Spectral studies and antibacterial activity of Garlic

(*Allium sativum L.*)

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Abstract : The spectral analysis and assessment of antibacterial and identification of phytocompounds by FTIR, HPLC analysis from garlic extract. Preliminary phytocompounds of phenolics and flavonoids clearly showed their presence in FTIR and HPLC analysis. The antibacterial activity, the minimum concentration of A. sativum required to inhibit the growth of Gram-positive bacteria (S. aureus and S. pneumoniae) and Gram-negative bacteria (E. coli, P. aeruginosa, P. vulgaris, K. pneumonia and S. dysenteriae) were 4 mg/ml. Based on the results, garlic shows a great importance to understand the pharmacological actions and therapeutic efficacy possessed by majority of active compounds.

Keywords: Spectral studies; FTIR; UV-Vis; PL; HPLC; antibacterial activity.

I Introduction

Garlic (*Allium sativum L.*) has been considered as a medicinal plant for a long time [1]. Garlic contains many organo-sulfur compounds that give the characteristic flavor and potent biological health benefits [2]. Several epidemiological studies have demonstrated that a garlic extract and its sulfur-containing compounds had anticancer activity [3-5] the consumption of garlic and related sulfur compounds has been reported to reduce carcinogen- induced mammary, colon, lung, stomach, skin and liver cancers [4-9]. The mechanisms of garlic for its biological activities have been ascribed to its potent antioxidative [10] antithrombotic [1] and lipid-lowering [11] activities, and to its stimulating ability for immunological responses [12].

A. sativum crude extract is prepared by methanol and cured characterizations weredone by FT-IR, UV-Visible, and Photoluminescence spectra and HPLC studies. The antibacterial studies were carried out Gram-positive bacteria (*S. aureus* and *S. pneumoniae*) and Gram-negative bacteria (*E. coli*, *P. aeruginosa*, *P. vulgaris*, *K. pneumonia* and *S. dysenteriae*) strains.

II Experimental methods

Bulbs of *Allium sativum* L. (*A. sativum* L.) were collected from the market and which is commonly known as garlic. Garlic cloves were dried under the shade condition at room temperature and dried garlic cloves were crushed into fine powder using electrical blender and stored in airtight container.

2.1 Preparation of extracts

The dried fine powder of *A. sativum* colves were fed in a soxhlet extractor and extracted with methanol solvent added in a 1:3 ratio of garlic and methanol (60-80°C) till completely exhausted. This was subjected to Soxhlet extraction for 72 h according to the prescribed method of Eidi *et al*. (2006) [13]. After extraction, the solvent was filtered. The extract was concentrated to eliminate the solvent. Then concentrated extract was kept into tightly closed container and stored at 4°C in the refrigerator for further use.*A. sativum* L. crude extract is shown in Fig. 1.

Figure 1 *A. sativum*L. crude extract

2.2 Antibacterial Activity

The antimicrobial activity of the *A. sativum* extract was investigated by the disc diffusion method against the test Gram positive bacteria (*S. aureus* and *S. pneumonia*) and Gram-negative bacteria (*K. pneumonia,E. coli, S. dysenteriae, P. vulgaris,* and *P. aeruginosa*) on Mueller Hinton according to the Clinical and Laboratory Standards Institute (CLSI) [13]. The media plates (MHA) were streaked with bacteria 2-3 times by rotating the plate at 60˚ angles for each streak to ensure the homogeneous distribution of the inoculums. After inoculation, disc loaded with 2, 3,4, 6 and 8 mg/ml of the test samples were placed on the bacteria-seeded plates. The plates were then incubated at 37 ˚C for a day. The inhibition zone around the disc was measured and recorded. Amoxicillin (Hi-Media) was used as the positive controls.

2.3 Characterization techniques

Fourier Transform Infra-Red (FT-IR) spectroscopic studies

A Perkin-Elmer Fourier Transform Infra-Red (FT-IR) spectrometer was used in transmission mode and the corresponding spectra were recorded in the range of 4000-400 cm^{-1} using the KBr pellet technique for *A. sativum L.* samples.

