

Full Length Research Paper

Comparative study of biological activity of glutathione, sodium tungstate and glutathione-tungstate mixture

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Glutathione (GSH) and sodium tungstate (Na_2WO_4) are important pharmacological agents. They provide protection to cells against cytotoxic agents and thus reduce their cytotoxicity. It was of interest to study the biological activity of these two pharmacological active agents. Different strains of bacteria were used and the zone of inhibition was determined for GSH, Na_2WO_4 and GSH+ Na_2WO_4 mixture. The results show high antibacterial activity of GSH as compared to Na_2WO_4 and Na_2WO_4 +GSH mixture. It was observed that GSH antibacterial activity was significantly lowered upon addition of Na_2WO_4 to GSH aqueous solution. The results conclude with the proposed formation of W-SG complex in aqueous solution.

Key words: Glutathione, sodium tungstate, antibacterial activity, strains of bacteria.

INTRODUCTION

Glutathione (GSH) has attracted the attention of biomedical scientists due to its important intra- and extracellular anti-oxidants properties (Pastore et al., 2003). Variations in content of GSH in the cells may cause oxidative stress or biomarker of pathogenesis of diseases (Schafer and Buettner, 2001). GSH also acts as a free radical scavenger and thus may increase the efficacy of antibiotics, which in certain cases are responsible for the production of free radical even in different bacterial strains (Albesa et al., 2004). Generation of free radicals can result in the damage of DNA, proteins and lipids (Albesa et al., 2004; Becerra and Albesa, 2002).

Glutathione (GSH) is one of the most important intra and extracellular antioxidant (Djordjevic, 2004; Pastore et al., 2003), which provides protection to cells (Griffith, 1999; Meister and Anderson, 1983; Schafer and Buettner, 2001). GSH has been observed to protect bacterial pathogens against certain antibiotics (Albesa et al., 2004; Cabiscol et al., 2000), and thus scavenge free radicals produced during antibiotic treatment (Goswami

et al., 2006). Glutathione has shown the ability to modify the susceptibility of *Staphylococcus aureus* to ciprofloxacin and gentamicin (Paulina et al., 2010). Moreover, the sensitivity of certain antibiotics increases by the use of GSH. GSH inhibits the growth of certain strains including *Pseudomonas aeruginosa*, and thus increases the efficacy of antibiotics as was observed in case of tetracycline (Zhang and Suan, 2009).

Sodium tungstate has also been shown to possess a variety of pharmacological properties, which include antidiabetic and B-cell regeneration (Jorge et al., 2003; Barbera et al., 1997; Rodrigues et al., 2000). Both glutathione and sodium tungstate are important pharmacological agents. Therefore, it was of interest to check and examine the comparative antibacterial activities of GSH and Na_2WO_4 and the mixture containing both GSH and Na_2WO_4 in aqueous solution. The results reveal that GSH had high, Na_2WO_4 had zero and the mixture of GSH and Na_2WO_4 had lowered antibacterial activity against six bacterial strains.

MATERIALS AND METHODS

Chemicals

L-Glutathione (GSH) (Fluka), sodium hydroxide (Fluka AG), sodium

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chloride (Merck), disodium edetate (Riedel Dehean AG Sneeze Hannover), potassium dihydrogen phosphate (Merck), HCl 35% (Kolchlight), sodium tungstate (Across, Belgium), distilled water (double refined), chloroform (Merck), ethanol (Merck), barium chloride (Merck), sulfuric acid (Merck), and Mueller-Hinton Broth (Oxide) were used in the experiments.

Equipments and glass wares

The equipments used included autoclave, incubator, freezer, pH meter: Model NOV-210 (Nova Scientific Company Ltd. Korea), and analytical balance AX 200 (Schimadzu, Japan). The glass wares included Petri dishes, pipette, test tubes, borers, beakers and conical flasks.

