The bactericidal activity of a medicinal plant, *Terminalia chebula* is enhanced upon addition of manganese salts

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Abstract: The aqueous extract of *Terminalia chebula*, a natural medicinal herb had been demonstrated to exert its bactericidal activity against *Salmonella* sp., *Shigella* sp., *Vibrio cholerae* and *Escherichia coli*: the bacteria that cause gastrointestinal. This activity was analyzed upon addition of different concentrations of salts of magnesium and manganese in the aqueous extract of the plant in order to observe the effect of the salts, if any. This study revealed that addition of manganese chloride at a concentration of about 80 μg/gm of dried leaf powder could further increase the existing antibacterial activity of the plant to enteric bacteria by 25 to 45%.

Keywords: Medicinal plant; *Terminalia chebula*; antibiotics; antibacterial activity; manganese.

Introduction

A common problem in the twenty first century is the high rates of antimicrobial resistance pattern of pathogens. This is so much on the rise that almost 70% of bacteria in the environment may have become resistant to common drugs used for treatment of different infections. This has resulted in difficulty in appropriate control of diseases. The development of microbial resistance as well as economic incentives, have resulted in research and development in the search for new antibiotics or alternate medicines in order to maintain a pool of effective drugs at all times. The development of resistant strains is inevitable since the ways that these drugs are administered in developing countries has greatly damaged the future of antibiotic therapy.

Antibiotics have been shown to cause adverse effects to the host including hypersensitivity, immune-suppression and allergic reactions occasionally (Ahmad et al. 1998). Contrasting to its effects, the traditional medicines are safe for the infections originated from both microbial and non-microbial origins (WHO 1978). Most people in Indian sub-continent and the tribal communities in particular, rely on traditional medicine for treatment of their ailments from ancient ages. Of them, the *Terminalia chebula* had been practiced as an important herbal drug due to its extraordinary potential of healing, for what it is listed first in the Ayurvedic materia medica, and is called the *king of medicines*. Known by its local name as Haritaki, *T. chebula* is reported to be antimicrobial (Ahmad et al. 1998; Malekzadeh et al. 2001; Aqil and Ahmad 2007), anticaries (Jagtap and Karkera 1999), inhibitor to cancer cell growth (Saleem et al. 2002), radioprotector (Naik et al. 2003), hepatoprotective (Tasduq et al. 2006) and antioxidant (Naik et al. 2003; Cheng et al. 2003, Lee et al. 2007).

Low-molecular-mass metal compounds (zinc, magnesium etc) are known to demonstrate bactericidal and/or bacteriostatic activities. *Staphylococcus* spp, for example, were found susceptible to solutions of metal salts, viz. CuSO$_4$, NiCl$_2$, ZnSO$_4$, and CoCl$_2$ in the range of 50 mol to 80 mmol amounts (Uğ and Ceylan 2005). These apparent antimicrobial effects of metal ions coupled to a lack of significant toxicity in human cells has led to their incorporation into a wide range of healthcare products from catheters to wound dressings (O’Neill et al. 2003; Strohal et al. 2005). Further, different metal ions have been reported to augment antibacterial activity of antibiotics (Sazawal et al. 1998). Metal ions play important role in various biochemical reactions by acting as a cofactor for many enzymes. After binding with antibiotics,
essential proteins of microorganisms become inactive resulting in death of the microorganisms. Therefore, if metal ions help to maintain the active conformation of the protein, more drugs will be able to bind with that particular protein paving the way for effectiveness of the antibiotics (Rahman et al. 2005).

Earlier, we demonstrated that the aqueous extract of the leaves of Terminalia chebula produced profound antibacterial activity to various enteric pathogens, and the activities were well ahead when compared with most of the traditionally-used chemotherapeutic drugs (Mostafa et al. 2011). Taking the idea of metal ions-mediated activity enhancement of antibiotics, the present study aims to observe whether the addition of various concentrations of salts: MnCl$_2$ and MgCl$_2$ could also enhance the existing biocidal activity of the extract to the same pathogens. The study also determines the dose of the additives required to elicit any effect on the plant extract upon addition of salts.

**Methods and Materials**

*Extraction of plant materials*

The aqueous extract of the leaves of Terminalia chebula was prepared as described previously (Mostafa et al. 2011), and the final concentration of the extract was estimated at 10 mg/ml of dried leaf powder.

*Evaluation of antimicrobial activity*

The assessment of antibacterial activity of the plant extract against *Salmonella* sp, *Shigella* sp, *E. coli* and *V. cholerae* was determined in vitro by using the Kirby Bauer method (Bauer et al. 1966). Test culture was prepared by inoculating a colony of organism to Mueller Hinton broth and allowed to grow until the McFarland standard 0.5 was obtained. Sterile blank discs (Oxoid, UK) soaked with 30 μl of aqueous extract of haritaki leaf dried powder, supplemented with or without 5 μl of magnesium chloride or manganese chloride (BDH Chemicals Ltd, UK) solutions at their different concentrations (0.025, 0.05, 0.1, 0.5, 0.8, 1.0 μg/ml) were placed on MHA (Mueller Hinton Agar) plates after they had been swabbed with the test organism. As negative control, sterile discs containing only salts of respective concentrations were used. The plates were then incubated at 37°C and the zone of inhibition of bacterial growth (in mm) around the disc was measured after a 24-hour incubation.

