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COMPARATIVE ANALYSIS OF THE CRUDE EXTRACT AND ACTIVE FRACTION OF *ALLIUM SATIVUM* AGAINST *STREPTOCOCCUS PNEUMONIAE* ISOLATE FROM CHRONIC ILLNESS PATIENTS

T.M.M. John Bastin[1], M. Boominathan[2]*

1. Department of Biotechnology, School of Life Sciences, Vels University (VISTAS), Chennai, Tamil Nadu, India

2. Department of Biotechnology, Marudhu Pandiyar College, Thanjavur-613 403, Tamil Nadu, India.

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For Correspondence:

M. Boominathan

Department of Biotechnology, Marudhu Pandiyar College, Thanjavur-613 403, Tamil Nadu, India

E-mail:

master.maniji@gmail.com

ABSTRACT

The present study deals with antimicrobial studies of different crude extracts and fractions of *Allium sativum*. The antimicrobial studies conformed that the combination of ethanol with methanol fraction against *Streptococcus pneumoniae* (12:8) with maximum inhibition zone diameter at 8 mm. While, soul crude extract and fraction of all solvents not posses significant activity as compare to standard drug. Phytotoconstituents included sterols, tannins and alkaloids, flavonoids, terpenoids, which was preliminarily investigation. The major components of extracts tested were identified by gas chromatography coupled with mass spectrometry (GC/MS) analysis. Present study might be useful to supplement information in regard to its active fraction identification as potent antimicrobial agent.

INTRODUCTION

Pneumonia is a major infectious disease associated with significant morbidity, mortality and utilisation of healthcare resources. *Streptococcus pneumoniae* is the predominant pathogen in community-acquired pneumonia (CAP), accounting for 20–60% of bacterial cases. It causes community-acquired pneumonia, sinusitis, otitis media, and meningitis. Several antibiotic treatment, pneumococci remain a major cause of morbidity and mortality worldwide (1). Emergence of multidrug-resistant *S. pneumoniae* has become a significant problem in the management of CAP as well as high cost and adverse side effects are commonly associated with popular synthetic antibiotics (2, 3). However, plant medicines and supplementations are considered less toxic than the synthetic compounds (4).

Allium sativum is a perennial bulb-forming plant that belongs to the genus *Allium* in the family Liliaceae, along with A. *porrum, A. cepa* and A. *schoenoprasum* (5). *Allium* family maximum species have antibacterial, antifungal, antiseptic, antiviral, antidiabetic and anti-inflammatory properties. Moreover, active phytoconstituents like alkaloid, flavonoids, triterpenoid, and polyphenols were reported by several studies (6). This work reports the comparative antimicrobial effect of various solvent crude extract and fraction of *Allium sativum* against hazardous bacteria *Streptococcus pneumoniae.*

MATERIALS AND METHODS

Plant material

Allium sativum fresh leaves were collected from various area of Tamil Nadu and analysis was carried out at Vels University, Chennai, Tamil Nadu, India.

Extraction

The shade dried plant material was chopped into small pieces and finally pulverized into fine powder. 500g of powdered plant material was soaked and then extracted successively water, ethanol, methanol, acetone, hexane and butanol solvent in separate Soxhlet extractor for 48h. The extract was concentration to dryness in rotary vacuum evaporator and stored -30˚C until further use.

Fractionation

500g of powdered plant material was soaked and then extracts with ethanol in a soxhlet apparatus. After, collected extracts were evaporated to dryness in desiccators. After, some amount of these crude extract was fractionated with water, ethanol, methanol, acetone,

hexane and butanol using column chromatography under reduced pressure over silica gel. These fractions were then stored in a refrigerator until used for the phytochemical and antimicrobial screening.

Micro organisms

The bacterial strain used in this test was *Streptococcus pneumoniae* which were clinical isolates of patients isolated from clinical patients at dental clinics in and around Thanjavur and Chennai, Tamil Nadu, India.

Determination of antimicrobial activity

Culture supernatants with fractions and extracts of the plants were used in the disc-diffusion method separately. *Streptococcus pneumoniae* swabbed on the surface of the sabouraud agar plates and discs (Whatman No.1 filter paper with 9 mm diameter) impregnated with the 50 µl of each plant sample was place on the surface individually. To compare the antibacterial activities, Nystatin (20 µg/disc) used as standard antibiotic and negative control, a blank disc impregnated with solvent followed by drying was used. The plates (triplicates) were incubated 28°C for 72 h. The antimicrobial potency of the test samples was measured by determining the diameter of the zones of inhibition in millimeter.