Microorganisms

Six bacterial strains namely *Escherichia coli* (*E. coli*), *Klebsiella pneumonia* (*KP*), *P. aeruginosa* (*PA*), *Enterobacter* (*Ent*), *S. aureus* (*SA*) and *Micrococcus luteus* (*ML*) were used in the antimicrobial assay. All strains were obtained from the Microbiology Research Laboratory (MRL), Microbiology Department, Quaid.e.Azam University, Islamabad, Pakistan, where these were identified and characterized. These strains were maintained on agar slants at 4°C in Gomal Center of Biochemistry and Biotechnology (GCBB) for antimicrobial tests. Microorganisms were incubated overnight at 37°C in Mueller-Hinton Broth (Oxoid) at pH 7.4. The reference antibiotics used was Cefipime (5 µg).

Inoculum preparation

A loopful of isolated colonies was inoculated into 4 ml peptone water and incubated at 37°C for 24 h. The turbidity of actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 McFarland units prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dihydrate with 99.5 ml 1% (v/v) sulphuric acid. The concentration of the suspension was standardized by adjusting optical density to 0.1 at 600 nm wavelength (Shimadzu UV 1700) (TereSchuk et al., 1997). This turbidity is equivalent to approximately 1 to 2×10^8 colony-forming units per milliliter (cfu/ml). This 24-h grown suspension was used for further testing.

Antibacterial activity

Disc diffusion method

The antimicrobial test was performed by the agar well diffusion method as described by Bauer et al. (1966) using a cell suspension of about 1.5×10^6 cfu/ml "colony forming units per milliliter" obtained following 0.5 McFarland turbidity standard. The concentration of the suspension was standardized by adjusting the optical density to 0.1 at 600 nm wavelength (SHIMADZU UV-1700 spectrophotometer) (Tereschuck et al., 1997). Petri dishes were filled with Mueller Hinton agar and inoculated with the test microorganism using sterilized cotton swab to ensure a uniform thick lawn of growth following incubation. Wells of 6 mm in diameter were formed on agar plates using a sterile cork borer. The wells were filled with the test agents (50 µL) and the plates were then allowed to stay for 1 to 2 h at room temperature. Finally, the plates were incubated at 37°C for 18 to 24 h and the resulting diameters of zones of inhibition were measured. The results are averages of triplicate tests.

The biological activities of all the drugs/complexes were also

examined. The samples for biological activity were prepared as follows: (1) Glutathione (1 mM) solution was prepared by adding 3.07 mg (Mol. weight 307.4) of glutathione in sufficient quantity of phosphate buffer (pH 7.6) to make 10 ml of final solution for determination of antimicrobial activity of GSH. The final concentration of GSH was 1000 µM; (2) sodium tungstate (ST): 1 mM solution of ST was prepared by dissolving 3.34 mg in sufficient quantity of distilled water to obtain a 10-ml solution. This solution containing ST was used for antimicrobial activity of ST. The final concentration of ST was 1000 µM; (3) ST-GSH mixture: briefly, 2 mM solution of ST was mixed with 2 mM solution of GSH in 1:1. The final concentration of ST and GSH in this mixture was 1000 µM.

Finally, 50 µL from each sample was poured into the microbial media to check antimicrobial activity of each sample.

RESULTS AND DISCUSSION

When the antibacterial activity of GSH, ST and GSH+ST were studied on *E. coli*, *KP*, *PA*, *Ent*, *SA* and *ML*, maximum inhibition was found by *PA* on GSH, no inhibition by *KP* and *E. coli* and minimum by *SA*, while no inhibition was found by all the strains on ST (Figures 2 to 7). Equal zone of inhibition was found by *PA* and *Ent* on GSH+ST and least inhibition was found by *ML*, while no inhibition was found by *MC* and no inhibition by *SA*, *KP* and *E. coli* was found as shown in Figure 1a to e. Table 1 shows the zone of inhibition of each sample in the concerned bacterial culture.

