Research Article

STUDY OF CHEMICAL COMPOSITION OF GARLIC OIL AND COMPARATIVE ANALYSIS OF CO-TRIMOXAZOLE IN RESPONSE TO *IN VITRO* ANTIBACTERIAL ACTIVITY

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DOI: 10.7897/2230-8407.050220**ABSTRACT**

During the last 20 years, traditional system of medicine has become a topic of global importance. *Allium sativum* also known as garlic, belong to the Genus *Allium* is one of the representatives of this system of medicine. In this study, the garlic oil was extracted by solvent extraction using n-hexane as solvent. The reported percentage yield of oil was 1.1 %, and the specific gravity was 0.90 g/ml. Gas Chromatography-Mass Spectrometry analysis of oil showed the presence of four compounds Diallylmonosulfide, Trisulfide methyl 2 propenyl, Diallyl trisulfide, Dimethyl tetrasulfide and their GC retention times were 6.2 minutes, 9.0 minutes, 5.9 minutes and 8.1 minutes respectively. The chemical equivalence of drugs plays an important role in their quality assurance. Five different brands of co-trimoxazole were studied for qualitative and quantitative analysis. The result of study showed that samples A-E had 101.29 %, 100.38 %, 99.35 %, 98.65 % and 97.31 % of sulfamethoxazole and 99.40 %, 98.69 %, 96.46 %, 89.25 % and 88.67 % of trimethoprim content. Only three brands A-C confirmed to official monograph for trimethoprim but two samples D and E showed lower values than standard values. This brings the need for drug agencies to monitor the quality of the drugs to check adulteration. The comparison of antibacterial activity of garlic extract and the drug was carried out *in-vitro* against *E-coli*, and the result of the study showed that garlic has good anti-bacterial activity with respect to co-trimoxazole.

Keywords: Garlic, GC-MS analysis, antibacterial activity, co-trimoxazole**INTRODUCTION**

Allium sativum is one of the medicinal herbs placed in the family Alliaceae¹. The important chemical constituents reported from Bulbus Allii Sativi are the sulfur compounds. The allicin, ajoenes and sulfides (e.g. diallyl disulfide, diallyl trisulfide), are not naturally occurring compounds. They are formed by naturally occurring alliin. When the garlic bulb is crushed, alliin is released and interacts with the enzyme alliinase to forms allicin.^{2,3} Allicin itself is an unstable product and undergo additional reactions to form different derivatives, depending on environmental conditions.⁴ Due to presence of compounds such as, sulfur's compounds, lipids, complex of fructosans, etheric oil, cellulose, minerals (Mg, Zn, Se, germanium), vitamins (C, A, from B complex), enzymes, amino acids, etc., it is particularly important in medicine.⁵ The presence of above chemicals in Garlic helps to inhibit bacteria, fungi, parasites. Cooked garlic or various aged extracts and oils can in some cases provide better protection against infection than raw garlic.⁶ Garlic extracts exhibited activity against gram negative (*E. coli*, *Enterobacter*, *Pseudomonas*, *Kilabsella*) and gram positive (*S.aureus*, *S. Pneumonia*, Group A *Streptococcus* and *Bacillus anthrax*).⁷ Allicin is the major biologically active component of garlic. This compound exhibits antibacterial and anti-fungal properties. Allicin is responsible for lipid-lowering, anti-cancer, antioxidant and anti-microbial effects⁸ Allicin contains the thiosulfinate functional group, R-S (O)-S-R. Allicin blocks the enzymes by reacting with one of their important components known as sulfhydryl (SH) groups, or thiols. Co-trimoxazole is an antibacterial drug, used for the treatment of a wide variety of infections caused by Gram-positive and Gram-negative organisms; particularly those of the gastro-intestinal tracts, urinary and respiratory tracts.⁹ Co-trimoxazole is the combination of Trimethoprim (TMP) and Sulfamethoxazole (SMX) in a 1:5 ratio. A synergy effect between the 2 drugs has been demonstrated both *in vitro* and

in most studies.¹⁰ The two agents are synergistic in inhibition of folate synthesis - the sulfamethoxazole inhibiting production of dihydrofolate from para-aminobenzoic acid and the trimethoprim inhibiting the next step in the pathway from dihydrofolate to tetrahydrofolate. In this study, selected brands of Co-trimoxazole tablets sourced from pharma stores of Vidisha were chemically evaluated.

