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ANTIMICROBIAL STUDIES OF ALLICIN AND AJOENE

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ABSTRACT

Garlic(.*allium sativum)* has been shown to inhibit the growth of variety of microorganisms.The antimicrobial activity of garlic is believed to be due to the effect of allicin and ajoene.The transformation of allicin to ajoene is summarized. The broad spectrum antimicrobial effects of allicin and ajoene are due to the multiple inhibitory effects on various thiol dependent enzymatic systems.. The antimicrobial activity of different concentration of allicin and ajoene were measured by determining the growth of test fungus and bacteria by dry weight increased method and by agar diffusion method against *Aspergillus flavus, Aspergillus niger,Cryptococcus neoformons* and *Alternaria alternate* fungi.*Streproproteus, Staphylococcus* & *E.Coli* bacteria. The results indicate that ajoene has more antimicrobial as compare to allicin. The activity index for different microbes have also been calculated.

KEY WORDS: **allium sativum,A.l.,antimicrobial activity,Richard's liquid medium**

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INTRODUCTION

Literature revealed that allicin,ajoene,thisulfinates and wide range of other organosulfurate compounds which are known to be the constituents linked to the garlic properties.Allicin is a thioester of sulfonic acid and exhibit antimicrobial[1-4],antiviral[5],antioxidant[1],anticancer [6],and has significant anticholesterol activity[7],so, used to prevent heart diseases including artherosclerosis (hardening of arteries), high blood pressure, sugar, digestive disorder, reduce platelet aggregation, hyperlipidermia[8], reduce the incidence of a multitude of chemically induced tumer and help for AIDS patient to treat cryptosporidium and toxoplasmosis,and used as antihypertensive[8].Allicin has a wide spectrum of antibacterial activity against numerous gram(+) and gram(-) bacteria such as *E.coli ,Salmonella enterica, Shigella,Enterococcusfaecalis,Staphylococcus aureous,Streptococcus,Klebsiellaaerogenes,Pseudomonasaeruginosa,Proteusvulgaris,Candida albicans* and *Aspergillus niger* [9-15] Pharmacokinetic studies indicate that allicin will reach a maximum level in the blood after 30-60 minutes and may still be present 72 hours later with more than 85% clearance through urine and faecal path-way[16]. Similarly allicin have significant enhancing effects on the immune system . Allicin can be synthesized by mild oxidation of diallyl disulphide and characterized by UV,FT-IR,MS,NMR. Naturally, it is extracted from garlic in which it is prepared by the interaction of alliin with enzyme allinase. Ajoene is more stable and has stronger agent than allicin and was first isolated and studied in1984[17,18].It is an unsaturated disulfite and form by the interaction of two molecule of allicin.Ajoene has been found as an antioxidant,antithrombotic agent[19],broad spectrum antimicrobial agent[20-21],antileukemia agent[22]for accute myeloid,leukemia therapy,decrease basal cell carcinoma tumor size by inducing apoptosis[23]of several human leukemia and non-leukemiamalignantcellsincludingbreast,bladder ,colorectal,hepatic,prostatic cancer,lymphoma and skin,antiparasitic and antimutagenic.ajoene inhibit yeast growth at concentration below 20 micro gm/ml,cholesterol and isoprenoid synthesis through inhibition of HMG-CoA reductase and the mevalonate pathway The present communication deals to calculate the antimicrobial activity of the test compound at different concentration against different bacteria and fungi by using standard method and compared. The MIC and activity index for different microbes have also been calculated.

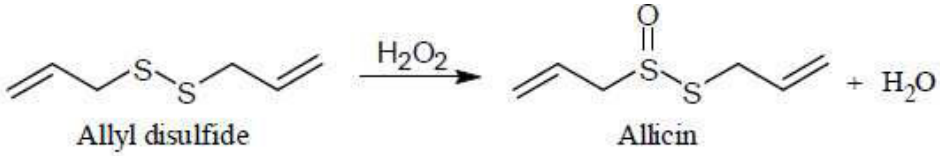
EXPERIMENT

Chemical composition of allium sativum

Naturally allicin extracted from clove of allium sativum, which also contain alliin, polysaccharides, protein, saponine, allinase enzyme, vitamin A,B,B3,B5,B6,C,E, minerals such as Se,Ca,Fe,Mg,Mn,K,Na,Zn, flavonoids, scardinine and antioxidant.

