Research Article **Biotechnology**

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

COMPARATIVE BIOLOGICAL STUDIES OF ALLICIN WITH PAEONOL.

F.REHMAN* AND SAMYA MAIRAJ

Deptt. of Analytical Chemistry Faiz-E-Aam Degree College,Meerut

ABSTRACT

It has been reported that Allium Sativum contains a large number of essential metal, carbohydrate, protein,vitamins and volatile oil and allicin is one of the active ingredient of freshly crushed garlic homogenates and has a variety of antimicrobial activities and used to prevent the heart diseases that include artherosclerosis, high cholesterol and high blood pressure. Antimicrobial activity of allicin is mainly due to S-S and S-O bond which has the ability to react with thiol containing enzyme to form S-thiolation product, the broad spectrum antimicrobial effects of allicin is due to the multiple inhibitory effects on various thiol dependent enzymatic systems. Anticholesterol activity was tested by standard method and results shows that cholesterol level (LDL) decrease significantly, when allicin is used for 8-12 week. The antimicrobial activity of different concentration of ligand and paeonol 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 neformons & Allternaria alternate fungi Streproproteus, staphylococcus & E.Coli bacteria. The results indicate that allicin has more antimicrobial properties as compare to paeonol. The activity index for different microbes have also been calculated.

Key words; **allium sativum,paeonol,A.I.,antimicrobial activity,Richard liquid medium**

F.REHMAN
Deptt.of Analytical Chemistry Faiz-E-Aam Degree College,Meerut

***Corresponding author**

INTRODUCTION

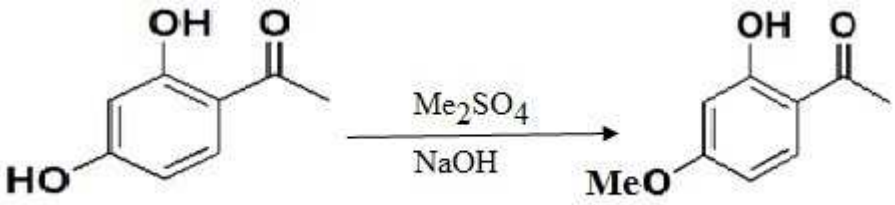
Literature revealed that the different type of phenones and their oxime are widely used as antiseptic, germicides, anthelmintics, analgesic,[1]antituberculosis[2], herbicides,[3]therapeutic against mycobacteria,[4] antibacterial[5-7] antifungal and antiviral[8] agent. Similarly allicin has wide application in biological field, mainly used as antimicrobial[9-11] , antioxidant [12] and anticarcinogenic agent[13]. Allicin shows significant anticholesterol activity, so, used to prevent heart diseases including artherosclerosis (hardening of arteries), high blood pressure, sugar, digestive disorder, reduce platelet aggregation, hyperlipidermia, reduce the incidence of a multitude of chemically induced tumer and help for AIDS patient to treat cryptosporidium and toxoplasmosis. 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[14]. 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 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 with paeonol.

The MIC and activity index for different microbes have also been calculated.

EXPERIMENT

Preparation of paeonol

2,4-dihydroxy acetophenone (1.0 mol) was dissolved in cold dil. sodium hydroxide solution in a three nacked flask fitted a thermometer and a dropping funnel. The solution was stirred with a mechanical stirrer and dimethyl sulphate (1.0 mol) was added drop wise. When the addition was completed. The mixture was stirred for a few minutes, heated to about 90°C and again stirred for about half an hour. A little more of alkali was now added and the process repeated. Finally, the solution was acidified with HCl and extracted with benzene. The solvent was then distilled off and the resulting oily product was distilled under reduce pressure, where upon the product separated as large transparent crystals, mp 50°C and characterized by elemental analysis, UV,FT-IR,NMR techniques.

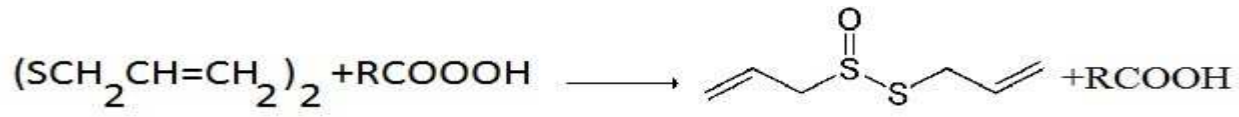


Chemical composition of allium sativum

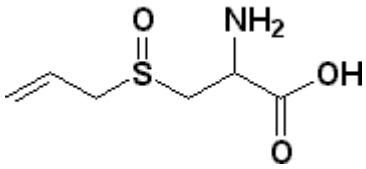
Naturally allicin extracted from clove of allium sativum, which also contain alliin, polysaccharides, protein, saponine, allinase enzyme, vitamin A,B,B_3,B_5,B_6,C,E, minerals such as Se,Ca,Fe,Mg,Mn,K,Na,Zn, flavonoids, scardinine and antioxidant.