MATERIALS AND METHODS

The Garlic cloves were collected from the Besnagar, Hansua, Kuakhedi villages of district Vidisha, Vidisha block and stored at room temperature. Dried garlic cloves were weighed and the grinded initially in a mortar pestle and then in a mixer grinder to obtained the garlic powder. The garlic powder was subjected to Soxhlet Apparatus and n-hexane (boiling point 50°-60° C) was used as solvent. The process of extraction was carried out for six hours. The oil was made free from hexane by refluxing at 70°C, to remove excess of solvent.¹¹ The extracted oil was stored in refrigerator below 2°C for further analysis.

GC-MS Conditions

The oil of *Allium sativum* was subjected to gas chromatography-mass spectra (GC/MS) analysis on an Agilent 7890A GC with Agilent 5975C MS with a dual detector mode (FID and MS together). Analysis was carried out at 70 eV and 20°C. The capillary column was a CP-Sil 8 CB LB with a 30 m long fused silica, an interior diameter of 0.25 mm and a film thickness of 0.25 µm. The carrier gas was helium at a flow rate of 1.2 ml/min. The oven temperature was programmed from 50 to 300°C with an initial increase of 10°C/ 1mn. 1 µl of oil sample prepared in acetone (1 % concentration) was injected into the apparatus.^{11,12} Five brands of co-trimoxazole tablets were commercially purchased from the pharma stores of Vidisha which contain trimethoprim and sulfamethoxazole in

different concentrations. The samples were named A to E, which were identified by HPTLC "CAMAG, LINOMAT 5. TLC was performed on 10 × 10 mm plates coated with layer of silica gel 60F254. Methanol was used as solvent and spray gas nitrogen. 2.0 µl volumes per spot were applied with 7 No. of tracks having 6.0 mm of band length. The percentage assay of trimethoprim and sulfamethoxazole was estimated according to the Indian Pharmacopeia monograph.¹³

Antimicrobial Activity of Garlic and the Drug

Aqueous garlic extract (AGE) was prepared in a sterile laminar air flow chamber. This extract was considered as the 100 % concentration of the extract. The next concentration, 50 %, was made by diluting the concentrated extract with appropriate volume of sterile distilled water. 1 mg of drug

sample A dissolved in 1 ml distilled water, and this concentration of the drug was considered as 100 % and from this solution, another concentration 50 % was prepared. The isolates of *E. coli* were used to check the antibacterial activity of the drug and the AGE.

Agar Well Diffusion Assay

E. coli was inoculated into 20 ml sterile broth and incubated for 24 hours at 37°C. The culture was then swabbed on Nutrient agar plates. Agar wells were prepared on both the plates and 100 µl of two different concentration (100 % and 50 %) of AGE and drug suspension were added to one well of each plate. Then plates were placed in incubator at 37°C for 24 hours. The zone of inhibition was measured for comparative antibacterial activity of drug and AGE.¹⁴

Table 1: Garlic Oil Components with their Molecular Masses and Retention Times

S. No	Compound	Molecular mass	GC Retention time
1.	Diallylmonosulfide	115.0	6.2 min
2.	Trisulfide methyl 2 propenyl	152.3	9.0 min
3.	Diallyl trisulfide	178.3	5.9 min
4.	Dimethyl tetrasulfide	157.1	8.1 min

Table 2: Comparison of R_f values of samples A-E with standard Trimethoprim and Sulfamethoxazole

Sample I.D.	Sample name	Max. R _f value		Result
		Trimethoprim	Sulfamethoxa-zole	
1.	Standard	0.29	0.74	
A	Septan	0.28	0.71	Present
B	Co-trimoxazole IP	0.29	0.71	Present
C	Bactrim	0.29	0.71	Present
D	Co-trimoxazole	0.29	0.71	Present
E	Antrima	0.29	0.74	Present

Table 3: Percentage of Trimethoprim and Sulfamethoxazole in tablets

Sample I.D	% TMP	% SMX
A	99.40	101.29
B	98.69	100.38
C	96.46	99.35
D	89.25	98.65
E	88.67	97.31

Table 4: Diameter of zone of inhibition exhibited by *E. coli* with AGE and Co-trimoxazole

S. No.	Test organism	Substance under study	Diameter of zone of inhibition	
			100 %	50 %
1.	<i>E. coli</i>	AGE	15 mm	14 mm
2.	<i>E. coli</i>	Co-trimoxazole	25 mm	20 mm

