinsically produced efflux inhibitors. However, bioactive compounds of plant origin when used together with antibiotics can increase the sensitivity of microbial cells to such antibiotics [9].

Earlier studies showed that a combination of fresh extracts of garlic (*Allium sativum*) and antibiotics has more antibacterial sensitivity [10]. This can be of immense value in combating infections caused by virulent strains of pathogenic and drug-resistant bacteria that are now causing enormous public health concerns in both rich and poor countries. Many studies have implicated *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* as leading causative agents of both community and hospital acquired infections [11] as has been reported earlier on major UTI causes, bacterial isolates were identify as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* [12]. In recent years, several reports from the scientific community have raised concerns that antibacterial drug development at its current pace will not adequately address the problems posed by antibiotic resistance among important bacterial pathogens leading to diseases [13].

Emerging antimicrobial resistance compels once again to look back into traditional medicines or herbal products, which may provide appropriate and acceptable alternative solutions. Plants derived products have made large contributions to human health and wellbeing [14]. This study aimed to investigate a comparative analysis of antibacterial activity of garlic aqueous and organic extract with antibiotic and a GC-MS analysis of garlic extract.

## Materials and Methods

The study was carried out duration 2016-2017 and using the local laboratories in Aden Gov, in addition for GC-MS in the laboratory of Higher Yemeni Agency for Specification and Measurements- Sana'a, Yemen.

### Preparing the garlic extract

The peeled and cleaned garlic bulbs were weighed [100g] and its cloves grounded thoroughly to obtain fine aqueous garlic by electrical extractor [MX-1515G2, Panasonic China], then the mixture was filtered through a sterile muslin cloth. The filtrate kept in 1.5 $cm^3$ tubes and was stored at – 20 °C until use. 100 g of raw garlic yielded 40 cm3 [2.5g/$cm^3$]. Each time concentrated aqueous garlic 100% [undiluted] was inoculated on nutrient agar media, incubated at 37 °C overnight, and was found to be sterile distilled water. The concentrated [100%] aqueous was further diluted to various concentrations [0.0015, 0.003,, 0.006, 0.012, 0.024, 0.048, 0.096, 0192, 0.384 and 0.768 g/$cm^3$] by mixing with appropriate sterile distilled water to study its bactericidal effect with nutrients and in 100 cm3 of nutrient broth to investigate the inhibitory or suppressive effect of studied plants extracts.

### Organic extraction of dried studied plants

The dried Studied Plants under a shade were ground by blender (MX-1515G2.Panasonic. China) into fine powder, 40g of the powdered Fresh Studied Plants were cold macerated in 100 $cm^3$ Methanol 96% (Al-noor Lab. Co. Aden, Yemen), stirred at intervals of four hours and kept at room temperature for forty eight hours. By using Whatman's filter paper NO.1, then sterilized by passing through a 0.22-µm filter membranes (Millipore, china) the solvent was filtered, and finally dried in oven in 40 °C for 24 hours. Moreover, in a sterile dark airtight bottle the methanolic extract kept at -4 °C in the refrigerator to avoid any microbial growth until required for further analysis. Each extraction was running in triplicate.

### Test microorganisms

The antibacterial assay was carried out using: -*Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. These species of bacteria were obtained from the Department of Bacteriology, Aden Hospital, Aden, Yemen.

### Identification of compounds using GC-MS analysis

Analysis of garlic samples extracts was performed under the same conditions with GC, using a Hewlett Packard 6890 GC, then gas chromatography coupled with mass-spectra were performed on HP 6972 mass selective detector agilent technologies, USA and HP-5MS column stationary phase (5% phenyl 95% dimethylsiloxane,30m X 0.25mm IDX0.2 micron film thickness).

Crude extracts with pentane dichloromethane of 4.0 g from each crushed local and imported garlic bulbs was extracted by adding to 5 $cm^3$ of dichloromethane. Then filtered the mixture after 5 min shaking in path. Then injected one µl from sample into the GC-MS.