**Statistical Analysis**

The effect of salts on the activity of the plant was measured as percentage of activity enhancement as per following equation:

\[
\text{Activity enhancement} = \left[ \frac{\text{Diameter of zone of inhibition with salt and extract} - \text{Diameter of zone of inhibition with extract only}}{\text{Diameter of zone of inhibition with extract only}} \right] \times 100\%
\]

**Results**

The effects of magnesium and manganese salts on the antibacterial activity of aqueous extract of *T. chebula* to *Salmonella* sp., *Shigella* sp., *E. coli* and *V. cholerae* were studied by disc diffusion method on a solid culture medium. Different concentrations of salts were mixed with the plant extract and were absorbed in blank discs before they were impregnated on Mueller-Hinton agar, previously swabbed with the specific bacterial pathogen. Both of the salts increased the antimicrobial activity of the plant extract against the pathogens tested (Figure 1) however; the salts alone did not produce any activity by their own. The manganese-mediated enhanced activity was recorded highest at its 25 ng (5 μl of 5 μg/ml concentrated solution of salts, dissolved in 30 μl plant’s aqueous extract per disc) however, the amount of the salt decreased the antibacterial activity of the extract (Figure 1A). Such a salt-mediated activity-enhancement was recorded for all the tested pathogens when compared to that of the extract alone (Figure 1C). MgCl$_2$, on the other hand, produced much weaker enhancement of antibacterial activity compared to that of the MnCl$_2$. Dissolved in 30 1 plant’s aqueous extract per disc, an amount of 0.5 ng of MgCl$_2$ (5 1 of 0.1 g/ml concentrated solution of salts) showed the peak antibacterial activity (Figure 1B), but only to *Salmonella* sp. and *Shigella* sp. when the activity was compared to the extract alone (Figure 1D).
Mn$^{2+}$ enhances antimicrobial activity of T. chebula

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Figure 1: Antibacterial activities of the aqueous extract of Terminalia chebula upon addition of various concentrations of salts of Mn$^{2+}$ (A) and Mg$^{2+}$ (B) to enteric pathogens of interest. The analyses were done by an agar-diffusion assay where zones of inhibition, produced around the discs, soaked with the extract and salts, were measured in mm scale to record the antibacterial activities. Antibacterial activity of the extract, changed upon addition of 25 ng of manganese (C), or 0.5 ng of magnesium (D) salts, the amounts that produced the highest antibacterial activities, were compared with the extract alone.

The amounts of the salts that helped produce the highest antibacterial activities of the plant extract were compared with that of the activities of the extract alone, i.e. extract without added salts. It was revealed that the activities of the plant extract was increased from 25% (against E. coli) to 45% (against Salmonella sp) after addition of MnCl$_2$, whereas the MgCl$_2$ produced only about 7% (Salmonella) to 8% (Shigella) enhancement of antibacterial activities (Figure 2). Therefore, it can be concluded that MnCl$_2$ upon addition of a suitable dose could further increase the inherent antibacterial activities of the leaf extract of T. chebula.

Figure 2: Comparison of the activity enhancement (%) of aqueous extract of Terminalia chebula upon addition of MnCl$_2$ or MgCl$_2$ to that of the extract without added salts.
Discussion

In an earlier study, we revealed that the water extract of *Terminalia chebula* dried leaf powder demonstrated antibacterial activity against the enteric pathogens, e.g. *E. coli*, *Shigella* sp., *Vibrio cholerae* and *Salmonella* sp (Mostofa et al. 2011). Quantitatively, this activity was measured to have an MIC and MBC values fall in the range of 5,000 to 8,000 μg/ml (Mostofa et al. 2011). The current study addresses whether the supplementation of mineral elements in the plant extract augments its antibacterial activity to the aforementioned pathogens. We chose magnesium and manganese for this study, for they are essential mineral nutrients, present in both naturally and as a result of contamination in soils, sediments and water; and more importantly they participate in many enzyme systems as an enzyme activator and as a component of metalloenzymes in the body (Risk assessment, manganese 2003). We report that the addition of manganese salts (calculated about 80 μg per gram of the plant’s dried leaf powder, Mostofa et al. 2011) could significantly enhance further the biocidal activity of the aqueous leaf extract of *T. chebula* against the same group of pathogens, having a bacterial population of 10⁸ cfu/ ml. Hence, the dose of the salts for increased activity was calculated about 80 g per gram of the plant’s dried leaf powder.

As far as the safe limits of manganese in humans are concerned, The EU Scientific Committee for Food (SCF) considered a ‘safe and adequate intake’ to be 1-10 mg/person/day. The US National Research Council (NRC) specified Estimated Safe and Adequate Daily Intakes (ESADDIs) of 0.3-1, 1-3 and 2-5 mg/day for infants, children and adults respectively (Risk assessment, manganese 2003). The estimated manganese level required to boost the antibacterial activity of *T. chebula* fell well below the standard level, as reported in the current study, and therefore should be considered safe for human system.

Conclusion

The aqueous extract of the leaves of *Terminalia chebula* together with suitable addition of manganese salts (calculated about 80 g/ gm of dried leaf powder) could be an effective dose for further increase of bactericidal activity to enteric bacteria.

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