Glutathione (GSH) is an endogenously synthesized tripeptide thiol, which plays an important role in intra and extracellular antioxidants defense (Pastore et al., 2003). Glutathione is exclusively found in its reduced form (GSH), and the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) within the cells is often used scientifically as a measure of cellular toxicity (Pastore et al., 2003). Glutathione deficiency contributes to oxidative stress, which plays a key role in aging and the pathogenesis of many diseases. Thus, glutathione has vital role in the maintenance of body defense system in fighting against diseases along with metal and drug poisoning. A considerable data on the chemistry and pharmacological properties of sodium tungstate is available (Tajima, 2001; Jelkic-Stankov, 2007).

Glutathione (GSH) and sodium tungstate (Na_2WO_4) are known pharmacological agents with varied pharmacological properties. To test the biological activities of these pharmacological agents and to check their mode of action alone and in combination, we selected six strains including *PA*, *Ent*, *SA*, *ML*, *KP* and *E. coli*, obtained from the Microbiology Research Laboratory, Department of Microbiology, Quid.e.Azam University, Islamabad. The antibiotic, Cefipime, was used as a standard drug for inhibition of growth or zone of inhibition of antimicrobial agent. Interestingly, GSH alone had the widest zone of inhibition representing maximum antimicrobial activity or the effect was more pronounced on *PA* strains. The antimicrobial effects of GSH on the remaining strains were found in the order of decreasing effect. The GSH