Figure 1: GC spectra of garlic oil components

Figure 2: Mass spectra of Diallylmonosulfide (M.W.115.0)

Figure 3: Mass spectra of Trisulfide methyl 2 propenyl (M.W.152.3)

Figure 4: Mass spectra of Diallyl trisulfide (M.W.178.3)

Figure 5: Mass spectra of Diallyl tetrasulfide (M.W.157.1)

Figure 6: (a) Inhibition zone showed by *E. coli* with 100 % concentration of AGE and drug, (b) Inhibition zone showed by *E. coli* with 50 % concentration of AGE and drug

RESULT AND DISCUSSION

In the study of garlic oil, extracted by solvent extraction, the calculated percentage yield of oil is 1.1 %, specific gravity is 0.90 g/ml. Analysis of the oil sample was carried out and important constituents of oil were identified with the help of their GC spectra (Figure 1) and MASS spectra (Figure 2, 3, 4 and 5). Four compounds were identified along with their GC retention times, (Table 1) which are – 1. Diallylmonosulfide 2. Trisulfide methyl 2 propenyl 3. Diallyl trisulfide and 4. Dimethyl tetrasulfide.

The presence of all four compounds in oil showed the degradation of allicin. Allicin is formed in garlic when garlic bulb is crushed; enzyme allinase released which convert alliin into allicin. Allicin itself is unstable compound and instantly undergo reactions to form other sulfur derivatives depending upon environmental conditions. In this study, the result showed that allicin got converted into disulfides and trisulfide, which confirmed the degradation of allicin. Strict experimental conditions should be maintained in order to protect allicin from degradation.

Qualitative and Quantitative Analysis of Co-Trimoxazole

The R_f values for standard trimethoprim after HPTLC separation was 0.29 and all samples A-E showed their R_f 0.29, whereas R_f value for standard sulfamethoxazole was 0.74 and samples A-E showed R_f between 0.71-0.74. Hence all samples are positively identified. (Table 2) In quantitative analysis, lower values than those found in monograph, were obtained for trimethoprim and sulfamethoxazole values were found to be within limits. Only 3 brands of co-trimoxazole A, B and C confirmed the amount of trimethoprim according to Indian Pharmacopeia limits i.e. not less than 92.5 %; but brands D and E, showed lower values of trimethoprim i.e. 89.25 % and 88.67 % respectively. On the other hand, amount of sulfamethoxazole in brands A-E were found to be within range as per official monograph i.e. not more than 107 % (Table 3). The lower values could be due to the reason that other excipients present in the formulation. The result also showed that there is a need for drug safety agencies to conduct regular monitoring of drugs, to check substandard medicines in the Vidisha.

Comparison of Antimicrobial Activity of Garlic and Co-Trimoxazole

The diameter of zone of inhibition showed by aqueous garlic extract at 100 % and 50 % concentration was 15 mm and 14 mm respectively whereas showed by drug was 25 mm and 20 mm respectively. (Table 4) The result showed good antibacterial activity of AGE, when compared with the activity of drug. In both the concentrations of AGE used, the inhibition zone diameter didn't show any considerable variation. Hence the AGE could be used at 50 %

concentration for antimicrobial activity (Figure 6). The sulfa drug on the other hand, is in highly pure form, showed large inhibition zone i.e. 25 mm and 20 mm at 100 % and 50 % of its concentration used. The sulfa drug performs their action by metabolic antagonism. They are antimetabolites competes with Para Amino Benzoic Acid (PABA) for incorporation into folic acid. All cells require folic acid for growth. Bacteria must synthesize folic acid from PABA. The sulfur group present in garlic performs the same action and has potential to act as an antimicrobial agent to substitute some of the sulfa drugs.

CONCLUSION

The allicin present in garlic is main antibacterial and antifungal agent. Garlic shows a good potential to act as an antibacterial agent. Garlic is least expensive and easily available natural herb can act as a drug. The chemical purity of any drug is very important, hence, different brands of co-trimoxazole, were tested qualitatively for identification and quantitatively for assay on as is basis. The action of co-trimoxazole was compared with that of garlic extract, on *E. coli* and by referring the experimental observations it can be concluded that garlic extract could be a better option for curing bacterial infections and the use of synthetic drug could be avoided to some extent to protect long term side effects originated from synthetic drugs.

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