Isolation of Allicin

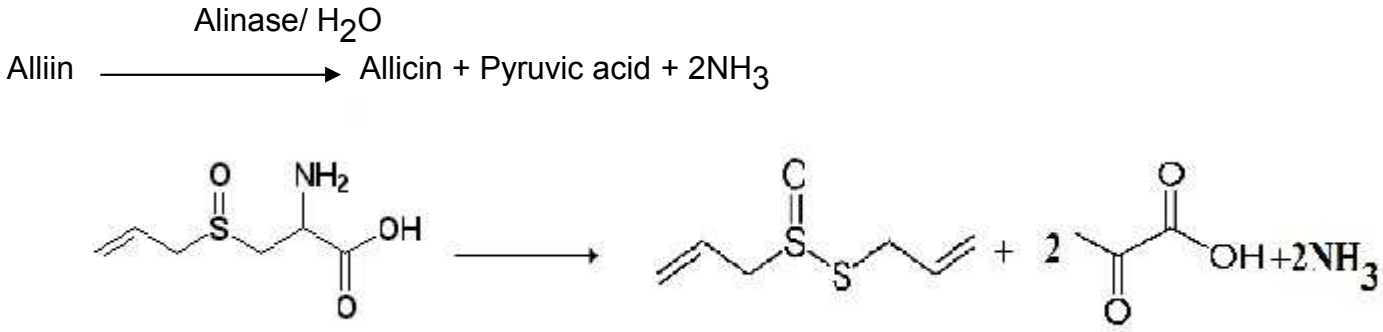
Allicin liquid made from fresh raw garlic. Clove of garlic are left unpealed and then subjected to filtration and a temperature controlled extraction process designed to produce pure liquid allicin dissolve in water. It could be synthesized by mild oxidation of diallyl disulphide [15] and characterized by UV,FT/IR,MS,NMR



Allicin (Diallyl thiosulphinate)does not occur in allium sativum naturally, instead it is found in the form of the amino acid- alliin(S-allyl cystein sulphoxide).



When it crushed, the alliin react with enzyme allinase located in bundle sheeth of garlic clove and converted into allicin [16].



Which degrates to diallyl disulphide and diallyl trisulphide.

It is colourless 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.

Biological studies

a. Antibacterial screening

The antibacterial activity of the test compound were measured by paper disc diffusion method[16], using agar nutrient medium and 5 mm diameter paper discs of whatman 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 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 [17]. 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

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

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

$$\text{The activity index was calculated as , AI} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}$$

Cholesterol screening – Cholesterol was tested by recommended method by taking blood sample from the vein of 14 hours fasted person.

RESULT AND DISCUSSION

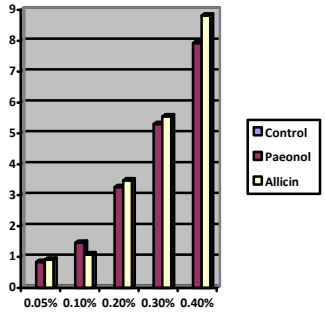
Antimicrobial Activities

The fungicidal and bactericidal data of the graded concentrations (0.05 to 0.40 %) and (0.40 to 0.60%) of paeonol and allicin against aspergillus flavus, aspergillus niger and cryptococcus neoformons, fungi and E. coli, staphylococcus and streproproteus bacteria were recorded in the table [1,2] Fig. [1-7]

Table-1

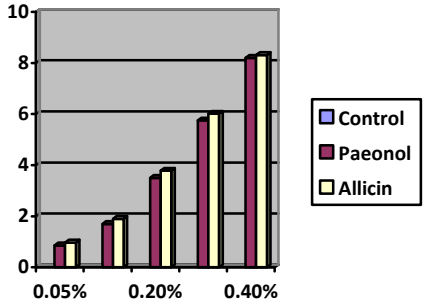
Antifungal Activity Data of Paeonol and Allicin against different fungus.

