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RESEARCH ARTICLE

A COMPARATIVE STUDY ON ANTIMICROBIAL PROPERTIES OF CERTAIN POLYMERIC NANOCOMPOSITES

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ABSTRACT

The objective of this investigation is the comparative study of antibacterial and antifungal potentials of citric acid and tartartic acid based nano composites on the growth inhibition of some important pathogenic fungi and bacteria *in vitro*. The study is to assess the antimicrobial activity and to determine the zone of inhibition of nanocomposites on some bacterial and fungal strains. In the present study, the microbial activity of (Tartartic acid + Glycerol + n-HAp) TAGH and (Citric acid + Glycerol + n-HAp) CAGH nanocomposites was evaluated for potential antimicrobial activity against medically important bacterial and fungal strains such as *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi* and fungi namely *Candida albicans, Trichoderma viride, Rhizopus microspores*. Antibacterial activity of the extracts was determined by disc diffusion method on Muller Hinton agar (MHA) medium. Antifungal activity of the extracts was determined by disc diffusion method on Sabouraud Dextrose agar (SDA) medium. The assay was performed by agar disc diffusion method. The results demonstrated that citric acid has more bactericidal and fungicidal activities than those of tartaric acid against all pathogenic bacteria and fungi tested. The small size of the Nanomedicine is very suitable for carrying out antimicrobial biological operations. Further research is needed to assess the efficacy of citric and tartaric acids as inhibitors of bacterial and fungal growth in clinical trials, especially in treatment of patients with microbial infections.

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INTRODUCTION

Despite numerous existing potent antibiotics and other antimicrobial means, bacterial and fungal infections are still major cause of morbidity and mortality. Consequently, attention has been especially devoted to new and emerging nanoparticle-based materials in the field of antimicrobial chemotherapy. Microbiological resistance to antifungals, particularly polyenes and azoles, has been increasingly reported. Despite the increase in frequency of resistance, reports of clinical antifungal failures or single stains becoming resistant to antifungal therapies remain distinctly uncommon (Diekema *et al*., 2003; Frosco and Barret, 1998). On the other hand, the number of serious invasive fungal infections has continued to increase due to the fact that more immunosuppressed patients are at risk for these infections. Organic acids are widely used as preservatives in foods and have been used as buffer agents in medical solutions (Cherrington *et al*., 1990; Dibner and Buttin, 2002). Several studies reported the inhibitory effect of these acids such as saturated fatty acids, formic and propionic acids, lactic acid and medium-chain fatty acids against different microorganisms (Maroune *et al*., 2003; Anders *et al*., 1989; Lee *et al*., 2002). In addition to their suppressing effect on the growth of food spoilage microorganisms, organic acids were shown to possess antibacterial activities against various infectious pathogens including *Staphylococcus aureus, Yersinia enterocolitica, Listeria monocytogenes, Escherichia coli*, as well as *Clostridium botulinum* (McWilliam Leitch and Stewart, 2002; Qvist *et al*., 1994; National Committee for Clinical Laboratory Standards, 1997). As far as we know, there is little information about the antimicrobial activity of organic acids, especially citric and tartaric acids against clinically important potential pathogens. The main objective of this study was to investigate the antibacterial and antifungal effects of two above mentioned acids against bacteria such as *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi* and fungi namely *Candida albicans, Trichoderma viride, Rhizopus microspores.*

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MATERIALS AND METHODS

Test Organisms

Bacteria and fungi involved in testing antimicrobial activity are *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi* and *Candida albicans, Trichoderma viride, Rhizopus microspores* respectively.

Bacterial Cultivation and Preparation of Inocula

Stock cultures were maintained at 4°C on Nutrient agar Slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing nutrient broth, that were incubated at 24hrs at 37ºC. The Assay was performed by agar disc diffusion method.

Antibacterial activity by Agar Disc Diffusion Method

Antibacterial of extracts was determined by disc diffusion method on Muller Hinton Agar (MHA) medium. Muller Hinton Agar (MHA) medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The disc were placed in MHA plates and add 20 µl of sample (Concentration: 1000µg, 750µg and 500 µg) were placed in the disc .The plates were incubated at 37ºC for 24 hrs. Then the antimicrobial activity was determined by measuring the diameter of zone of inhibition.

Fungal Cultivation and Preparation of Inocula

Stock cultures were maintained at 4°C on Sabouraud Dextrose agar Slant. Active cultures for experiments were prepared by transferring the stock cultures into the test tubes containing Sabouraud Dextrose broth that were incubated at 48 hrs at room temperature. The assay was performed by agar disc diffusion method.

Antifungal activity by Agar Disc Diffusion Method

Antifungal activity of the extracts was determined by disc diffusion method on Sabouraud Dextrose Agar (SDA) medium. Sabouraud Dextrose Agar (SDA) medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. Amphotericin-B is taken as positive control. Samples and positive control of 20 µl each were added in sterile discs and placed in SDA plates. The plates were incubated at 37ºC for 24 hrs. Then antifungal activity was determined by measuring the diameter of zone of inhibition.