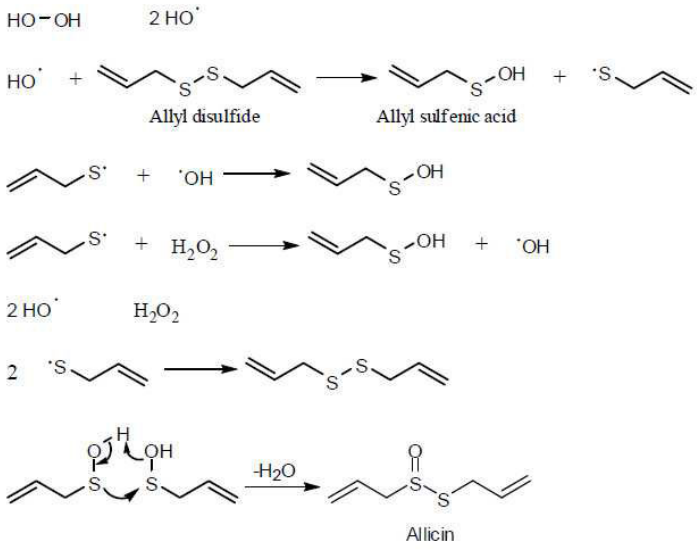
Isolation of Allicin

Allicin was extracted from garlic bulbs and synthesized by the oxidation of diallyl disulfide with H2O2 in acidic medium at low temperature(0-25°C) due to the sufficient instability of allicin, and transformation was proposed by free radical mechanism



Allyl disulfide Allicin

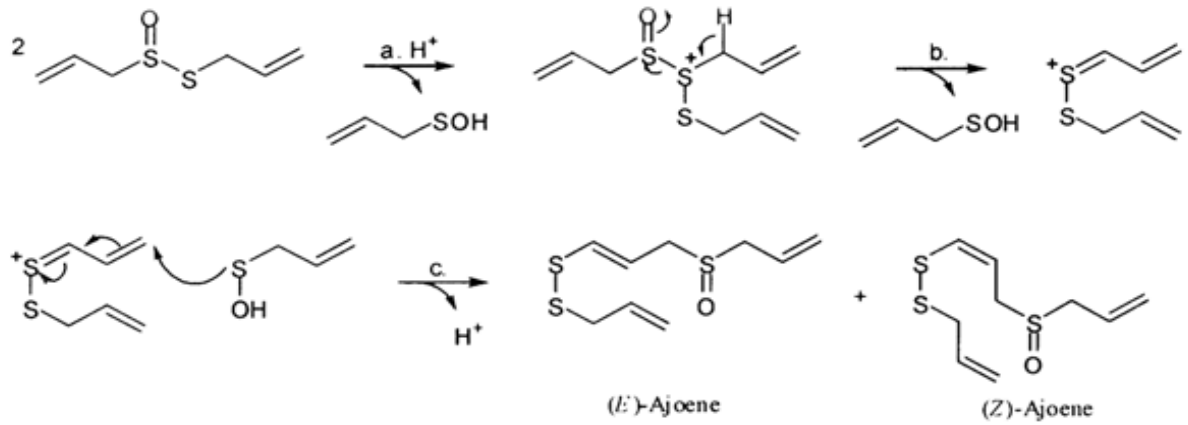
Mechanism



It is an yellow oily liquid,density1.112gcm-3,mp<25°C.Allinase is irreversibly deactivated below a pH of 3, so allicin is generally not produced in the body from the consumption of allium sativum

Isolation of ajoene

Ajoene was prepared by heating acidic solution of allicin or diallyl disulfide below pKa 7,for a sufficient period of time.The structure of ajoenes were determined by U.V, FTIR, NMR,and MS technique. The complete transformation of allicin to ajoene is shown in the scheme