		Aspergillus flavus			Aspergillus niger			Cryptococcus neoformons			Alternaria alternata		
Con.	% of inhibition	Control	Paeonol	Allicin	Control	Paeonol	Allicin	Control	Paeonol	Allicin	Control	Paenol	Allicin
0.05%	Wt	1.089	1.798	1.0790	1.046	1.037	1.0358	1.068	1.058	1.055	1.099	1.089	1.0864
	%		0.84	0.9156		0.86	0.9754		0.89	1.0234		0.92	1.145
	Al			1.090			1.134			1.15			1.244
0.10%	Wt	1.082	1.066	1.064	1.041	1.023	1.0212	1.062	1.042	1.039	1.090	1.066	1.062
	%		1.46	1.58		1.70	1.897		1.92	2.15		2.15	2.61
	Al			1.082			1.1159			1.120			1.214
0.20%	Wt	1.072	1.083	1.034	1.030	0.994	0.9910	1.053	1.0143	1.0108	1.084	1.0430	1.0366
	%		3.26	3.48		3.50	3.787		3.68	4.01		3.780	4.370
	Al			1.067			1.082			1.09			1.156
0.30%	Wt	1.060	1.004	1.001	1.008	0.961	0.9462	1.038	0.98	0.974	1.076	1.0116	1.0052
	%		5.30	5.554		5.75	6.014		5.87	6.193		5.98	6.578
	Al			1.048			1.0459			1.055			1.10
0.40%	Wt	1.047	0.964	0.961	1.005	0.923	0.9214	1.018	0.932	0.930	1.068	0.975	0.9712
	%		7.92	8.182		8.20	8.315		8.40	8.57		8.65	9.065
	Al			1.033			1.014			1.02			1.048



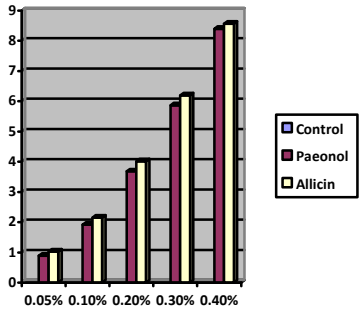
Antifungal Activity of Paeonol and Allicin Allicin against Aspergillus flavus

Figure. 1



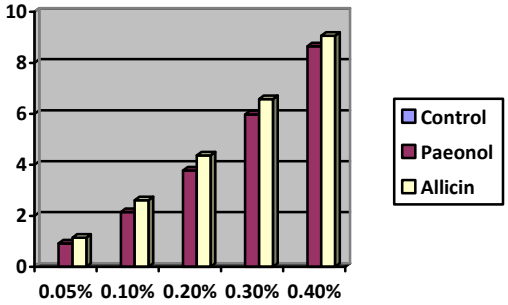
Antifungal Activity of Paeonol and against Aspergillus niger

Figure.2



Antifungal Activity of Paeonol and Allicin against Cryptococcus neoformons. alternate.

Figure.3



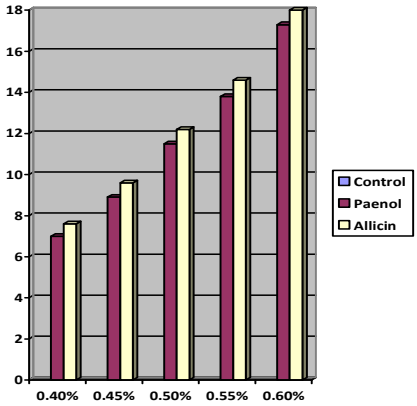
Antifungal Activity of P aeonol and Allicin against Alternaria

Figure.4

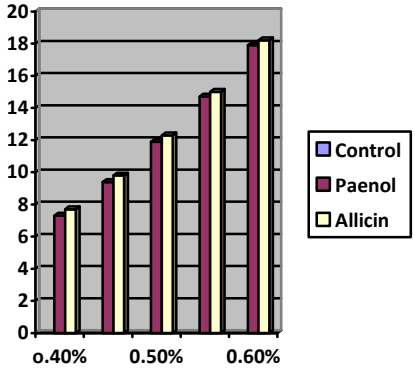
Table-2

Antibacterial Activity Data of Paeonol and Allicin against different Bacteria.

