Int J Pharm Bio Sci 2013 Oct; 4(4): (B) 1071 - 1076

Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ANTIBACTERIAL ACTIVITY OF *ALLIUM SATIVUM* (GARLIC) AND IDENTIFICATION OF ACTIVE COMPOUND BY GC-MS ANALYSIS

KARWAN SEDEEQ MOHAMMED

Microbiology Department Indian academy Degree College Hennur cross, Hennur Main Road Kalyan Nagar, Bangalore 560043 INDIA

ABSTRACT

In this study, different solvent extracts of garlic such as Ethanol, Methanol, Butanol, Acetone and Hexane was used for screening of antibacterial activity. The extracts were tested against both gram positive and gram negative bacteria such as *B. subtilis, S. aureus, P.auregunisa,* and *S. typhi*. The antibacterial activity was determined by agar well diffusion method on Mueller-Hinton Agar plates. Also, the inhibitory activity was tested against fungal species *A. niger.* All extracts showed inhibitory action against all four bacteria as well as fungi. The bioactive compound was identified by GC-MS analysis as {2-Furancarboxaldehyde, 5-(hydroxymethyl)}. These findings claim capacity and future use of this compound for drug development. The future work will be to determine toxicity, side effects and pharmaco-kinetic properties of this compound.

KEYWORDS: Garlic, *Allium sativum*, Antibacterial compound, GC-MS

KARWAN SEDEEQ MOHAMMED
Microbiology Department Indian academy Degree College Hennur cross, Hennur Main Road Kalyan Nagar, Bangalore 560043 INDIA

INTRODUCTION

In India medicinal plants are used in various tribal medicine due to minimal side effect and cost effectiveness[1-3]. Since long time Garlic (*Allium sativum*) family *Liliaceae* is a common spice used asflavoring agent and infolk medicine[4].Literatures show use of garlic for preventing common cold and cough and biological properties like antimicrobial, anti-cancer, antioxidant, immunomodulatory andanti-inflammatory effects[5, 6].Garlic is known to exhibit inhibitory action against both Gram-positive and Gram-negative bacteria[7]. Allicin is a key component of Garlic responsible for antimicrobial activity and its characteristicodour[8]. In recent years antibiotic resistance in pathogenic microorganisms has increased due to disorganized use of antimicrobial drugs which created serious threat to human health[9-11].Therefore, reevaluation of the therapeutic use of plants can be used as alternative strategiesto combat microbial resistance[12]. Most of the modern drugs are derived from plant sources and their extracts dominate in ayurvedic medicines[13].Identification of particular bioactive compound of plants has become easier due to the development of modern analytical tools such as especially gas chromatography–mass spectrometry (GC-MS). In this study we tested the antibacterial activity of five Garlic extracts against four bacteria (*B. subtilis, S. aureus, P. auregunisa* and *S. typhi*) and fungi *A. niger.* Furthermore, the bioactive compound was identified by GC-MS analysis.

MATERIALS AND METHODS

Plant material

Garlic was obtained from the local market area of Bangalore, India. It was dried at 40 ^{0}Cand powdered for extraction procedure.

Solvent extraction

Five gram of garlic powder was mixed with 50 ml of different solvents like Ethanol, Methanol, Butanol, Acetone and Hexane. The mixture was kept on rotary shaker for 48 hrs. The concentrated extract was used for antibacterial activity determination.

Collection of microorganisms

The bacteria were isolated from clinical samples and identified according to Bergey's manual on the basis of morphological, biochemical and physiological characteristics. The isolated bacteria were found to be *B. subtilis, S. aureus, P. auregunisa* and *S. typhi.*

Determination of antimicrobial activity

The antimicrobial activity was determined by the agar well-diffusion method. Overnight grown bacterial culture was spread on Mulleher Hinton agar plates. Wells were punched and approximately 50 *µ*l of the extract was added in to the wells, incubated at 4ºC for 2 hrs and then at 37°C for 24 hrs. The diameter for the zone of inhibition was measured and antibacterial activity was determined.