UV-Vis spectroscopic studies

The UV-Visible measurements were performed for *A. sativum L.* was studied in the range between 200 and 1100 nm by Lambda 35 spectrometer.

Photoluminescence (PL) studies

Room temperature PL measurements were performed for *A. sativum L.* samples with excitation wavelength of 300 nm, 320 nm, 340 nm and 360 nm using Perkin Elmer instruments. The emission spectra were recorded in the UV and visible range (350-800 nm).

High Performance Liquid Chromatography system (HPLC)

The *A. sativum* extract samples fraction were analyzed usingHPLC, it was performed on aWaters modular system consistingof two modelpumps, an automated gradientcontroller, a injector, an in-line solvent degasser,and a modelPDA detector. HPLC apparatus equipped with a Photo Diode Array (PDA – 2998)detector (Waters, USA). C18 reverse phase column (4.6 × 250 mm, 5 µm, SYMMETRY). The solvent system consisted of water with 0.1% formic acid (A) andAcetonitrile (B). Elution was carried at 25°C at an initial flow rate of 1.0 mL/min.

III Results and discussion

3.1 FT-IR spectroscopic studies

FT-IR spectra of *A. sativum* crude extract is shown in Fig. 2. The wide band observed at 3307 $cm^{-1}$is assigned to the N-H stretch of proteins and O-H stretch of polysaccharides and water [14]. The bands at 2,844 and 2,979 cm^{-1} are attributed -CH_2 symmetric and anti-symmetric stretch of methyl group mainly from lipids [15].The vibration spectra bands range between 1800 and800 cm^{-1} reflected the chemical compositions of plant extracts,specifically lipids, proteins, polysaccharides, and polyphenols.The intense peak at 1636-1635 cm^{-1} is assigned to C=C stretching vibration of the allyl group. From the FT-IR result C=C pecks observed at 1636 cm^{-1}, which is due to the garlic oil and is assigned to the conjugated double bond of the dithiin ring. The symmetric CH_3bending modes of the methyl groups of proteins are observed at 1401 cm^{-1} for *A. sativum* extract [16].The skeletal vibration of diallyl sulfide molecule causes the double peak found at 1192 cm^{-1}.The stretching vibration (CC) ring observed at1120 cm^{-1} is ascribed topolysaccharides andcellulose [17]. Thecompounds, such as sulfides containing C-S and S-S bonds, show stretching bands at700-600 $cm^{-1}$and near 500 cm^{-1}, respectively. FTIR result shows that the sulfides groups observed at 655 and 602 cm^{-1} for *A. sativum* crude extract.

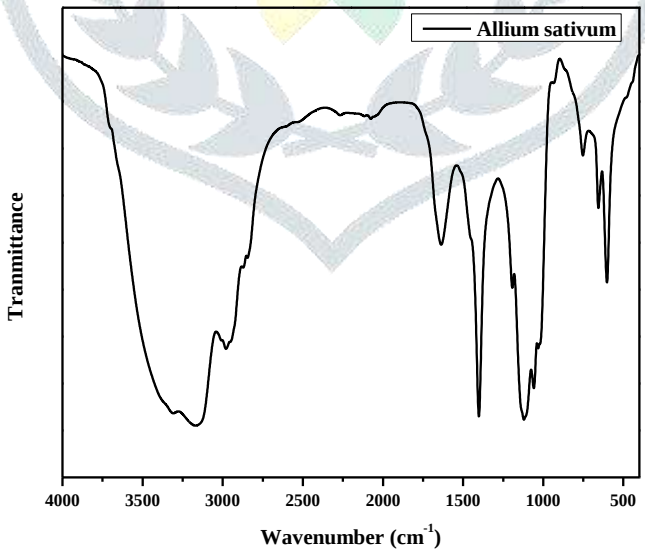


Figure 2 FT-IR spectra of *A. sativum* crude extract

3.2 UV-Vis Spectroscopic Studies

The UV-Vis absorbance spectra *A. sativum* crude extract have been recorded in the range 200-1100 nm are shown in Fig. 3. From the absorbance spectra, the absorption edge peak is observed at 277 nm for *A. sativum* crude extract; this reaction product may be sulfides containing C-S and S-S bonds. The estimated indirect and direct band gaps are observed at (5.5 eV and 5.515 eV) for *A. sativum* extract (in Fig. 4).