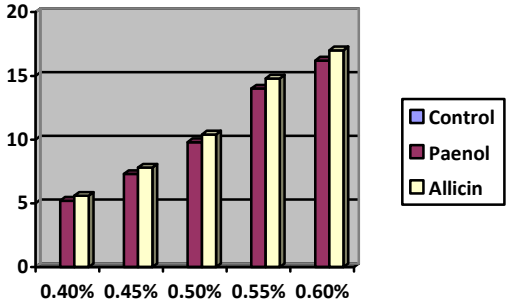
Conc.	Zone of inhibition	Streproproteus			Staphylococcus			E.Coli		
		Control	Paeonol	Allicin	Control	Paeonol	Allicin	Control	Paeonol	Allicin
0.40%	Zone of inhibition	-	7.0	7.6	-	7.3	7.7	-	5.2	5.6
	A.I			1.085			1.055			1.077
0.45%	Zone of inhibition		8.9	9.6		9.4	9.8		7.3	7.8
	A.I			1.07			1.04			1.068
0.50%	Zone of inhibition		11.5	12.2		11.9	12.3		9.8	10.4
	A.I			1.06			1.03			1.061
0.55%	Zone of inhibition		13.8	14.6		14.7	15.0		14.0	14.8
	A.I			1.05			1.02			1.057
0.60%	Zone of inhibition		17.3	18.3		17.9	18.2		16.2	17.0
	A.I			1.04			1.016			1.049



Antibacterial Activity of Paeonol and Allicin against Streproproteus.
Figure.5



Antibacterial Activity of Paeonol and Allicin against Staphylococcus.
Figure.6



Antibacterial Activity of Paeonol and Allicin against E.Coli.
Figure.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 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 strain resistant to antibiotics such as methicillin resistant staphylococcus aureus [causes eczema and acne] as well as multidrug resistant enterotoxico genic strains of Echerichia Coli, Enterococcus etc have been found to be allicin sensitive.

The biological activity of allicin is to be related to combination of 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.

Mechanism of action of allicin

Antimicrobial agent act to control disease in one of the following three ways –

1. They may kill or inactivate the pathogen in the host.
2. They may increase the host resistance.
3. They may inhibit the production of microbial products which are responsible for malignity and block the development of symptoms in the invaded organism.

Antimicrobial agent exert their action on pathogen by the following three different ways.

1 Inhibition of energy production or ATP production.
2 Interference with bio-synthesis
3. Disruption of cell structure.

Antimicrobial activity of allicin is 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- allylmercaptocysteine 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.[18] 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[19]. 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[20].

It conclude that the broad spectrum antimicrobial effects of allicin is due to the multiple inhibitory effects on various thiol dependent enzymatic systems. It could be noted that allicin effect is not same for all target. Thiol protease could be inhibited at the lowest concentrations.

Inhibition of these enzyme could not be lethal only at lower concentrations but block the microbes virulence sufficiently. Enzymes like dehydrogenases or thioredoxin reductases could be effected at slighty higher concentrations.

Anticholesterol activity

Artherosclerosis causes hardening of arteries developing heart disease, The body requires only small amounts of cholesterol function normally. According to American Heart Association, for adult, cholesterol below 200mg/dl[5.18mmol/L] is desirable and causes low risk of heart diseases. Cholesterol of 200 to 239 mg/dl [5.18 to 6.18 mmol/L] is consider borderline high and causes moderate risk and above 239 is considered high risk.

Table-3.

Cholesterol is tested by recommended method and the effect of the allicin on cholesterol shown in

Investigation	Before using garlic	After 2 months	Unit	Normal
Serum cholesterol	311	245.6	mg/dl	130-200
Serum Triglycerides	201	193.7	mg/dl	40-150
HDL cholesterol	60.7	58.4	mg/dl	30-130
LDL cholesterol	224	148.46	mg/dl	<100

Good cholesterol or HDL [high density lipoprotein] flow through blood stream much more easily and are not responsible for dangerous hardening of arteries and help to remove LDL and other substances that are blocking the arteries. While bad cholesterol or LDL [low density lipoprotein] do not flow through the blood stream very well and tend to get left behind and can clog up the arteries, which contribute to artherosclerosis. If blood clot

occurrs in the blocked region resultantly heart attack or stroke take place.

On the experimental basis it conclude that total cholesterol decrease 19 mg/dl, LDL cholesterol 6.7 mg/dl and triglyceride level 21.1 mg/dl, when allicin is used 8-12 week and it has been reported that allicin show a significant anticholesterol activity.

It has been reported that allicin show a significant anticholesterol activity, A 12 week study compairing the effect of standarized garlic powder tablet [900 mg daily] with that of bezafibrate [commonly used for blood lipid lowering drugs 600 mg/day]. The multi centre double blical study was performed with 94 patients having cholesterol or triglyceride value exceeding 250 mg/dl, after four week of treatment the decreases in cholesterol LDL cholesterol and triglyceride level were all statistically highly significant and there were no differences between the effect of allicin and benzafibrate, HDL cholesterol values in the course of four weeks also increased significantly.

CONCLUSION

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

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