Sheme

The transformation of allicin to (E)-and(Z)-ajoene in low polar solvents

BIOLOGICAL STUDIES

a. Antibacterial screening

The antibacterial activity of the test compound were measured by paper disc diffusion method, using agar nutrient medium and 5 mm diameter paper discs of what man No.1 filter paper discs were soaked in a solution of known amount(0.4 to0.6% w/v) of test compound dried and laid on the surface of petri-plates which were already seeded with the test organism- *Echerichia coli, staphylococcus, streproproteus.* All the agar dishes were then incubated in an incubator at 27± 1°C for about 48 hours. After the incubation period, the growth of the microorganism was studied as inhibition zone(

mm), around each disc in the form of turbid layer, except in the region where the concentration of antibacterial agent is above the MIC and the zone of inhibition is seen. The size of the zone of inhibition depends upon sensitivity of the organism, nature of the culture medium, incubation condition, rate of diffusion of the agent and the concentration of the antibacterial agent on the filter paper.

b. Antifungal screening

The antifungal activity of different concentrations (0 .05 to .40% w/v) of test compound was measured by determining the growth of test fungi *aspergillus flavus, aspergillus niger* and *cryptococcus neoformons* by dry weight increase method and Richard liquid medium used as culture medium [25]. The test compounds of varying concentration (0.05 to 0.40% w/v) were directly added in a Richard liquid medium having interested fungus in a sterilized chamber and was kept for seven days in an incubation chamber at 27 ± 1°C. Media with test solution served as treated while without them as check. The resultant mycelial mats in each set were carefully removed, washed, dried and then weighed separately. The percentage of inhibition was calculated by the following formula

$$\text{Percentage inhibition of fungal growth} = \frac{(Cg - Tg) \times 100}{Cg}$$

Where, Cg = Average growth in the check set
Tg = Average growth in the treated set

RESULT AND DISCUSSION

Antimicrobial Activities

The fungicidal and bactericidal data of the graded concentrations (0.05 to 0.40 %) and (0.40 to 0.60%) of ajoene and allicin against *aspergillus flavus, aspergillus niger* and *cryptococcus neoformons, fungi* and *E. coli, staphylococcus* and *streproproteu*s bacteria were recorded in the table [1,2] Fig. [1-7] The observed results reveal that the antimicrobial activity of the compound is directly proportional to the concentration of the test compound and differ from fungus to fungus and bacteria to bacteria. The ajoene is more antimicrobial than allicin itself, as the presence of electronegative group in the side chain enhances the toxicity. The antibacterial effect of allicin is of a broad spectrum. In most cases the 50% lethal dose concentration were same what higher than those required for some of the newer antibiotics. It has been noted that various bacterial strains resistant to antibiotics such as methicillin resistant staphylococcus aureus [causes eczema and acne] as well as multidrug resistant enterotoxicogenic strains of *Echerichia Coli, Enterococcus* etc have been found to be allicin sensitive.

Mechanism

Antimicrobial agents interfere chemically with the synthesis of function of vital components of microorganism in the different ways. Inhibitors of cell wall synthesis, inhibitors ofcellmembrane,inhibitorsofbiosynthesis(i.e.productionof purines,pyrimidine,A.A.,Vitamins,protein, DNA,RNA), inhibitors of energy production (inhibit the respiration or by uncoupling of oxidative phosphorylation). The biological activity of allicin and ajoene are to be related to a combination of the following factors.

1. Its activity as an antioxidant.
2. Its ability to attack the sulpher [SH] group in enzymes and proteins and modify their activities.
3. Its ability to rapidly penetrate into cells through the cell membrane.