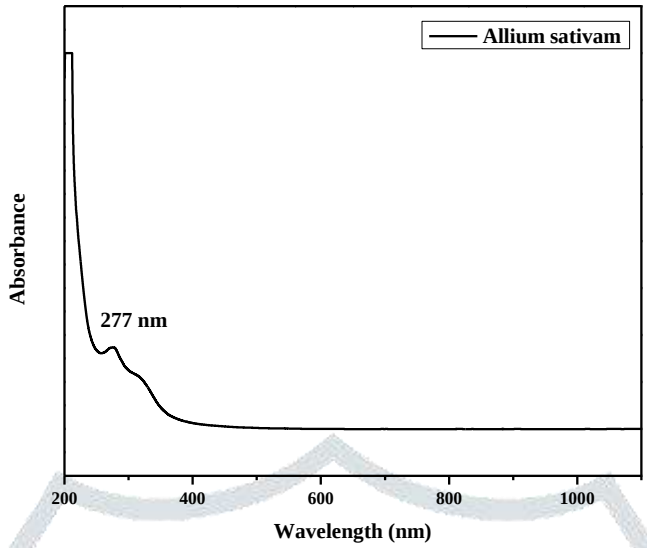


Figure 3 UV-Vis absorbance of spectra of *A. sativum*curdeextract.

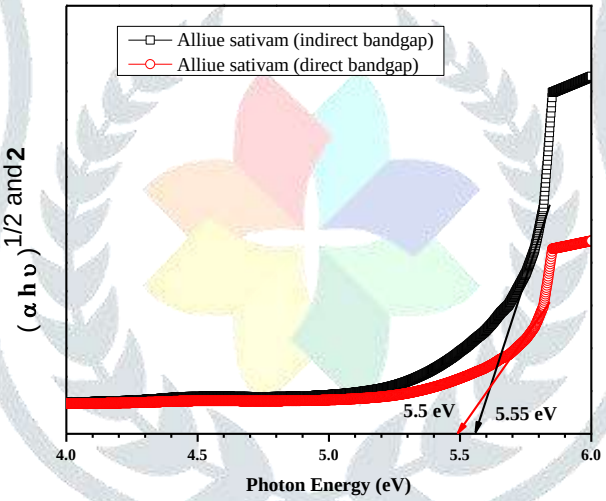


Figure 4 Photon energy level indirect and direct band gap of *A. sativum* curde extract.

3.3 Photoluminescence spectroscopic studies

The photoluminescence of spectra of *A. sativum* curde extract is shown in Fig. 5(a-d) and excitation wavelength observed at 300 nm, 320 nm, 340 nm and 360 nm respectively. A good fit of five peaks are made using Gaussian function. The solid lines represent the linear combination of five Gaussian peaks 349 nm has the lowest wavelength and 725 nm have the highest wavelength. In the PL spectra of *A. sativum*, the five emission peaks are observed in the UV and Visible region (Table -1).