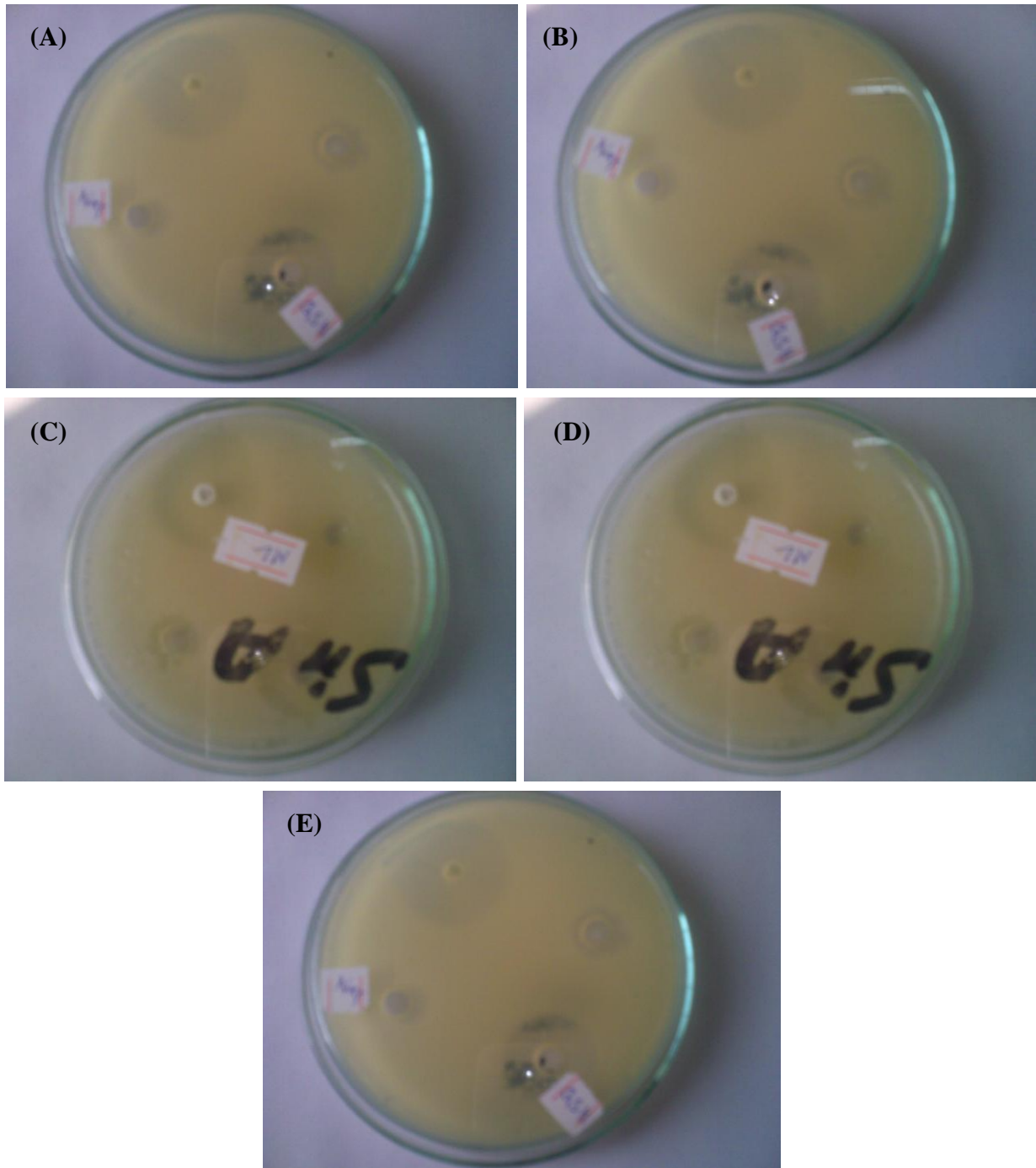


Figure 1. Antimicrobial samples with zones of inhibitions of the bacterial cultures (mm). (A) *P. Aeruginosa* (9, 0, 5, 16). (B) *Enterobacter* 7, 0, 5, 17). (C) *Staphylococcus aureus* (6, 0, 0, 19). (D) *Micrococcus luteus* (8, 0, 4, 18). (E) *Escherichia coli* (0, 0, 0, 17).