Antimicrobial activity of allicin and ajoene are mainly due to S-S and S-O bond which has the ability to react with thiol containing enzyme [L-cysteine] to form the S-thiolation product S-allyl mercaptocysteine which is characterized by NMR and Mass spectroscopy. It has been noted that in amoeba parasite, allicin was found to strongly inhibit the cysteine proteinases,alcohol hydrogenases[26] Inhibition of these enzymes was observed at

rather low concentrations [< 10 µg/ml]. Allicin also irreversibly inhibited the well known thio-protease papain. Allicin also inhibits other bacterial enzymes such as the acetyl-co-A forming systein consisting of acetate kinase and phosphotransacetyl-co-A synthetase[27]. Allicin was found to partially inhibit the DNA and protein synthesis but the effect on RNA was immediate, suggesting that this could be a primary target of allicin action[28].The mechanism of antifungal effect in lower eukaryotes is considered to be inhibition of phosphotidyl chloride biosynthesis[29]. It concludes that the broad spectrum antimicrobial effects of allicin and ajoene are due to the multiple inhibitory effects on various thiol dependent enzymatic systems. It could be noted that allicin effect is not same for all targets. Thiol protease could be inhibited at the lowest concentrations.

CONCLUSION

It may be concluded from the study that ajoene has more antimicrobial activity as compaired to allicin against Aspergillus flavus,Aspergillus niger,Cryptococcus neoformons and Alternaria alternate fungi, Streproproteus,Staphylococcus and E.Coli bacteria and show significant antimicrobial activity. It is essential That research should continue to isolate and modify the allicin and ajoene by chemical process to form more Potent against different studies.

Table-1
Antifungal Activity Data of Allicin and Ajoene against different fungus.

		Aspergillus flavus				*Aspergillus niger*				*Cryptococcus neoformons*				*Alternaria alternata*			
Conc	**%of Inhibition**	**Control**	**Paeonol**	**Allicin**	**Ajoene**	**Control**	**Paeonol**	**Allicin**	**Ajoene**	**Control**	**Paeonol**	**Allicin**	**Ajoene**	**Control**	**Paeonol**	**Allicin**	**Ajoene**
0.05%	Wt	1.089	1.798	1.079	1.0748	1.046	1.037	1.0358	1.0318	1.068	1.058	1.055	1.0528	1.099	1.089	1.0864	1.0814
	%		.84	0.9156	1.30		.86	0.9754	1.366		0.89	1.0234	1.432		0.92	1.145	1.60
	Al			1.09	1.547			1.134	1.588			1.15	1.61			1.244	1.740
0.10%	Wt	1.082	1.066	1.064	1.058	1.041	1.023	1.0212	1.0134	1.062	1.042	1.039	1.030	1.090	1.066	1.062	1.0502
	%		1.46	1.58	2.22		1.70	1.897	2.65		1.92	2.15	3.01		2.15	2.61	3.65
	Al			1.082	1.52			1.1160	1.558			1.120	1.567			1.214	1.697
0.20%	Wt	1.072	1.083	1.034	1.0198	1.030	0.994	0.9910	0.9754	1.053	1.0143	1.0108	.996	1.084	1.0430	1.0366	1.0176
	%		3.26	3.48	4.87		3.50	3.787	5.30		3.68	4.01	5.62		3.780	4.370	6.12
	Al			1.067	1.494			1.082	1.514			1.09	1.527			1.156	1.620
0.30%	Wt	1.060	1.004	1.001	.978	1.008	0.961	0.9462	.9232	1.038	0.98	.974	.948	1.076	1.0116	1.0052	.9768
	%		5.30	5.554	7.76		5.75	6.014	8.42		5.87	6.193	8.67		5.98	6.578	9.21
	Al			1.048	1.464			1.0459	1.464			1.055	1.477			1.10	1.540
0.40%	Wt	1.047	0.964	.961	.927	1.005	0.923	0.9214	.888	1.018	0.932	.930	.8958	1.068	0.975	0.9712	.9324
	%		7.92	8.182	11.45		8.20	8.315	11.64		8.40	8.57	12.0		8.65	9.065	12.70
	Al			1.033	1.445			1.014	1.420			1.02	1.428			1.048	1.468

Standard = Paeonol

Table-2
Antibacterial Activity Data of Allicin and Ajoene against different Bacteria