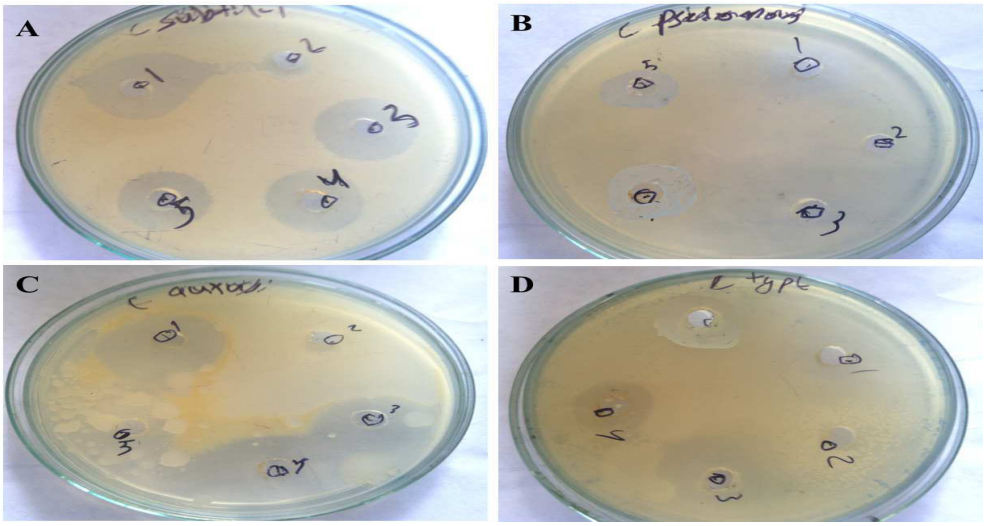
GC-MS Analysis

The bioactive compound was identified in three extracts Ethanol, Methanol and Acetone. The extracts were sent to VITTAL MALLYA SCIENTIFIC RESEARCH FOUNDATIONfor GC-MS analysis and results with identity of compound were collected.

RESULTS AND DISCUSSION

The antibacterial activity was determined against both gram positive and gram negative bacteria. Zone of inhibition was observed for all the extracts (Ethanol, Methanol, Butanol, Acetone and Hexane) against all four bacteria. The observed zone of inhibition was showed in figures 1. The measured zone of inhibition is summarized in table 1.

Figure 1
Antibacterial activity of Garlic Extract against bacteria



A- B. subtilis, B- P. aureginosa, C- S. aureus,D- S. typhi, 1-Ethanol , 2-Methanol, 3- Butanol, 4- Acetone, 5- Hexane.

Table 1
Zone of inhibition (mm) produced by garlic extract

Extract	*P. aureginosa*	*S. typhi*	*S. aureus*	*B. subtilis*
Ethanol	19	18	27	20
Methanol	37	24	19	22
Butanol	15	08	20	10
Acetone	17	26	27	26
Hexane	17	20	25	21

The tested bacteria responded in a different way to garlic extracts. The methanol extract was most active against *P. aureginosa* and produced maximum 37 mm zone of inhibition. *S. aureus* was most sensitive and showed highest zone of inhibition for ethanol, butanol, acetone and hexane extract. Earlier studies showed that antimicrobial activity of garlic against variety of gram positive and gram negative organisms as well as fungi and viruses[14-17]. Our results were in accordance with these earlier studies. We also tested the inhibitory action of garlic extract against fungus *A. niger*. The observed zone of inhibition was showed in figure 2.

Figure 2
Antibacterial activity of Garlic Extract against A. niger

For identification of bioactive compounds in solvent extracts GC-MS analysis was performed for three extracts. The Ethanol extract showed presence of [2-Furancarboxaldehyde 5-(hydroxymethyl)] as main compound with other 12 compounds in trace quantity (figure 3). In the same way the Methanol and Acetone extracts also showed presence of 2-Furancarboxaldehyde, 5-(hydroxymethyl) as main compound with other 14 compounds in methanol extract and 12 compounds in acetone extract in trace quantity (figure 4 and 5). From the results it can be interpret that the identified compound {2-Furancarboxaldehyde, 5-(hydroxymethyl)} is responsible for the Antimicrobial activity of Garlic.

