

**ANTIMICROBIAL STUDIES OF ALLICIN WITH PAEONOL AND ITS OXIME****F.REHMAN* AND SAMYA MAIRAJ***Department 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 alliin 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 arteriosclerosis, high cholesterol and high blood pressure. Antimicrobial activity of alliin 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 alliin 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 alliin is used for 8-16 week. The antimicrobial activity of different concentration of alliin, paeonol and its oxime 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 *Streptoproteus*, *staphylococcus* & *E.Coli* bacteria. The results indicate that oxime of paeonol has more antimicrobial properties as compare to paeonol and alliin. The activity index for different microbes has also been calculated.

KEY WORDS: *Allium sativum*, paeonol, A.I., antimicrobial activity, Richard's liquid medium**F.REHMAN**

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INTRODUCTION

Literature revealed that the different type of phenones and their oxime are widely used as antiseptic, germicides, anthelmintics, analgesic[1], antituberculosis, herbicides[2], therapeutic against mycobacteria[3] antibacterial, antifungal[4-6] and antiviral agent. Paeonol which is naturally found in paeonia suffruticosa [7], arisaema erubescens [8] and dioscorea japonica [9] plant is widely used to reduce cerebral interaction involving the superoxide anion and microglia activation in isochemia-reperfusion injured rat [10]. It shows antimutagenic activity[9]. It also has anti-inflammatory and analgesic effect in carrageenan-evoked thermal hyperalgesia[11]. It inhibits anaphylactic reaction by regulating histamine and TNF- α [12]. It improves blood circulation through its inhibitory effects on both platelet aggregation and blood coagulation [13]. It reduced histopathological scores and attenuated myeloperoxidase-reactive cells as an index of polymorphonuclear neutrophils accumulation[14]. Similarly alliin has wide application in biological field, mainly used as antimicrobial[15-16], antioxidant [17] and anticarcinogenic agent. Alliin shows significant anticholesterol activity, so, used to

prevent heart diseases including atherosclerosis (hardening of arteries), high blood pressure, sugar, digestive disorder, reduce platelet aggregation, hyperlipidemia, reduce the incidence of a multitude of chemically induced tumor and help for AIDS patient to treat cryptosporidium and toxoplasmosis. Pharmacokinetic studies indicate that alliin 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 [18]. Similarly alliin have significant enhancing effects on the immune system. Alliin can be synthesized by mild oxidation of diallyl disulphide and characterized by UV, FT-IR, MS, and NMR. Naturally, it is extracted from garlic in which it is prepared by the interaction of alliin with enzyme alliinase. 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 and its oxime. The MIC and activity index for different microbes have also been calculated.

the product separated as large transparent crystals, mp 50°C and characterized by elemental analysis, UV, FT-IR, NMR techniques.

Preparation of Paeonol oxime

A mixture of paeonol (0.1 mol), hydroxyl amine, hydrochloride (0.2 mol) and anhydrous sodium acetate (0.3 mol) was refluxed in ethanol for about an hour. The product separated on concentration and subsequent dilution. It was filtered, washed with water, dried and recrystallised from alcohol into pure paeonol oxime, m.p. 101°C and characterized by elemental analysis, UV, FT-IR, NMR techniques.

Chemical composition of allium sativum

Naturally alliin extracted from clove of allium sativum, which also contain alliin,

EXPERIMENT

Preparation of paeonol

2, 4-dihydroxy acetophenone (1.0 mol) was dissolved in cold dil. sodium hydroxide solution in a three necked 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 and 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

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

As reported earlier [18].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, 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, streptoproteus. 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 neoformans by dry weight increase method and Richard liquid medium used as culture medium [19]. 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

$$\text{Where, } C_g = \text{Average growth in the check set} \\ T_g = \text{Average growth in the treated set}$$

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,it's oxime and allicin against aspergillus flavus, aspergillus niger and cryptococcus neoformans, fungi and E. coli, staphylococcus and streptoproteus bacteria were recorded in the table [1,2] Fig. [1-7]

Table1
Antifungal Activity Data of Paeonol, Its Oxime and Allicin against different fungus.