The excitation 300 nm has emission peaks (349 nm, 383 nm, 427 nm, 496 nm and 608 nm), excitation 320 nm has emission peaks (406 nm, 434 nm, 445 nm, 511 nm and 687 nm), excitation 340 nm has emission peaks (405 nm, 431 nm, 445 nm, 460 nm and 707 nm) and excitation 360 nm has emission peaks (401 nm, 425 nm, 443 nm, 464 nm and 725 nm) respectively. The Near band edge emission range at 349-400 nm, Violet emission range at 401-420 nm, Blue emission range at 425-485, Blue-green emission range at 485-499 nm, Green emission range 500-550 nm, Yellow emission range at 600-650nm, orange emission range at651-700 nm and Red emission at 700-725 nm for UV and Visible color emissions. *A. sativum* curde extract combination of product of thiosulfate (R-S (O)-S–R', R = allyl, R' = (E) or (Z)-propenyl; or R = (E)-propenyl, R' = allyl) with glycine moleclues[18]. Organic constituents from examined samples were found to be complex mixtures of hydrocarbons, alcohols, aldehydes, acids, phenols, and pigments.

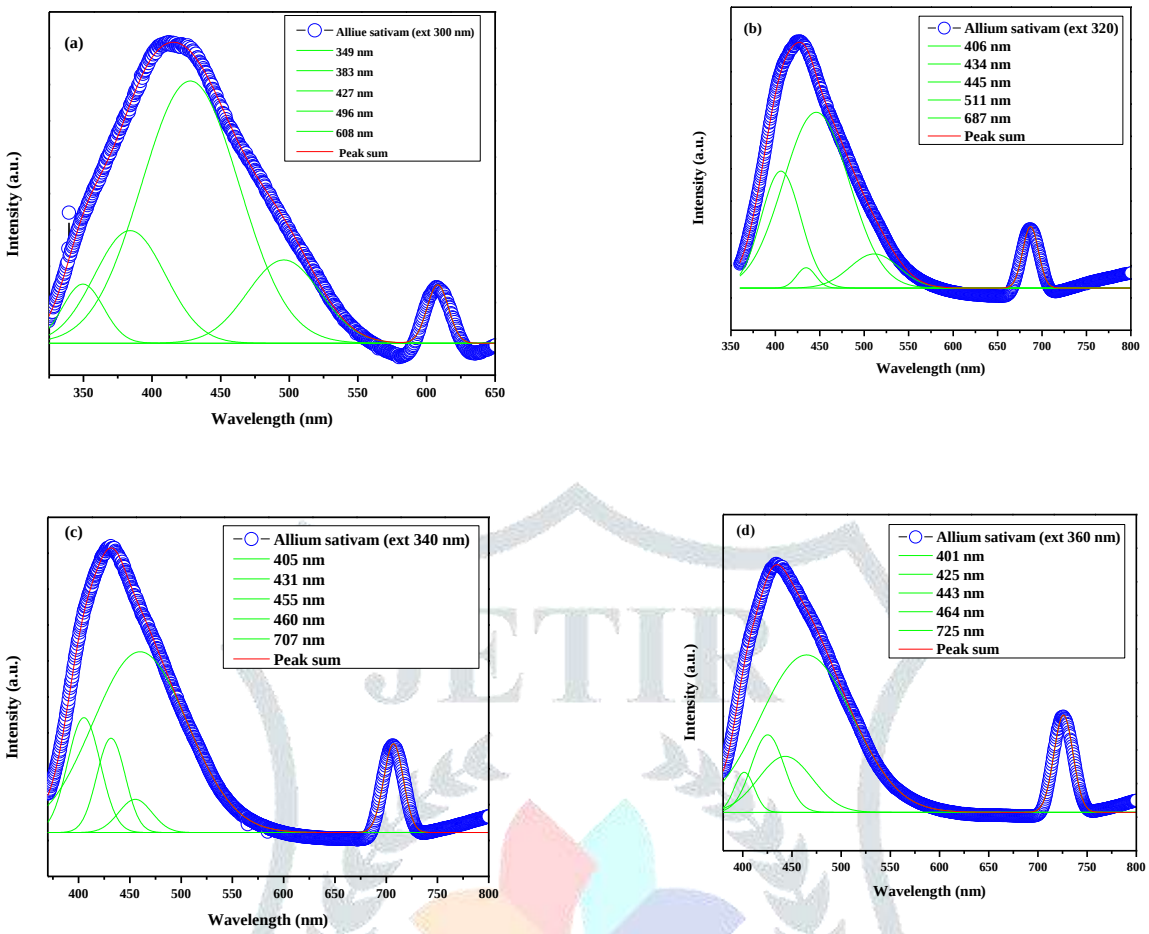


Figure 5 (a-d) PL spectra of different excitation wavelength 300 nm, 320 nm, 340 nm and 36 nm for *A. sativum* crude extract.