Figure 3
Chromatogram and compound identification for ethanol extract

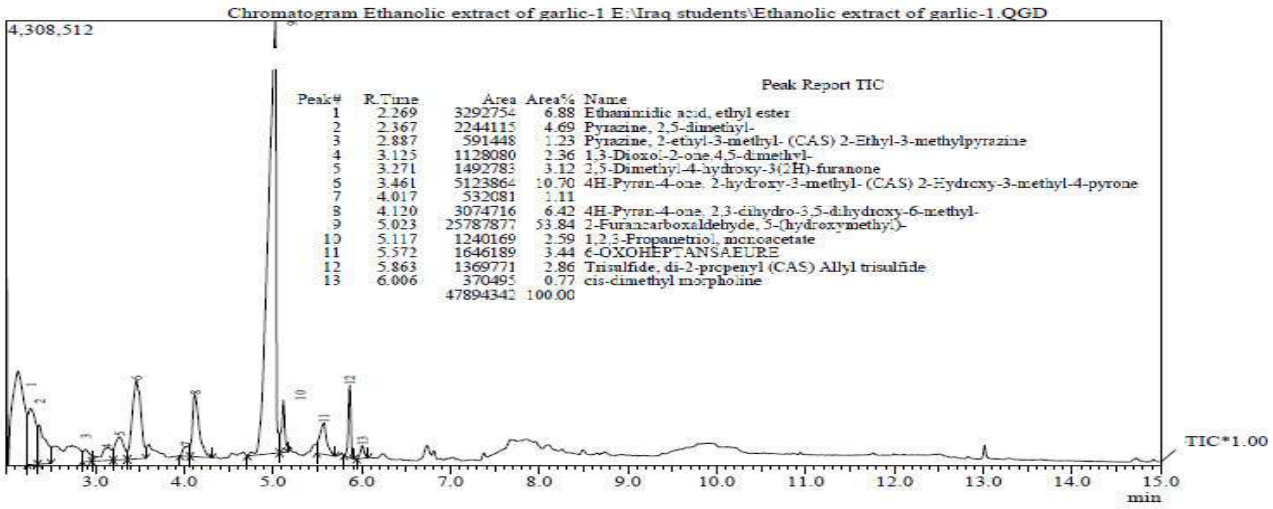


Figure 4
Chromatogram and compound identification for methanol extract

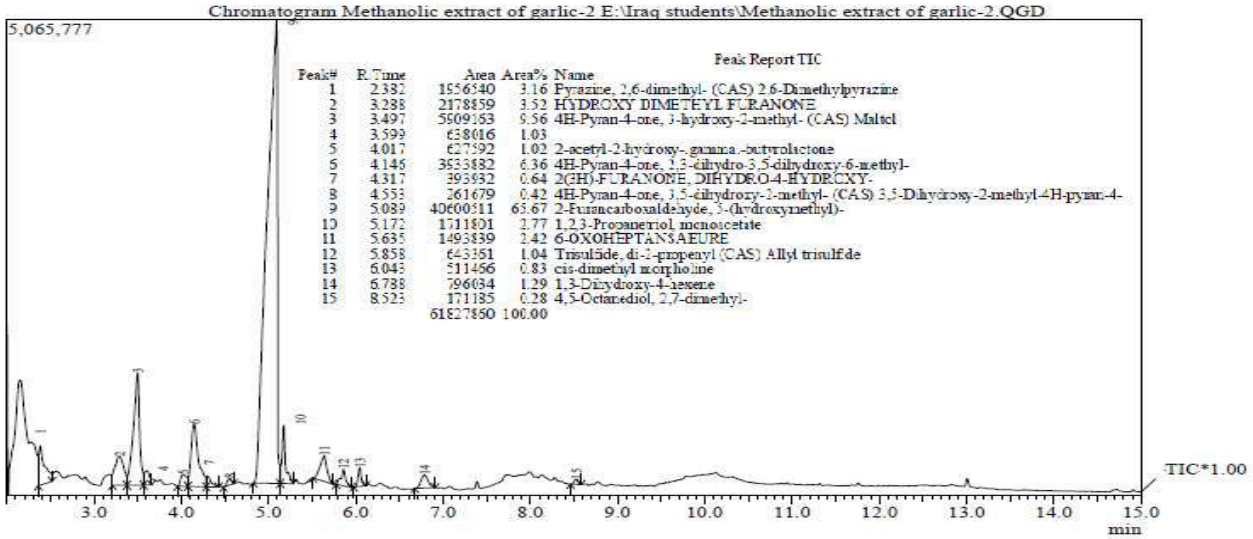
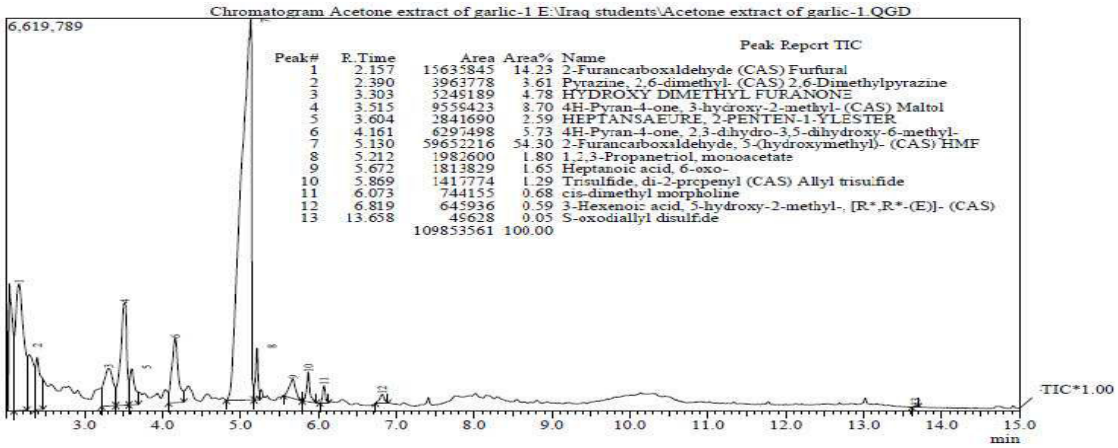