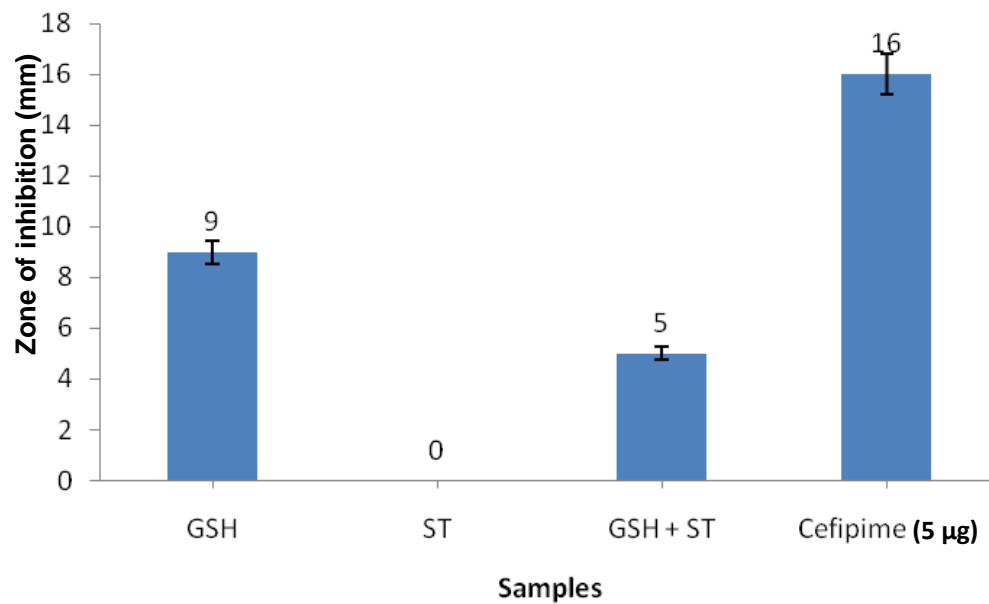
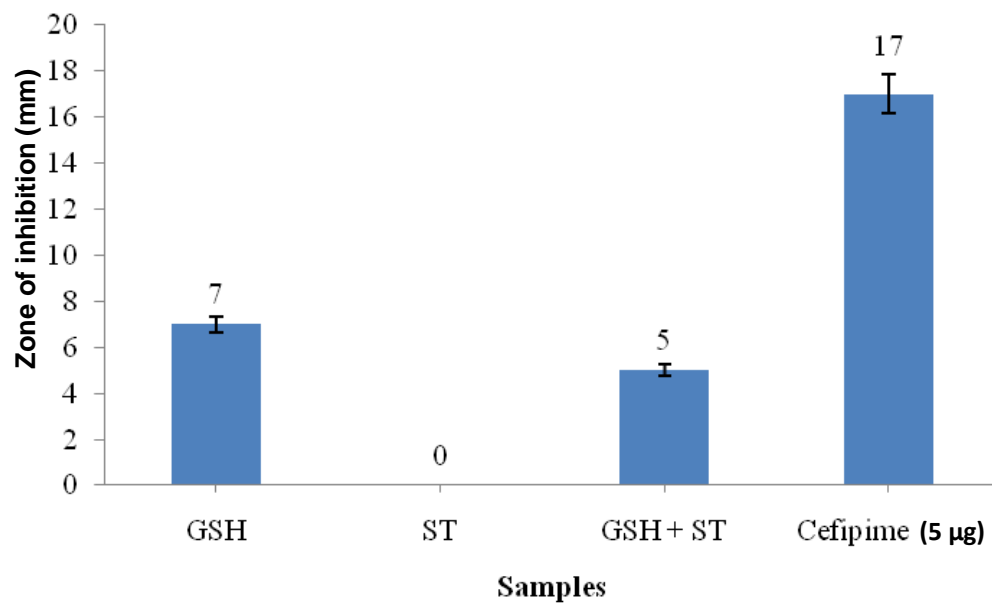
showed noticeable effect on *Ent*, *ML* and *SA*, but surprisingly no effect of GSH was observed on *KP* and *E. coli* under this concentration and experimental condition. Na_2WO_4 antimicrobial activity on these strains was also examined and very interestingly Na_2WO_4 indicated no antimicrobial activity in terms of its effect on those strains.

A mixture of GSH and ST was tested for zone of inhibition for those strains. Surprisingly, a decreased inhibition zone was found by the mixture in cases of *PA*, *Ent*, *ML*, and no effect or zero inhibition in case of *SA* strains was found. The effect of GSH and the mixture of GSH and ST on these cultured strains indicated the

Table 1. Zones of Inhibition of samples used in the selected bacterial cultures.

| Sample | Strain | | | | | |
|-----------------|--------|------|----|----|----|---------|
| | PA | Ent. | SA | ML | KP | E. coli |
| GSH | 9 | 7 | 6 | 8 | 0 | 0 |
| ST | 0 | 0 | 0 | 0 | 0 | 0 |
| GSH + ST | 5 | 5 | 0 | 4 | 0 | 0 |
| Cefipime (5 µg) | 16 | 17 | 19 | 18 | 16 | 17 |

PA = *Pseudomonas aeruginosa*; Ent. = *Enterobacter*; SA = *Staphylococcus aureus*; ML= *Micrococcus luteus*; KP = *Klebsiella pneumoniae*; E. coli = *Escherichia coli*.

**Figure 2.** Zone of inhibition of different samples against *Pseudomonas aeruginosa* (PA).**Figure 3.** Zone of Inhibition of different samples against *Enterobacter* (Ent).