Table 1 PL spectra of different emission wavelength of *A. sativum* curde extract

S.No	Excitation (300 nm)	Excitation (320 nm)	Excitation (340 nm)	Excitation (360 nm)	Assignment
1	349	406	405	401	Near Band Edge and Violet emission
2	383	434	431	425	Near Band Edge and Blue emission
3	427	445	445	443	Blue emission
4	496	511	460	464	Blue and Green emission
5	608	687	707	725	Yellow, Orange and Red emission

3.4 High Performance Liquid Chromatography system (HPLC) studies

HPLC mobile phase, in this analysis, was set with Methanol (Fig. 6). UV wavelength was set at 254 nm. Initial volume of 20 micro liters, flow of 1 ml per min and maximum pressure of 350 Barr at ambient temperature was set as the conditions for the HPLC analysis. The HPLC conditions were set to be as best to accommodate the available chemicals/materials as well as allicin's stability. The *A. sativum* fractions consisted of several peaks observed (Fig. 6). The highest retention time of 1.972 min was found at organosulfur compounds for *A. sativum* crude extract.

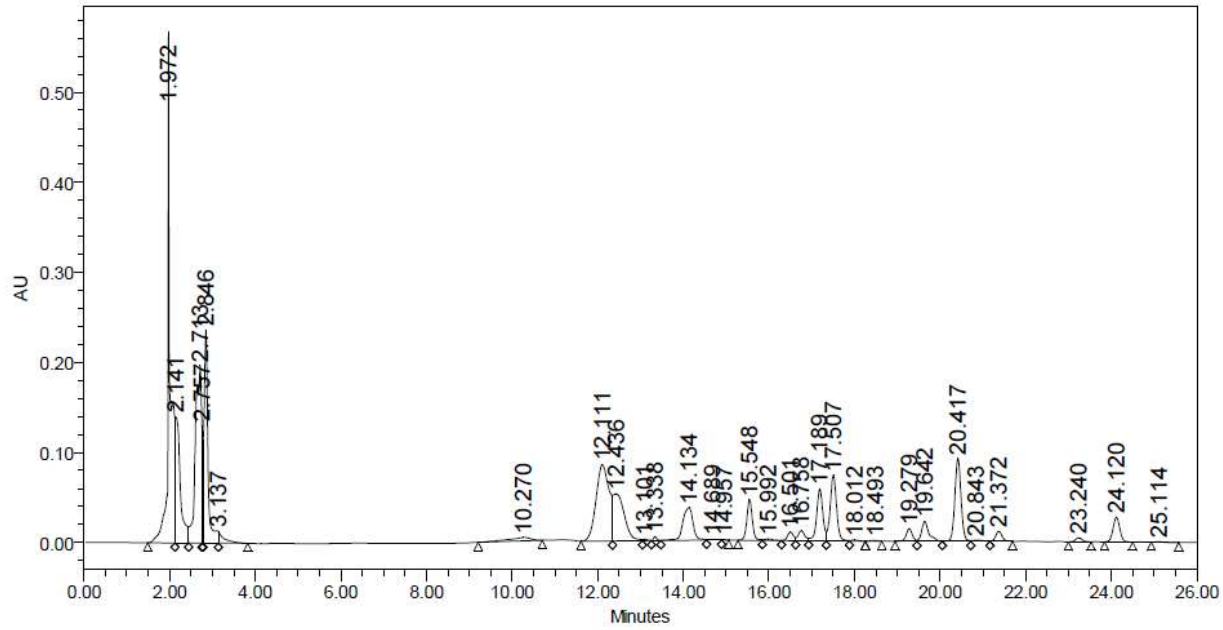


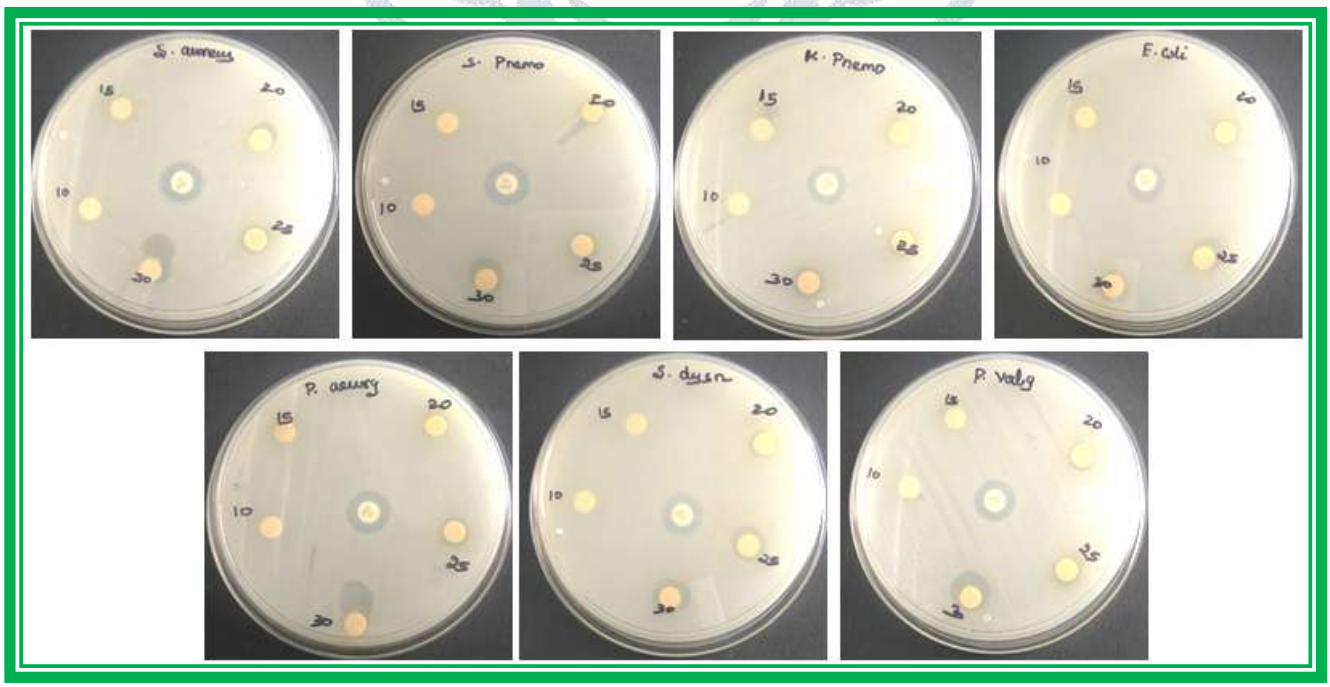
Figure 6 HPLC graph and peaks of retention times for *A. sativum* crude extract

3.5 Antibacterial activity

Antibacterial activity of *A. sativum* cured extract is investigated against Gram-positive bacteria (*S. aureus* and *S. pneumoniae*) and Gram-negative bacteria (*E. coli*, *P. aeruginosa*, *P. vulgaris*, *K. pneumonia* and *S. dysenteriae*) are studied by disc diffusion method as shown in Fig. 7.

A. sativum has been known to have anti-bacterial, anti-fungal, and anti-viral activity [18]. The present results are in fair correlation with the study above and the study carried out by Reuter et al., 1996 in which Garlic has been reported to inhibit the growth of Staphylococcus and many other species [19]. In another study crude juice of Garlic has been found to be high active against *E. col*i and *Salmonella typhi* [20]. Sasaki *et al*., (1999) have reported that the Garlic activity against *methicillin-resistant Staphylococcus aureus* and *candida albicans* [21]. Garlic extract possesses anti-bacterial activity against *Helicobacter pylori* at moderate concentration, thus it has protective effect against stomach ulcer [22].