GC-MS analysis

30 g powdered sample of *Allium sativum* were soaked and dissolved in 75 ml of methanol for 24 h. Then the filtrates were collected by evaporated under liquid nitrogen. The GC-MS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µm df capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised upto 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

Table 1: Antimicrobial activity of individual extract and fraction of *Allium sativum* tested against *Streptococcus pneumoniae* by disk diffusion method.

Plant sample / Solvent	Zone of inhibition (mm)					
	Water	Ethanol	Methanol	Acetone	Hexane	Butanol
***Allium sativum* extract**	1	5	6	3	1	0.5
***Allium sativum* fraction**	2	7	3	2	2	0.5

Table 2: Antimicrobial activity of ethanol and methanol combined fractions of *Allium sativum* tested against *Streptococcus pneumoniae* by disk diffusion method.

Plant sample/ Fraction concentration	Zone of inhibition (mm)								
	18:2 (E/M)	16:4 (E/M)	14:6 (E/M)	12:8 (E/M)	10:10 (E/M)	8:12 (E/M)	6:14 (E/M)	4:16 (E/M)	2:18 (E/M)
Allium sativum	1	5	3	8	2	0.5	2	4	7

Table 3: The main compounds identified by GC-MS in the extracts of *Allium sativum*

S.No.	Peak name	Retention time	Peak Area	%Peak Area
1.	Name: Propanal, 2,3-dihydroxy- Formula: $C_3H_6O_3$ MW: 90	3.32	2269563	0.7754
2.	Name: Butanimidamide Formula: $C_4H_{10}N_2$ MW: 86	3.67	2650035	0.9054
3.	Name: o-Methylisourea hydrogen sulfate Formula: $C_2H_6N_2O$ MW: 74	3.82	646611	0.2209
4.	Name: N'-Isopropylureidoacetic acid Formula: $C_6H_{12}N_2O_3$ MW: 160	4.08	3335755	1.1397
5.	Name: 2-Furanmethanol Formula: $C_5H_6O_2$ MW: 98	5.24	480925	0.1643
6.	Name: Pyrazinol Formula: $C_4H_4N_2O$ MW: 96 CAS	5.78	419570	0.1433
7.	Name: 2(3H)-Furanone, 3-bromodihydro- Formula: $C_4H_5BrO_2$ MW: 164	6.33	1467627	0.5014
8.	Name: 1,3-Dioxepin, 4,7-dihydro- Formula: $C_5H_8O_2$ MW: 100	6.49	344955	0.1179
9.	Name: 2-Cyclopenten-1-one, 2-hydroxy- Formula: $C_5H_6O_2$ MW: 98	6.59	1123614	0.3839
10.	Name: Dimethyl trisulfide Formula: $C_2H_6S_3$ MW: 126	7.45	189412	0.0647
11.	Name: 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one Formula: $C_6H_8O_4$ MW: 144	7.53	725959	0.2480
12.	Name: 2-Hydroxy-gamma-butyrolactone Formula: $C_4H_6O_3$ MW: 102	8.03	2621380	0.8956

13.	Name: Cyclopentanone, 2,4-dimethyl- Formula: $C_7H_{12}O$ MW: 112	8.53	806573	0.2756
14.	Name: Piperazine, 1,4-dimethyl- Formula: $C_6H_{14}N_2$ MW: 114	8.99	2147846	0.7338
15.	Name: 2,5-Dimethyl-4-hydroxy-3(2H)-furanone Formula: $C_6H_8O_3$ MW: 128	9.23	1296404	0.4429
16.	Name: Diallyl disulphide Formula: $C_6H_{10}S_2$ MW: 146	9.54	2371260	0.8102
17.	Name: Cyclopentane, 1-acetyl-1,2-epoxy- Formula: $C_7H_{10}O_2$ MW: 126	9.71	8661705	2.9593
18.	Name: Butanal, 3-methyl- Formula: $C_5H_{10}O$ MW: 86	9.99	7293549	2.4919
19.	Name: Trisulfide, methyl 2-propenyl Formula: $C_4H_8S_3$ MW: 152	10.78	1815177	0.6202
20.	Name: Ethyl N-(2-methoxyethyl)alaninate Formula: $C_8H_{17}NO_3$ MW: 175	10.86	826914	0.2825
21.	Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Formula: $C_6H_8O_4$ MW: 144	10.96	2731779	0.9333
22.	Name: N,N'-Diacetylethylenediamine Formula: $C_6H_{12}N_2O_2$ MW: 144	11.96	1016917	0.3474
23.	Name: Heptanoic acid, 6-oxo- Formula: $C_7H_{12}O_3$ MW: 144	13.59	2168060	0.7407
24.	Name: Trisulfide, di-2-propenyl Formula: $C_6H_{10}S_3$ MW: 178	13.78	3332813	1.1387
25.	Name: 2-Methoxy-4-vinylphenol Formula: $C_9H_{10}O_2$ MW: 150	13.97	805977	0.2754