Con.	% of Control	Aspergillus flavus			Aspergillus niger			Cryptococcus			Alternaria alternata						
		Paeonol	Oxime	Allicin	Control	Paeonol	Oxime	Allicin	Control	Paeonol	Oxime	Allicin	Control	Paeonol	Oxime	Allicin	
inh.																	
0.05%	Wt	1.089	1.798	1.0722	1.0790	1.046	1.037	1.0204	1.0358	1.068	1.058	1.0498	1.055	1.099	1.089	1.0778	1.0864
	%		0.84	1.5426	0.9156		0.86	1.60	0.9754		0.89	1.70	1.0234		0.92	1.930	1.145
	AI			1.836	1.090			1.86	1.134			1.91	1.15			2.098	1.244
0.10%	Wt	1.082	1.066	1.0532	1.064	1.041	1.023	1.0084	1.0212	1.062	1.042	1.0236	1.039	1.090	1.066	1.0416	1.062
	%		1.46	2.662	1.58		1.70	3.131	1.897		1.92	3.61	2.15		2.15	4.440	2.61
	AI			1.823	1.082			1.84	1.1159			1.88	1.120			2.065	1.214
0.20%	Wt	1.072	1.083	1.0098	1.034	1.030	0.994	0.9652	0.9910	1.053	1.0143	0.9817	1.0108	1.084	1.0430	1.0008	1.0366
	%		3.26	5.810	3.48		3.50	6.291	3.787		3.68	6.771	4.01		3.780	7.6658	4.370
	AI			1.782	1.067			1.797	1.082			1.840	1.09			2.028	1.156
0.30%	Wt	1.060	1.004	0.9640	1.001	1.008	0.961	0.9064	0.9462	1.038	0.98	0.9282	0.974	1.076	1.0116	0.9482	1.0052
	%		5.30	9.232	5.554		5.75	10.079	6.014		5.87	10.578	6.193		5.98	11.876	6.578
	AI			1.742	1.048			1.752	1.0459			1.802	1.055			1.986	1.10
0.40%	Wt	1.047	0.964	0.9076	0.961	1.005	0.923	0.991	0.9214	1.018	0.932	0.868	0.930	1.068	0.975	0.8806	0.9712
	%		7.92	13.314	8.182		8.20	13.930	8.315		8.40	14.73	8.57		8.65	16.798	9.065
	AI			1.681	1.033			1.699	1.014			1.753	1.02			1.942	1.048

Table 2
Antibacterial Activity Data of Paeonol, Its Oxime and Allicin against different Bacteria.

Conc.	Zone of inhibition	Streptoproteus			Staphylococcus				E.Coli				
		Control	Paeonol	Oxime	Allicin	Control	Paeonol	Oxime	Allicin	Control	Paeonol	Oxime	
Allicin													
0.40%	Zone of inh.	-	7.0	8.3	7.6	-	7.3	9.12	7.7	-	5.2	6.76	5.6
	A.I			1.86	1.085			1.25	1.055			1.30	1.077
0.45%	Zone of inh.		8.9	10.4	9.6		9.4	11.6	9.8		7.3	9.2	7.8
	A.I			1.168	1.07			1.234	1.04			1.26	1.068
0.50%	Zone of inh.		11.5	13.18	12.2		11.9	14.28	12.3		9.8	11.8	10.4
	A.I			1.146	1.06			1.20	1.03			1.20	1.061
0.55%	Zone of inh.		13.8	15.6	14.6		14.7	17.6	15.0		14.0	16.38	14.8
	A.I			1.130	1.05			1.197	1.02			1.17	1.057
0.60%	Zone of inh.		17.3	19.4	18.3		17.9	20.8	18.2		16.2	18.6	17.0
	A.I			1.121	1.04			1.162	1.016			1.14	1.049