		Streproproteus				*Staphylococcus*				*E.Coli.*			
Conc.	**Zone of inhibition**	**Control**	**Paeonol**	**Allicin**	**Ajoene**	**Control**	**Paeonol**	**Allicin**	**Ajoene**	**Control**	**Paeonol**	**Allicin**	**Ajoene**
	ZI		7.0	7.6	8.1		7.3	7.7	8.16		5.2	5.6	6.2
0.40%	AI			1.085	1.157			1.055	1.118			1.077	1.19
	ZI		8.9	9.6	10.12		9.4	9.8	10.24		7.3	7.8	8.4
0.45%	AI			1.07	1.137			1.04	1.089			1.088	1.151
	ZI		11.5	12.2	12.80		11.9	12.3	12.72		9.8	10.4	10.96
0.50%	AI			1.06	1.113			1.03	1.069			1.081	1.120
	ZI		13.8	14.6	15.16		14.7	15.0	15.46		14.0	14.8	15.41
0.55%	AI			1.05	1.098			1.02	1.051			1.57	1.10
	ZI		17.5	18.3	18.85		17.9	18.2	18.68		18.2	17.0	17.65
0.60%	AI			1.04	1.077			1.016	1.043			1.049	1.089

Standard = Paeonol

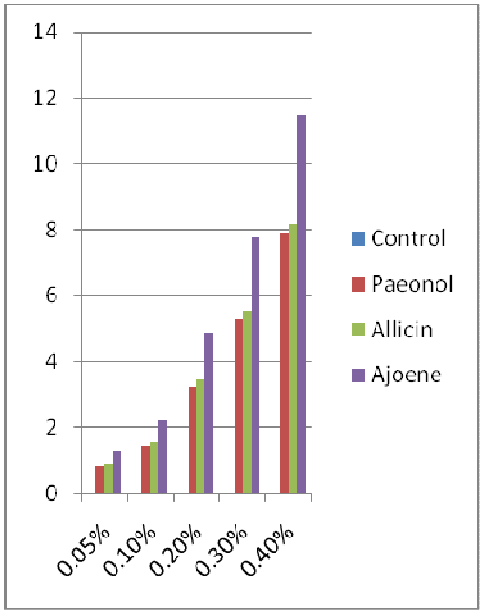


Figure 1
Antifungal Activity of Allicin and Ajoene against Aspergillus flavus.

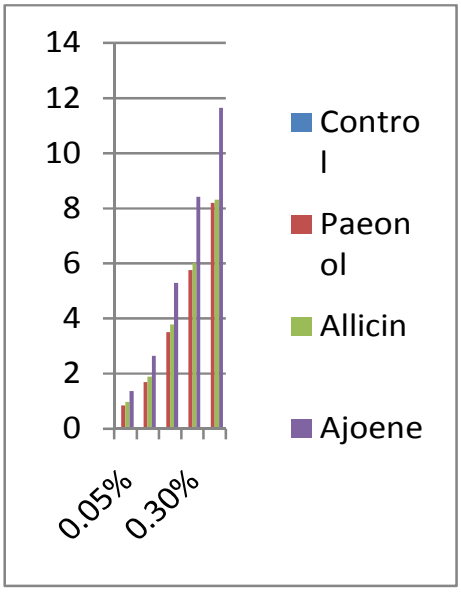


Figure 2
Antifungal Activity of Allicin and Ajoene against against Aspergillus niger.

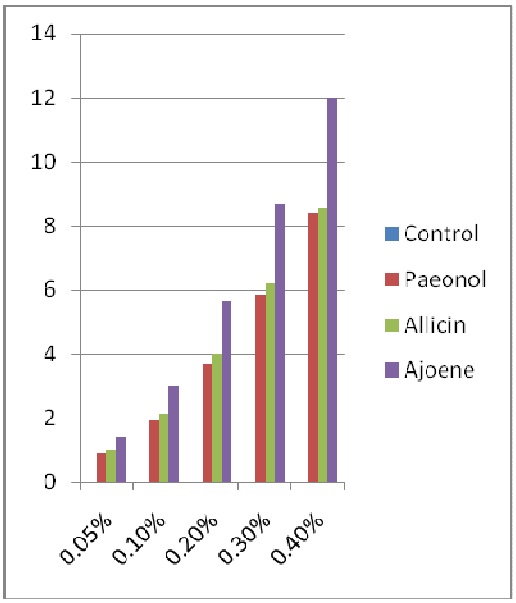


Figure 3
Antifungal Activity of Allicin and Ajoene against Cryptococcus neoformons.