Figure 5
Chromatogram and compound identification for acetone extract



CONCLUSION

The present findings demonstrate the antibacterial activity of garlic against four bacteria. The GC-MS analysis identified the compound as {2-Furancarboxaldehyde, 5-(hydroxymethyl)}.These results stipulate significant capacity and future scope for the use of this bioactive compound for new drug development. The future work will be to determine toxicity, side effects and pharmaco-kinetic properties of this compound.

REFERENCES

1. Nair R, Chanda SV. Antibacterial activity of some medicinal plants of Saurashtra region. *J Tissue Res.* 2004; 4: 117-120.
2. Nair R, Kalariya T, Chanda S. Antibacterial Activity of Some Selected Indian Medicinal Flora. *Turk J Biol*. 2005; 29: 41-47.
3. Rajlakshmi D, Banerjee SK, Sood S, Maulik SK. In-vitro and in-vivo antioxidant activity of different extracts of the leaves of Clerodendron colebrookianum Walp in the rat. *J. Pharm. Pharmacol*. 2003;55:1681-1686.
4. Rivlin RS (2001).Historical perspective on the use of garlic. *J. Nutr.*,131: 951S-954S.
5. CORZO-MARTINEZ M, CORZO N, VILLAMIEL M. Biological properties of onions and garlic. Trends Food Sci Technol 2007; 18: 609-625.
6. Reuter HD, Koch HP and Lawson LD (1996).Therapeutic effects and applications of garlic and its preparations. *In*: Garlic. The Science and Therapeutic Application of *Allium sativum* L. and Related Species (Koch HP, Lawson LD, eds.), Williams and Wilkins, Baltimore, pp.135-512.
7. Whitemore B.B., Naidu A.S. (2000) Thiosulfinates. In: Naidu A.S. (Ed.), *Natural food antimicrobial systems*. Boca Raton, FL: CRC Press, pp. 265-380.].
8. Jabar, M. A. and Al- Mossawi, A. 2007. Susceptibility of some multiple resistant bacteria to garlic extract. *Afr. J. Biotechnol.* 6: 771-776.
9. Levy, S.B., Marshall, B. (2004) Antibacterial resistance worldwide: causes, challenges and responses. Nature Medicine, 10, pp. 122-129.
10. Mandal, S., Pal, N.K., Chowdhury, I.H., and Deb Mandal, M. (2009) Antibacterial activity of ciprofloxacin and trimethoprim, alone and in combination, against Vibrio cholerae O1 biotype El Tor serotype Ogawa isolates. Polish Journal of Microbiology, 58, pp. 57-60.
11. Parekh J, Chanda S. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *Afr J Biol Res.*2007; 10: 175-181.

12. Mandal, S., Deb Mandal, M., and Pal, N.K. (2010) Synergistic anti-Staphylococcus aureus activity of amoxicillin in combination with Emblica officinalis and Nymphaeodorata extracts. Asian Pacific Journal of Tropical Medicine, 3, pp. 711-714.
13. Murugesan, S., Pannerselvam, A., and Tangavelou, A.C. (2011) Phytochemical screening and antimicrobial activity of the leaves of *Memecylonum bellatum*burm. F. Journal of Applied Pharmaceutical Science, 1, pp. 42-45.
14. Daka D, Awole M. Assessment of the Antibacterial Effect of Crude Preparation of Garlic (Allium Sativum) on Diarrhea Causing Bacteria: An In Vitro, study. asian Journal of Medical Sciences. 2009;1(1):12-4.
15. Durairaj S, Srinivasan S, Lakshmanaperumalsamy P. In vitro Antibacterial Activity and Stability of Garlic Extract at Different pH and Temperature. Electronic Journal of Biology. 2009;5(1):5-10.
16. Goncagul G, Ayaz E. Antimicrobial Effect of Garlic (Allium sativum) and Traditional Medicine. Journal of animal and Veterinary Advances. 2010;9(1).
17. Yin M-C, Chang H-C, Tsao S-M.Inhibitory Effects of Aqueous Garlic Extract, Garlic Oil and Four Diallyl Sulphides against Four Enteric Pathogens. Journal of Food and Drug Analysis. 2002;10(2):120-6.