Preparation of Polymeric Nanocomposites

The polyester, poly (Glycerol-Co-Citrate) (PGC) and Poly (Glycerol- co- tartrate) (PGT) were synthesised by catalyst free melt polycondensation method (Chandra Kumari and Jaisankar, 2017). Equimolar amounts of both glycerol with Citric acid and glycerol with Tartaric acid were added to a round bottom flask and melted together at 160-165ºC followed by mixing at 140-145ºC for 1h under constant steam of nitrogen to obtain pre-polymer. The pre-polymer was then mixed to incorporate 5% by weight n-HAp. PGC and PGT pre-polymers were dissolved in methanol and mixed with the desired amount of n-HAp powder. The n-HAp/PGC and n-Hap/PGT mixture was stirred to get (Citric acid + Glycerol + n-HAp) CAGH and TAGH (Tartartic acid + Glycerol + n-HAp) homogeneous solution and cast into Teflon dishes and left in an oven at 110ºC for 2 days for post-curing.

RESULTS AND DISCUSSION

Antibacterial activity of TAGH and CAGH

Anti-bacterial are the agent that interferes with the growth and reproduction of the bacteria. Anti-bacterial is used to disinfect surfaces and to potentiate harmful bacteria. Heat, chemicals such as chlorine, phenols etc. and anti-bacterial drugs have anti-bacterial properties.

Table 1. Sample: TAGH

Organisms	Zone of inhibition (mm) Concentration(µg/ml)			Antibiotic (1mg/ml)
	1000	750	500	
Staphylococcus aureus-3160	13	11	9	37
Bacillus subtilis – 2763	20	12	8	41
Salmonella typhi- 1169	10	9	9	28

Table 2. Sample: CAGH

Organisms	Zone of inhibition (mm) Concentration(µg/ml)			Antibiotic (1mg/ml)
	1000	750	500	
Staphylococcus aureus-3160	21	17	15	37
Bacillus subtilis – 2763	20	12	8	41
Salmonella typhi- 1169	11	10	10	28

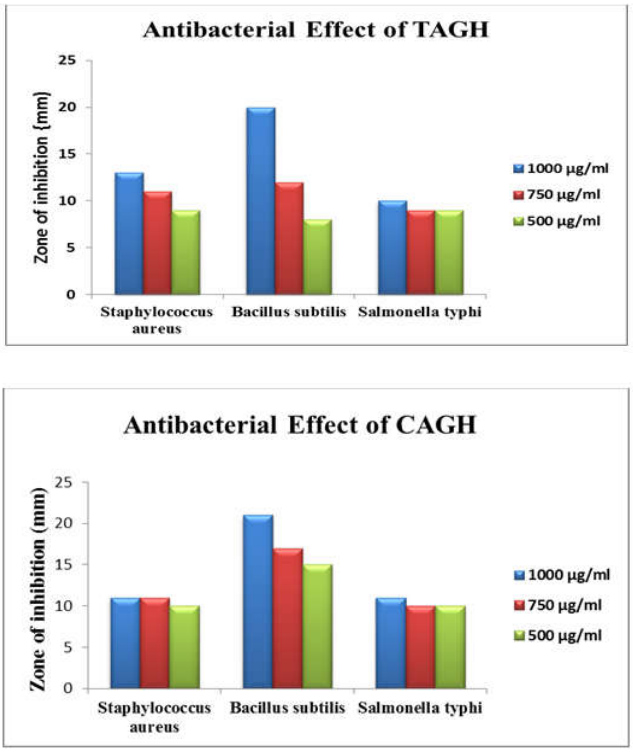


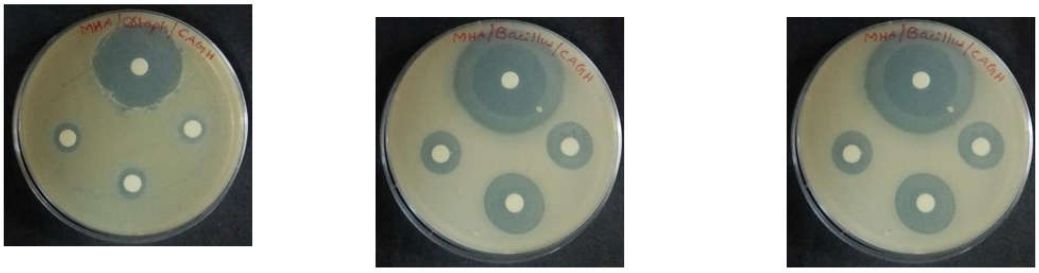
Fig.1. Antibacterial activity of (a) TAGH and (b) CAGH

Antifungal activity of TAGH and CAGH

An antifungal agent is a fungicide used to treat and prevent mycoses diseases. Antifungal Agents are the substances which