Figure 7result shows the zone inhibition was observed 4, 6 and 8 mg/ml concentration loaded disc and other 2 and 3 mg/ml load disc there is no antibacterial activity.Generally, bactericidal agents are much preferred in theclinical field because bactericides lead to rapid and betterrecovery from bacterial infections and also minimize thepossibility of the emergence of drug resistance. The minimum concentration of *A. sativum* required to inhibit the growth of Gram-positive bacteria (*S. aureus* and *S. pneumoniae*) and Gram-negative bacteria (*E. coli*, *P. aeruginosa*, *P. vulgaris*, *K. pneumonia* and *S. dysenteriae*) are 4 mg/ml.



The antibacterial activity of the *A. sativum*extract testedagainst Gram-positive (*S. aureus* and *S. pneumonia*) and Gram-negative (*K. pneumonia, E. coli, P. aeruginosa, S. dysenteriae and P. vulgaris*) bacterial strains.

IV Conclusions

A. sativum crude extract was prepared by methanol and cured werecharacterizationsdone by FT-IR, UV-Visible, Photoluminescence spectra, and HPLC carried out. From the FT-IR result, the sulfides groups were observed at 655 and 602 cm^{-1} for *A. sativum* crude extract. UV-Vis spectra, the absorption edge peak was observed at 277 nm for *A. sativum* crude extract; this reaction product may be sulfides containing C-S and S-S bonds. PL spectra of visible emission peak results might be, combination of product of thiosulfate (R-S(O)-S–R', R = allyl, R'= (E) or (Z)-propenyl; or R = (E)-propenyl, R' = allyl) with glycine molecules. HPLC results showed thathigh retention time of 1.972 min was found at organo sulfur compounds for *A. Sativum* crude extract. The antibacterial activity, the minimum concentration of *A. sativum* required to inhibit the growth of Gram-positive bacteria (*S. aureus* and *S. pneumoniae*) and Gram-negative bacteria (*E. coli*, *P. aeruginosa*, *P. vulgaris*, *K. pneumonia* and *S. dysenteriae*) were as 4 mg/ml. The garlic extracts have rich contents of alkaloids, flavonoids, phenolics and various bioactive compounds are responsible for drug-resistant microorganisms and the need to produce more effective antimicrobial agents.