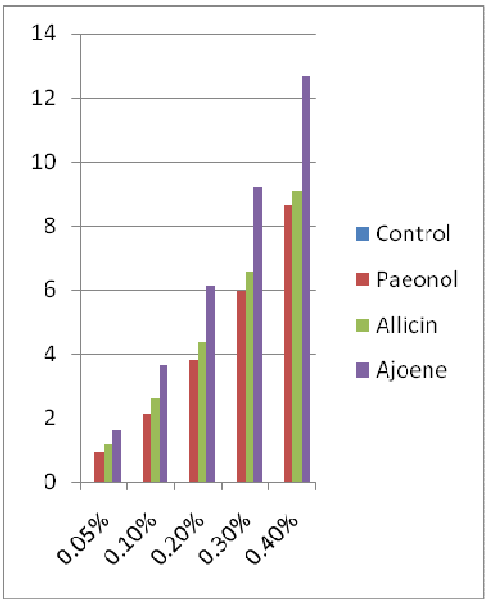


Figure 4
Antifungal Activity of Allicin and Ajoene against Alternaria Alternata.

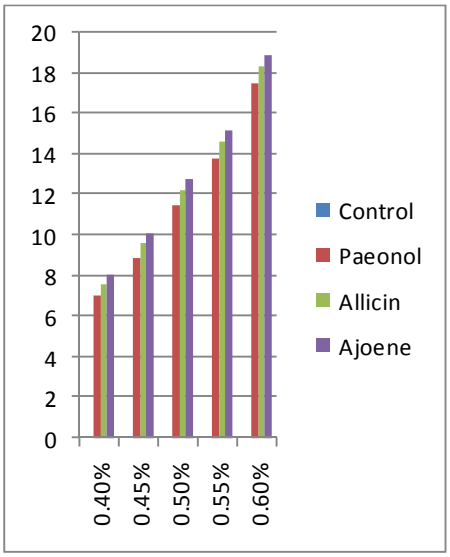


Figure 5
Antibacterial Activity of Allicin and Ajoene against Streproproteus.

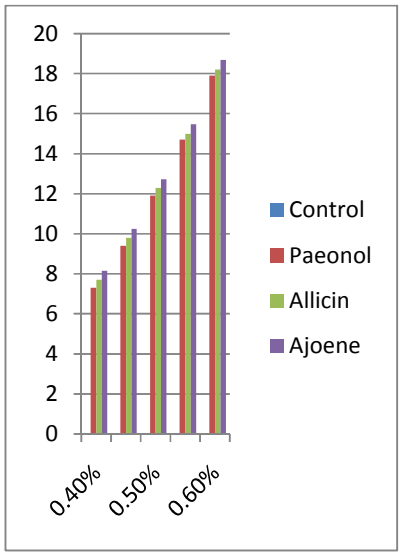


Figure 6
Antibacterial Activity of Allicin and Ajoene against Staphylococcus.

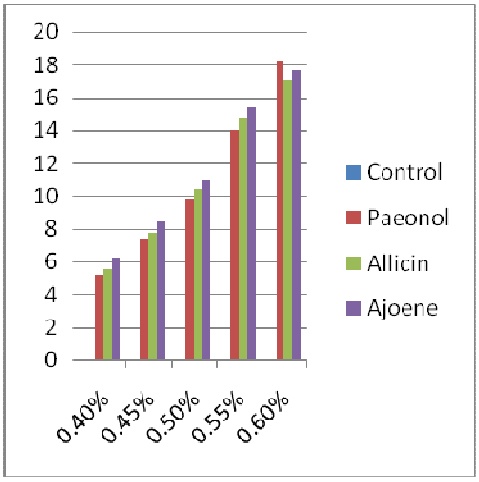


Figure 7
Antibacterial Activity of Allicin and Ajoene against E.Coli.

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