Staphylococcus aureus *Bacillus subtilis* *Salmonella typhi*

Fig. 2. Antibacterial study of TAGH



Staphylococcus aureus *Bacillus subtilis* *Salmonella typhi*

Fig. 3. Antibacterial study of CAGH

Table 3. Sample: TAGH

Organisms	Zone of inhibition (mm) Concentration(µg/ml)			Antibiotic (1mg/ml)
	1000	750	500	
Candida albicans - 4748	12	10	10	20
Trichoderma viride – 1763	15	10	10	28
Rhizopus microspores- 3934	7	7	7	8

Table 4. Sample: CAGH

Organisms	Zone of inhibition (mm) Concentration(µg/ml)			Antibiotic (1mg/ml)
	1000	750	500	
Candida albicans - 4748	17	16	12	19
Trichoderma viride – 1763	22	21	16	28
Rhizopus microspores- 3934	8	7	7	8

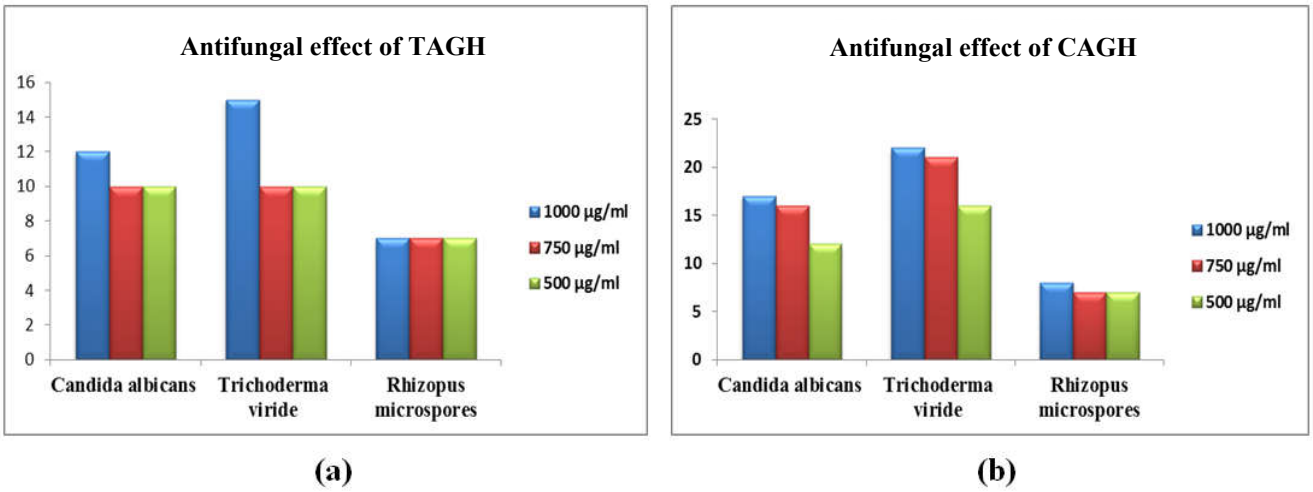


Fig. 4. Antifungal activity of (a) TAGH and (b) CAGH

Candida albicans *Trichoderma viride* *Rhizopus microspores*

Fig. 5. Antibacterial study of TAGH

Candida albicans *Trichoderma viride* *Rhizopus microsporus*

Fig. 6. Antifungal study of CAGH

destroy or prevent the growth of fungi. The most common types are mycoses such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as Cryptococci meningitis. The antibacterial and antifungal activity of citric and tartaric acid nano composites against all tested Bacteria and fungi, as measured by agar dilution test, was presented in Table 1,2,3,4. All of the Bacterial and fungal organisms tested were affected by citric and tartaric acid nanocomposites. Citric acid was active against all pathogenic bacteria and fungi tested. Conversely, tartaric acid showed modest activity against all bacteria and fungi tested. The higher activity of citric acid may be attributed to have several inhibitory mechanisms such as depression of internal pH of microbial cell by ionization of undissociated acid molecules and disruption of substrate transport by altering cell membrane permeability or reduction of proton motive force (Jay, 2000; Atabay and Corry, 1997). Conversely, tartaric acid, as an antimicrobial agent, is believed to act only by lowering the pH of the cell (Blaszyk and Holley, 1998). In addition to the inhibition of energy production, tartaric acid prevents the production of malic acid, which is a key intermediate in the production of glucose in the process of gluconeogenesis, the principal fuel for the cells (Anaissie *et al*., 2003). Several studies showed that citric acid and its salts inhibit the growth of the most common bacterial pathogens such as *Arcobacter spp., Campylobacter spp., lactobacilli, E. coli O157:H7 and L. monocytogenes* (Coste *et al*., 2007). This study showed that citrate salt was active against Gram-positive species but showed little activity against Gram-negative species; acetate salt showed the opposite results.

Conclusion

Citric acid and tartaric acid are active antibacterial and antifungal agents in vitro. However, further research is required to assess the correlation between antibacterial and antifungal activity in vitro and in vivo studies. The successful results might be applied in the future treatment of patients with bacterial and fungal infections.

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