The analysis of plant samples were carried by adding 2 $cm^3$ of pentane to prepared organic extracts. Dissolved 1 $cm^3$ of pentane with 5 µl of the filtered resultant mixture that was left overnight. [1µl] of organic samples were injected into the GC-MS for analysis [15, 16].

### Data analysis of studied plants bulbs

Statistical analysis was conducted using the SPSS software version [SPSS Inc]. By HP Chemstation software, each peak was analyzed for the most abundant compound that contains active constituents. The compounds were identified by direct comparison of their mass spectra to the Wiley NBS and NIST database.

## Results and Discussion

### The antibacterial activity of local and imported aqueous garlic extract

The inhibitory effect of local and imported garlic extract is compared with conventional antibiotics against some common hospital acquired pathogens. After incubation, the diameter of inhibition zone was measured for garlic extracts and antibiotics, the results are demonstrated in table (1).

The inhibition zones of aqueous local and imported garlic on gram positive and gram-negative bacteria were determined using filter paper disc diffusion method [17] [Mount *et al*., 2000] and the results are indicated in below table.

**Table 1:** Efficacy of aqueous extract and its synergism with antibiotics on test bacteria

| Aqueous extracts & its combinations | Inhibition Zones diameter [mm][a] | | | |
|---|---|---|---|---|
| | *P. aeruginosa* | *E. coli* | *S. aureus* | *Salmonella typhi* |
| LQ | 7.0±0.0 | 13.4±0.03 | 15.8±0.05 | 20.5±0.03 |
| IQ | 8.0±0.1 | 9.6±0.06 | 11.8±0.02 | 13.3±0.06 |
| NC | 6.0±0.0 | 6.0±0.0 | 6.0±0.0 | 6.0±0.0 |
| LQA | 12.0±0.0 | 16.7±0.3 | 16.0±0.0 | 23.7±0.06 |
| IQA | 10.±0.0 | 13.7±0.03 | 12.0±0.0 | 20.7±0.06 |
| Amp | 6.0±0.0 | 11.7±0.01 | 6.0±0.0 | 18.7±0.06 |
| Cipro. | 41.7±0.06 | 30.7±0.03 | 17.3±0.02 | 33.7±0.06 |

[LQ]: Local aqueous garlic, [LQA]: LQ+Ampicillin, [IQ]: Imported aqueous garlic, [IQA]: IQ +Ampicillin, [AMP]: Ampicillin, [NC]: +ve control, Ciprofloxacin [Cipro.]:-ve control, [a] =[0.6 cm] disk diameter.

The results of antibacterial activity revealed that aqueous extracts of local garlic (*LQ*) had a higher inhibitory effect on *Salmonella typhi* (20.5±0.03 mm) and a lower inhibitory effect on *P. aeruginosa* (7.0±0.0 mm).The *LQ* extract was effective than Ampicillin antibiotic (18.7±0.06 mm), although combination of LQ with ampicillin showed a higher inhibitory effect between aqueous extract, it was about (23.7±0.06). The aqueous extracts of imported garlic (*IQ*) revealed less effect than *LQ*, it was between 8.0±0.1 mm and 13.3±0.06 mm on *P. aeruginosa* and *Salmonella typhi* respectively.

Local aqueous extracts alone or with combination with ampicillin were more effective than imported aqueous extracts alone or with combination with ampicillin on tested bacteria. Local and imported aqueous garlic had low inhibition activity against P. aeruginosa [7.00 and 8.0±0.1] respectively.

The high antibacterial activity exhibited by local garlic as compared to imported garlic may be attributing to more quantity of sulphur-based compounds such as alliin, which possess strong antibacterial activities [18]. These compounds are present in the intact bulbs, flavorants formed on cutting or crushing the bulbs, substances derived from further reactions of these flavorants or metabolic degradation of these types of compounds [19].