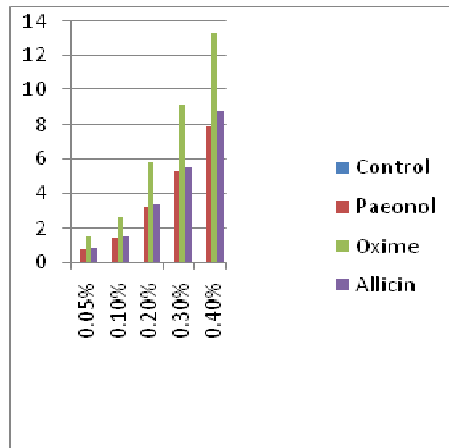


Figure 1
Antifungal Activity of Paenol, its oxime and Allicin against Aspergillus flavus.

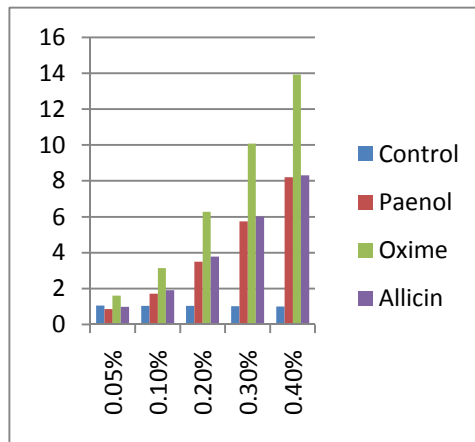


Figure 2
Antifungal Activity of Paenol, its oxime Allicin against Aspergillus niger

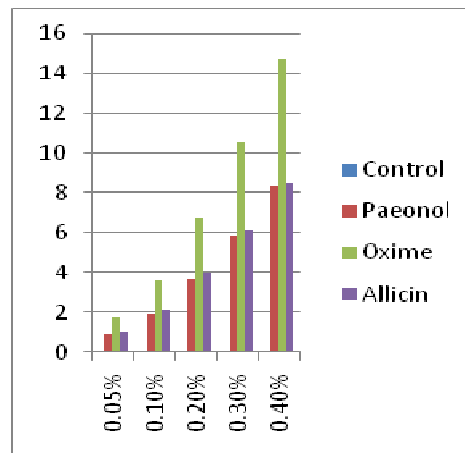


Figure 3
Antifungal Activity of Paenol, its oxime and Allicin against Cryptococcus neoformans.

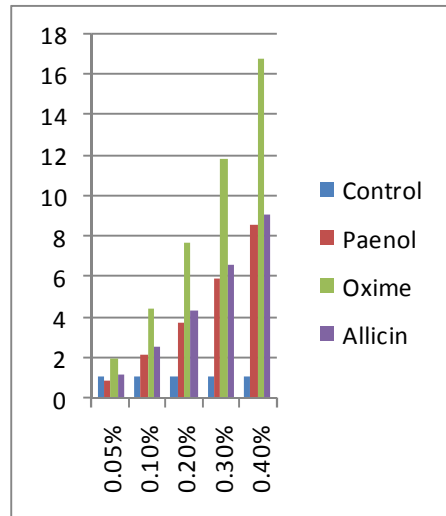


Figure 4
Antifungal Activity of Paenol, its oxime and Allicin against Alternaria alternate

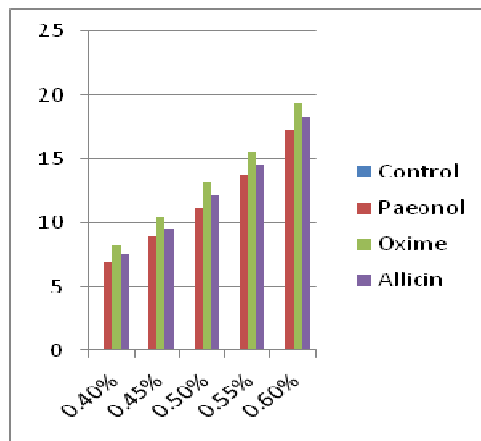


Figure 5
Antibacterial Activity of Paenol, its oxime and Allicin against Streptoproteus.