Reference

[1] Block, E., (1985). The chemistry of garlic and onions. Sci. Am., 252, 114–119.
[2] Amagase, H., (2006). Clarifying the real bioactive constituents of garlic. J. Nutr., 136, 716S–725S
[3] Amagase, H., and Milner, J. A., (1993) Impact of various sources of garlic and their constituents on 7,12-dimethylbenz [a]anthracene binding to mammary cell DNA. Carcinogenesis, 4, 1627–1631.
[4] Schaffer, E. M., Liu, J. Z., Green, J., Dangler, C. A., and Milner, J. A., (1996) Garlic and associated allyl sulfur components inhibit N-methyl-N-nitrosourea induced rat mammary carcinogenesis. Cancer Lett., 102, 199–204.
[5] Wargovich, M. J., (1987). Diallyl sulfide, a flavor component of garlic (Allium sativum), inhibits dimethylhydrazineinduced colon cancer. Carcinogenesis, 8, 487–489.
[6] Hong, J. Y., Wang, Z. Y., Smith, T. J., Zhou, S., Shi, S., Pan, J., and Yang, C. S., (1992) Inhibitory effects of diallyl sulfide on the metabolism and tumorigenicity of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3- pyridyl)-1-butanone (NNK) in A/J mouse lung. Carcinogenesis,
13, 901–904.
[7] You, W. C., Blot, W. J., Chang, Y. S., Ershow, A., Yang, Z. T., An, Q., Henderson, B. E., Fraumeni, Jr., J. F., and Wang, T. G., (1989). Allium vegetables and reduced risk of stomach cancer. J. Natl. Cancer Inst., 81, 162–164.
[8] Belman, S., (1983), Onion and garlic oils inhibit tumor promotion. Carcinogenesis, 4, 1063–1065.
[9] Hayes, M. A., Rushmore, T. H., and Goldberg, M. T., (1987), Inhibition of hepatocarcinogenic responses to 1,2-dimethylhydrazine by diallyl sulfide, a component of garlic oil. Carcinogenesis, 8, 1155–1157.
[10] Borek, C., (2001). Antioxidant health effects of aged garlic extract. J. Nutr., 131, 1010S–1015S.
[11] Silagy, C., and Neil, A., (1994), Garlic as a lipid lowering agent-a meta-analysis. J. R. Coll. Physicians Lond., 28, 39-45.
[12] Kyo, E., Uda, N., Kasuga, S., and Itakura, Y., (2001). Immunomodulatory effects of aged garlic extract. J. Nutr., 131, 1075S–1079S.
[13] Eidi A, Eidi M, Esmaeili E (2006) Antidiabetic effect of garlic (Allium sativum L.) in normal and streptozotocin-induced diabetic rats. Phyto Med 13:624–629.

[14] Wright GD. (2000) Resisting resistance: new chemical strategies for battling superbugs. Chem. Biol.; 7: R127-R132.

[15] Lu, X., Rasco, B.A., Jabal, J.M., Aston, D.E., Lin, M. and Konkel, M.E., (2011). Investigating antibacterial effects of garlic (Allium sativum) concentrate and garlic-derived organosulfur compounds on Campylobacter jejuni by using Fourier transform infrared spectroscopy, Raman spectroscopy, and electron microscopy. Applied and environmental microbiology, 77(15), pp.5257-5269.

[16] Naumann, D. (2001) FT-infrared and FT-Raman spectroscopy in biomedical research. Appl. Spectrosc. Rev., 36, 239–298.

[17] Movasaghi, Z.; Rehman, S.; Rehman, I. (2008) Fourier transform infrared (FTIR) spectroscopy of biological tissues. Appl. Spectrosc. Rev., 43, 134–179.

[18] Kubec R, Hrbáčová M, Musah RA, Velíšek J. (2004) Allium discoloration: precursors involved in onion pinking and garlic greening. Journal of agricultural and food chemistry. Aug 11;52(16):5089-94.

[19] Reuter, H.D., Koch, H.P. & Lawson, D.L. (1996) Therapeutic effects and applications of Garlic and its preparations in: Garlic: The Science and therapeutic actions of Allium sativum L. and realted species, 2 ndEdn , Koch , H.P. and D.L. Lawson (Eds): Wilkins, Baltamare , MD.; PP135-213.
[20] Abdou, A., Abou-Zaid, A.A., Al-Sherneeny, M.R., &Abou- AlGheat, Z.H. (1972) Anti-microbial activities of Allium sayivum, Allium cepa, Rephanussayivus, Capsicum frutescens, Eruca sativa and Allium kurret on bacteria " Plant foods for Humen Nutrition, 22:29-35. Agents, pp. 485-499. In A. Gennadios (ed.).
[21] Sasaki, J.T., Kita, K., Ishita, H., Uchisasawa, H. &Matuse, H. (1999) Anti-bacterial activity of garlic powder against Escherichia coli 0-157, J. Nutr. Sci.Vitaminol. 45:785-790.
[22] Setiawan, V.W., Yu, G.P., Lu, Q.Y., Yu, S.Z., Mu, L., hang, Z., Kutz, R.C., Cai, L., Hsich, C.C. & Zhang, Z.F. (2005) Allium vegetables and stomach cancer risk in china , Asian Pac. Cancer Prev. 6 : 387-395 .