Earlier studies showed that antimicrobial activity of garlic against variety of gram positive and gram-negative organisms as well as fungi and viruses [20]. Our study reveals that local garlic is active against resistant organisms to conventional antibiotics, which is in agreement to that reported in previous studies like [21]. The aqueous extract of garlic from the results obvious to be more efficacy than organic extracts, which is in agreement to that reported in previous studies [22, 23]. During aqueous extraction a number of phenolases, hydrolases are released, and these enzymes might serve to modulate the active compounds in the extract of plant materials [24].

Aqueous extracts contain active and non-active constituents that may result in dilution and lower concentration of samples of the potential content of liquefied extracts, so its activity appear less than Ciprofloxacin due to the pure form so no interferences from other compounds of the standards [25].

**The antibacterial activity of local and imported methanolic garlic extract**

The results of methanolic extracts efficacy on tested bacteria are summarizing in table (2):

**Table 2:** Efficacy of methanolic extracts and its synergism with antibiotics on tested bacteria

| Methanolic extracts & its combination | Inhibition Zones diameter [mm][a] | | | |
|---|---|---|---|---|
| | *P. aeruginosa* | *E. coli* | *S. aureus* | *Salmonella typhi* |
| LM | 10.00±0.0 | 7.5±0.06 | 9.00±0.0 | 6.3±0.06 |
| IM | 8.00±0.06 | 7.3±0.05 | 6.00±0.0 | 7.3±0.03 |
| NC | 6.00±0.0 | 6.00±0.0 | 6.00±0.0 | 6.00±0.0 |
| LMA | 11.00±0.0 | 12.4±0.1 | 10.3±0.0 | 19.6±0.01 |
| IMA | 8.8±0.0 | 11.9±0.06 | 8.00±0.0 | 19.1±0.00 |
| Amp | 6.00±0.0 | 11.7±0.03 | 6.00±0.0 | 18.7±0.06 |
| Cipr. | 41.7±0.06 | 30.7±0.06 | 17.3±0.21 | 34.7±0.06 |

[LM**]:** Local methanolic garlic**, [**LMA**]:** LM+Ampicillin, **[**IM**]:** Imported methanolic garlic, [IMA]**:** IM+Ampicillin**, [**Amp**]:** Ampicillin, [NC]**:**+ve control**,** Ciprofloxacin, [Cipr**]**:-ve control, [a]: [0.6 cm] disk diameter.

From the above results, imported methanolic garlic extracts [IM] recorded an inhibition zone less than the active limit [9.00 mm] against all tested bacteria. The factors associated with reduced activities of methanol extracts of imported garlic are not clear put believed the cause was due to drying interaction between methanol and constituents of studied plants and therefore require further work to explain. The activity of local methanolic garlic extract [LM] was [10.00±0.0 mm] against *P. aeruginosa*, and [9.00±0.0 cm] against *S. aureus* respectively. LM extract had slight effectively against tested bacteria. Study indicates combination of garlic extracts with conventional ampicillin leads to partial or total synergism, as found in local garlic as organic extract combination [LMA] gave its activity [11.00±0.0 cm].

IM extracts exhibit inhibition zone less than 9.00 mm against all the testedbacteria, which consider inactive values.

The result of antibacterial susceptibility assay showed promising slightly effects of local methanolic garlic extract against *P. aeruginosa and Staphyloccus aureus*. This was agreement with a study conducted by which showed organic extract of *Trema guineensis* and *Morinda Iucidaused* to be effective at high concentration against *P. aeruginosa* with inhibition zone about (19 mm) [26].

**Qualitative and quantitative analysis of studied garlic extract**

The two garlic extracts were analyzed by GC-MS to get the chromatograms, by using qualitative and quantitative analysis methods, and with the aid of computer retrieval and NIST library, calculating the result by peak area normalization method.