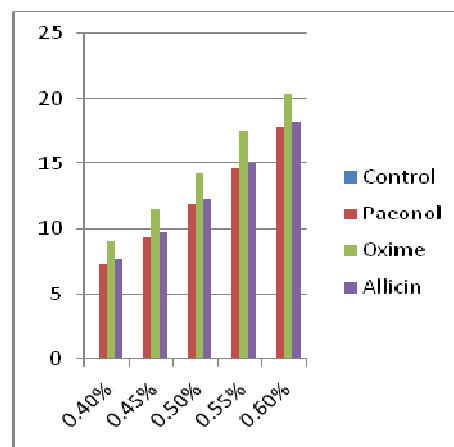


Figure 6
Antibacterial Activity of Paenol, its oxime and Allicin against Staphylococcus.

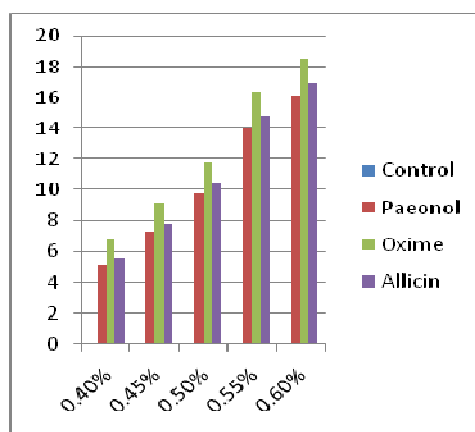


Figure 7
Antibacterial Activity of Paeonol, its oxime and Allicin against E. coli.

The observed results reveals that the antimicrobial activity of the compound is directly proportional to the concentration of the test compound and differs from fungus to fungus and bacteria to bacteria. The oxime of paeonol is more antimicrobial than paeonol 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 strain resistant to antibiotics such as methicillin resistant staphylococcus aureus [causes eczema and acne] as well as multidrug resistant enterotoxigenic 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 of cell membrane, inhibitors of biosynthesis (i.e. production of purines, pyrimidine, A.A., Vitamins, protein, DNA, RNA), inhibitors of energy production (inhibit the respiration or by uncoupling of oxidative phosphorylation). Oximes have high antimicrobial activity as compare to

paeonol this is due to the formation of dimeric and pseudomacrocyclic species by inter molecular H-bond. The biological activity of allicin is to be related to a combination of following factors.

1. Its activity as an antioxidant.
2. Its ability to attack the sulphur [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 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.[20] 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 system consisting of acetate kinase and phosphotransacetyl-co-A synthetase[21]. 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[22] 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 affected at slightly 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

Investigation	Before using garlic	After 2 months	Unit	Normal	after 4 Months
Serum cholesterol	311	245.6	mg/dl	130-200	220
Serum Triglycerides	201	193.7	mg/dl	40-150	185
HDL cholesterol	60.7	58.4	mg/dl	30-130	57.6
LDL cholesterol	224	148.46	mg/dl	<100	110

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 occurs in the blocked region resultanty heart attack or stroke take place. On the experimental basis it conclude that total cholesterol,LDL cholesterol and triglyceride level decreses sufficiently, when allicin is used 8-16 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 bezafibrate, HDL cholesterol values in the course of four weeks also increased significantly.

CONCLUSION

It may be concluded from the study that oxime of paeonol has more antimicrobial activity as compaired to paeonol and allcin against *Aspergillus flavus*, *Aspergillus niger*, *Cryptococcus neoformans* 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 and paeonol derivatives by chemical process to form more potent against different studies

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