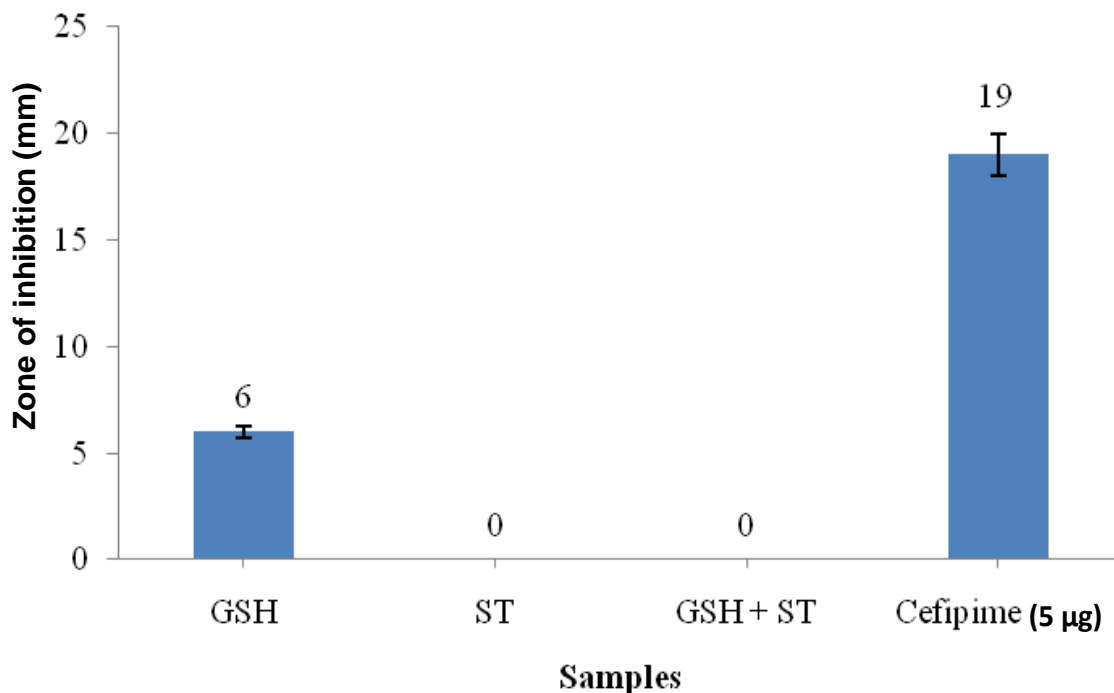


Figure 4. Zone of inhibition of different samples against *Staphylococcus aureus* (SA).