26.	Name: 1,2-Dithiolane Formula: $C_3H_6S_2$ MW: 106	15.33	620347	0.2119
27.	Name: Sucrose Formula: $C_{12}H_{22}O_{11}$ MW: 342	16.53	46301244	15.8192
28.	Name: 12-Oleanen-3-yl acetate, (3à)- Formula: $C_{32}H_{52}O_2$ MW: 468	29.96	10979310	3.7512
29.	Name: Cholesta-4,6-dien-3-ol, (3á)- Formula: $C_{27}H_{44}O$ MW: 384	31.66	7093176	2.4234
30.	Name: Stigmastan-3,5-diene Formula: $C_{29}H_{48}$ MW: 396	33.11	6458307	2.2065
31.	Name: Urs-12-en-3-ol, acetate, (3á)- Formula: $C_{32}H_{52}O_2$ MW: 468	36.67	169686944	57.9750

RESULTS AND DISCUSSION

Significant anti-bacterial activity was found against *Streptococcus pneumoniae* by the crude extract and fractions of the leaf of *Allium sativum* were described. The water, ethanol, methanol, acetone, hexane and butanol solvents crude extract were showed moderate anti-bacterial activity with the average zone of inhibition of 1, 5, 6, 3, 1, 0.5 mm, respectively. Whereas, 2, 7, 3, 2, 2, 0.5 mm zone of inhibition was observed same solvent fraction (Table 1). Above the study, the peak activity was discovered ethanol and methanol fractions than crude extracts; hence these solvent fractions were once more examined at various combinations against novel strain of *Streptococcus pneumoniae* (Table 2). Significant inhibitory activity of 8 mm was present in the methanol combining ethanol fraction of the leaf of *Allium* sativum, which reveal fraction are good anti-bacterial agents than crude extract and are so effective against *Streptococcus pneumoniae*.

In GC-MS analysis, totally 31 compounds identified from the methanol fraction of the *Allium sativum* are presented in Table 3. The plant samples revealed the synthesis of Propanal, 2,3-dihydroxy-; Butanimidamide; o-Methylisourea hydrogen sulfate; N'-Isopropylureidoacetic acid; 2-Furanmethanol; Pyrazinol; 2(3H)-Furanone, 3-bromodihydro-; 1,3-Dioxepin, 4,7-dihydro-; 2-Cyclopenten-1-one, 2-hydroxy-; Dimethyl trisulfide; 2,4-Dihydroxy-2,5-

dimethyl-3(2H)-furan-3-one; 2-Hydroxy-gamma-butyrolactone; Cyclopentanone, 2,4-dimethyl-; Piperazine, 1,4-dimethyl-; 2,5-Dimethyl-4-hydroxy-3(2H)-furanone; Diallyl disulphide; Cyclopentane, 1-acetyl-1,2-epoxy-; Butanal, 3-methyl-; Trisulfide, methyl 2-propenyl; Ethyl N-(2-methoxyethyl)alaninate; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; N,N'-Diacetylethylenediamine; Heptanoic acid, 6-oxo-; Trisulfide, di-2-propenyl; 2-Methoxy-4-vinylphenol; 1,2-Dithiolane; Sucrose; 12-Oleanen-3-yl acetate, (3à)-. All these compounds are of pharmacological importance as they possess the properties such as analgesic, anti-diabetic, antibacterial, and anti-bacterial. In conclusion, the results of this study have provided scientific justification for the use of *Allium sativum* fraction in health products and herbal remedies and that have immense bacteria toxic effect to bacterial pathogens. Therefore, complementary and alternative medicine practices with plant fraction as a means of decreasing the burden of drug resistance and reducing the cost of management of diseases would be of clinical and public health.

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