**Table 3:** The Percentage of chemical compound in local and imported aqueous garlic extract analyzed by GC-MC

| Compound name | Percentage (%) in | |
|---|---|---|
| | Local garlic | Imported garlic |
| 3-chlorothiophene | 7.35 | 6.35 |
| Diallyl disulphide | 13.93 | 9.84 |
| 3-vinyl-1,2-dithiacyclohex-4-ene | 18.40 | 18.45 |
| 3-vinyl1,2dithiacyclohex-5-ene | 4.12 | 17 |
| Ethyl trifluoromethyltrisulphide | 1.78 | 1.57 |
| Amidinothiourea | 1.04 | 0.77 |

| | | |
|---|---|---|
| 3,3‟thiobis1-propene | 3.34 | 2.70 |
| 1,4-diathiane | 2.75 | 3.28 |
| N, N‟-dimethyl-thiourea | 0.72 | 0.72 |
| 3-hydroxy, methyl-esteroctadecanoic acid | 1.34 | ND* |
| 2-chlorobutoxyethylester acetic acid | 2.73 | ND* |
| 2-methoxy-n-tetrahydrofurfuryl acetamide | 1.54 | 1.35 |
| 3-methyl Cyclohexen-1-ol | 00 | 0.72 |

*not detect

The high antibacterial activity exhibited by local garlic as compared to imported garlic may be attributing to more quantity of sulphur-based compounds such as alliin, which possess strong antibacterial activities [18]. These compounds are present in the intact bulbs, flavorants formed on cutting or crushing the bulbs, substances derived from further reactions of these flavorants or metabolic degradation of these types of compounds [19].

Earlier studies showed that antimicrobial activity of garlic against variety of gram positive and gram-negative organisms as well as fungi and viruses [20]. Our study reveals that local garlic is active against resistant organisms to conventional antibiotics, which is in agreement to that reported in previous studies like [18].

The aqueous extract of garlic from the results obvious to be more efficacy than organic extracts, which is in agreement to that reported in previous studies [22, 23].

During aqueous extraction a number of phenolases, hydrolases are released, and these enzymes might serve to modulate the active compounds in the extract of plant materials [24].

Aqueous extracts contain active and non-active constituents that may result in dilution and lower concentration of samples of the potential content of liquefied extracts, so its activity appear less than Ciprofloxacin due to the pure form so no interferences from other compounds of the standards [25].

The procedure yielded inconsistent results and poor resolution of bands. This may be due to the assertion that sulphoxides and thiosufinates are thermally unstable and may decompose into several sulfur compounds at high temperature that corresponding with reports of [26, 27].

As shown in (Tab. 3) All compounds are sulphur-containing compounds, except 2-methoxy n-tetrahydrofurfuryl, which might be responsible for antibacterial activity of aqueous garlic [28]. [Doglas.2008]. The obtained results from GC-MS analysis of imported aqueous garlic was showed that the procedure yielded inconsistent results and poor resolution of bands. This may be due to the assertion that sulfoxides and thiosufinates are thermally unstable and may decompose into several sulfur compounds at high temperature that corresponding with reports of [26, 27].

## Conclusion

The present study is consider a new report on the identification and characterization of bioactive compounds of Yemeni garlic bulbs as natural and important food sources, analysis allowed us to validate the experimental results and to distinguish the Yemeni garlic and Chinese garlic based on their antibacterial activity and chemical composition.

Garlic extracts showed a powerful inhibitory effect against *pathogens* compared with Ampicillin; the bacteriostatic activity of Ampicillin dramatically enhanced by addition of Liquefied garlic extract that mean exhibited a good synergism against *pathogens*.

The factorial analysis of Ampicillin and Liquefied garlic extract indicated intense positive interaction effects [$P$<0.05]. Suggest that the main content Allicin could enhance the activity of Ampicillin against *pathogens in vitro* and *in vivo* tests.

The differences of cultivation region have influence on garlic volatile flavor components, especially tremendous effects on the relative percentage content.

Climatic, geographic and varietal differences might also play an important role in the composition of photochemical components of local garlic and Chinese garlic. Therefore, the use of local garlic will reduce the side effects and cost associated with the applications of synthetic antibiotics and will be an ecofriendly measure.

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