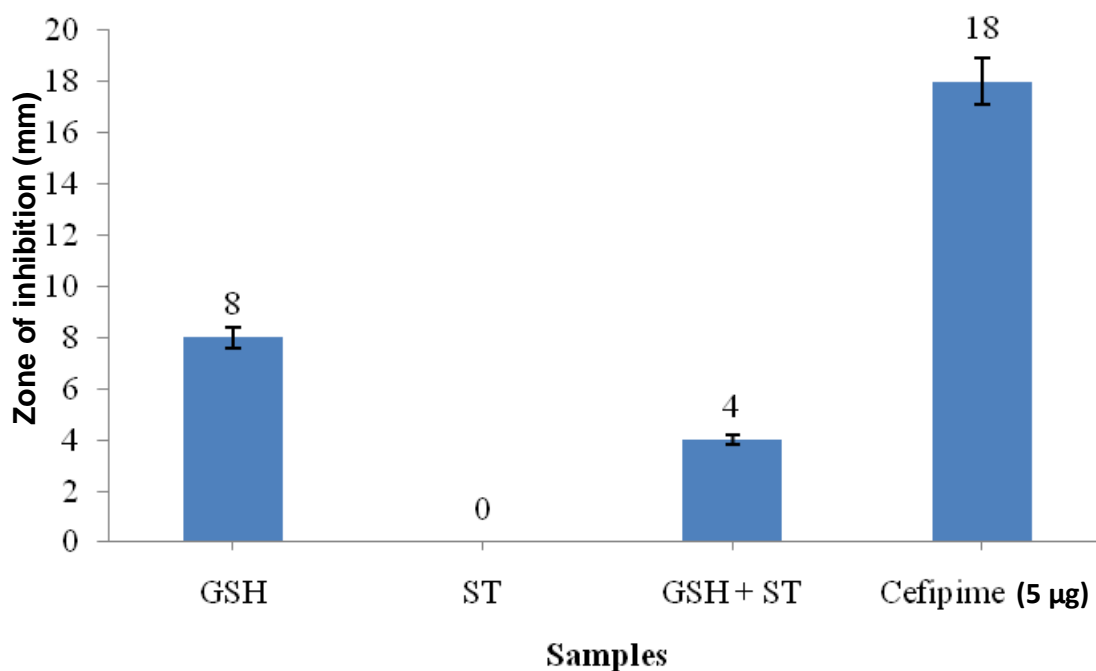


Figure 5. Zone of inhibition of different samples against *Micrococcus luteus* (ML).

altered chemical status of GSH in the aqueous solution. In our previous study, the addition of ST to GSH in aqueous solution, and the measurement of GSH concentration spectrophotometrically indicated the low concentration of GSH in the mixture aqueous solution. It

was proposed that ST had either converted the GSH to oxidized glutathione (GSSG) or to W-SG complex. There are two possibilities of conversion of GSH by Na_2WO_4 :

1) Conversion of reduced glutathione (GSH) to oxidized

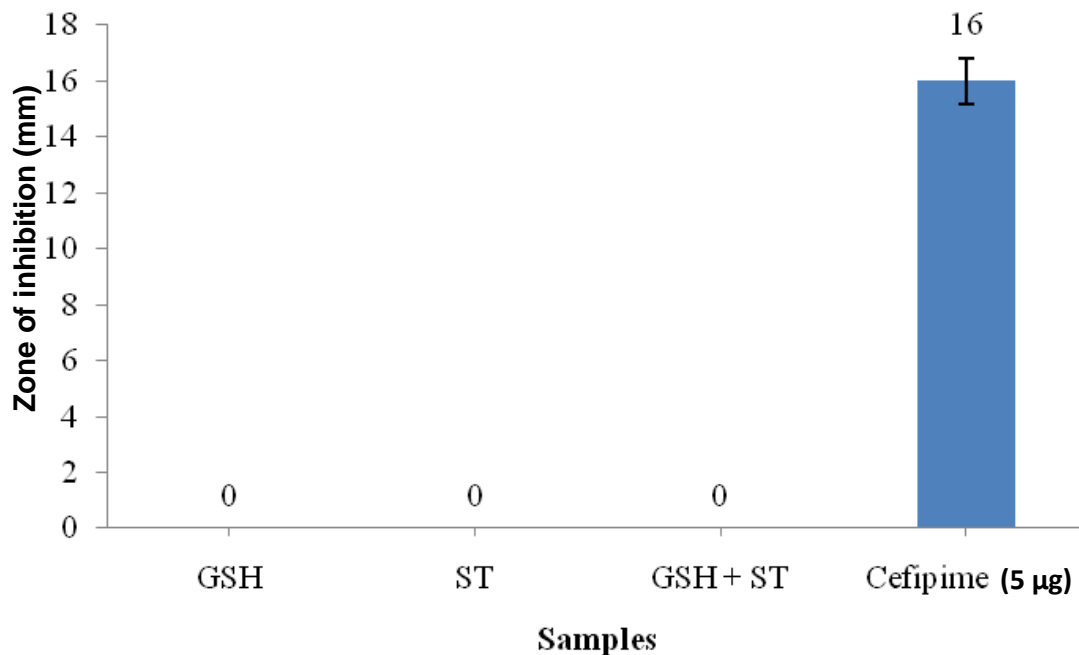


Figure 6. Zone of inhibition of different samples against *Klebsiella pneumonia* (KP).

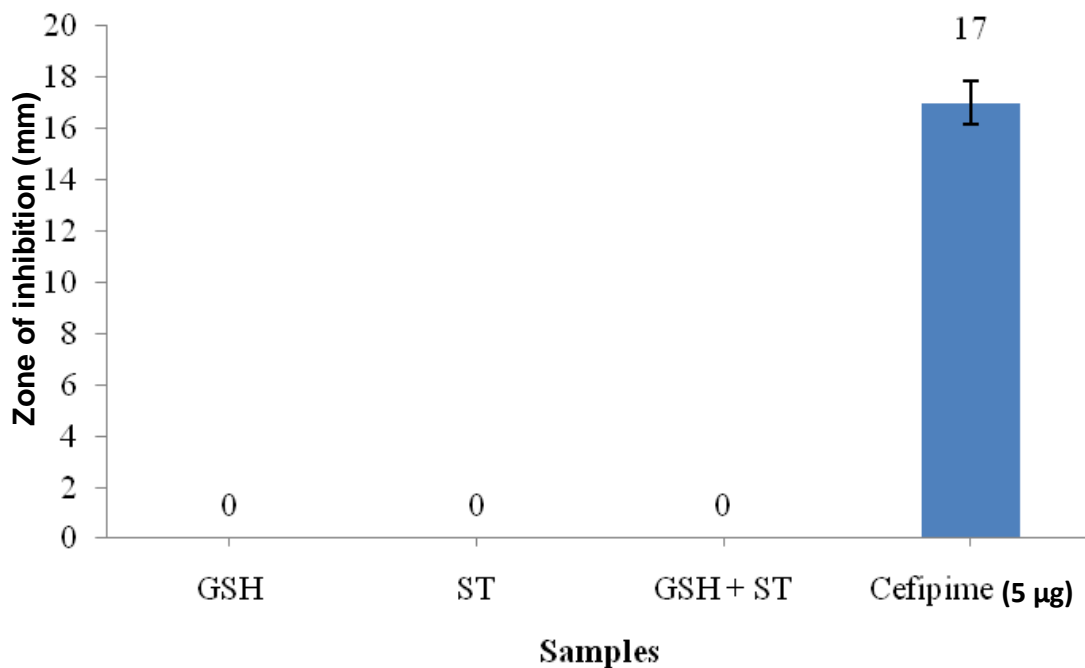
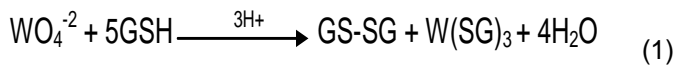
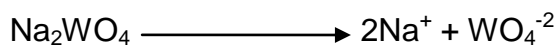


Figure 7. The zone of inhibition of different samples against *Escherichia coli* (E. coli).

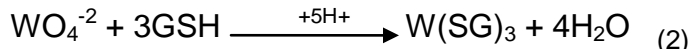
form GSSG and
 2) Conversion of GSH to tungsten-glutathione complex (W(SG)₃).

Such proposed reaction through a mechanism involving tungstate with S-H group of GSH with the formation of

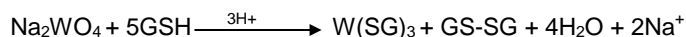
GSSG or W-SG complex is given as:



A second possibility is that:



The overall reaction between tungsten (W^{+6}) ion in Na_2WO_4 and GSH is given as:



The formation of GSSG in the mixture was not proposed because during the biological activity experiment, the ST and GSH mixture had low biological activity indicating no formation of GSSG, and most probably formed W-SG complex. This proposed mechanism is in agreements with the result obtained by Zhang and Duan (2009), whereas both GSH and GSSG have the same inhibitory activity against cultured strains including PA.

In this present experiment, the mixture of ST and GSH had low inhibitory activity, indicating no formation of GSSG. Therefore, the reduced inhibitory activity of the mixture of ST and GSH may be due to the formation of W-SG complex, as ST has no inhibitory activity. In conclusion, *in vitro* examination on the inhibitory effect of GSH and ST and ST-GSH mixture indicate that upon the addition of ST to GSH aqueous solution, the clinical status of GSH changed to W-SG complex.

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