Antimicrobial Activity of Food Grade Glucosamine

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Abstract

Being naturally derived from the cellular glucose metabolism, glucosamine is present in the hyaluronic acid present in the synovial fluid and also the fluids that help in lubrication of the cartilage in the joint cavities. This monomer, derived from the shell-fish waste or shell-fish chitosan by hydrolysis, it is widely accepted as a dietary supplement by FDA with its increasing commercial potential as a nutraceutical in many countries including USA, European countries and etc. Present in variable commercial food grade forms like glucosamine hydrochloride, glucosamine sulphate, N-acetyl glucosamine, etc. this amino-sugar is reported to be an effective oral supplement.

The purpose of the current study is to assess the antimicrobial activity of glucosamine. The study takes into account the effect of antimicrobial activity at variable concentrations of glucosamine to evaluate the range at which glucosamine is able to exhibit its antimicrobial properties. Food grade glucosamine was dissolved in acetic acid at variable concentrations range with stock solution being 1mg/mL. The antimicrobial activity was assessed against four different bacterial strains namely Bacillus subtilis, Micrococcus luteus, Staphylococcus saprophyticus and Pseudomonas fluorescens. Disc diffusion assay was used to test the antimicrobial properties wherein it allows preliminary screening of antimicrobial agents and has been used for wide range of microbes. This study was able to demonstrate a new angle of this dietary supplement wherein it is able to provide resistance against particular microbes. The result of the study shows that glucosamine possess antimicrobial properties and can be further researched upon as a potential antimicrobial agent.
Keywords: Nutraceuticals, NAG, glucosamine hydrochloride, dietary supplement.

1. Introduction
Glucosamine (2-amino-2-deoxy-alpha-D-glucose) is a naturally occurring amino monosaccharide present in the synovial fluid of a healthy human cartilage. Its requirement in the body is met by normal cellular glucose metabolism, and is also a primary component for synthesis of various complex molecules in the cartilage matrix and other fluid matrixes which are responsible of lubrication of the cartilage joints in the joint cavities. Glucosamine is found in various tissues and secretions and is primarily a major substrate for macromolecule synthesis inhyaluronic acid which is a component of synovial fluid required in collagen formation (1, 2). This being the primary reason for its supplementation in various dietary forms and as nutraceuticals, glucosamine is widely marketed in United States, major parts of Europe and many other continents with a global market of >$2 billion as per 2009 attests (3). It is commercially extracted from the hydrolysis of shell-fish waste or shell-fish derived chitin/chitosan, which is a major component of crabs, shrimps and other shell-fishes. As a dietary supplement for arthritic conditions, particularly osteoarthritis, FDA approved glucosamine supplements are presently marketed in its three food grade forms namely; glucosamine hydrochloride (GLnC.HCl), glucosamine sulphate (GS) and N-acetyl-glucosamine (NAG) (4).

Chitosan being the parent molecule, along with chito-oligosaccharides possesses well reported antimicrobial effect which is reported to be greatly dependent on its degree of polymerization (DP). Chitosan with high degree of deacetylation (%DD) i.e. constituting higher glucosamine units, exhibit higher antimicrobial properties than the rest chitosan forms. It can be further hypothesized that, the antimicrobial activity of the polymer is notably dependent on glucosamine units (5, 6, 7). The present study is aimed to focus on the antimicrobial property of Glucosamine Hydrochloride (GLnC.HCl) on common food-borne spoilage microorganisms namely *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus saprophyticus* and *Pseudomonas fluorescens*.

2. Materials and Methods
2.1 Chemicals
Glucosamine Hydrochloride was procured from HiMedia Laboratories®, India. Glacial Acetic Acid, Luria-Broth and Bacteriological Grade Agar were obtained from CDH® (Central Drug House), India.

2.2 Cultivation of the microorganism strain
*Bacillus subtilis* (MTCC 1427), *Micrococcus luteus* (MTCC 106), *Pseudomonas fluorescens* (MTCC 2797) and *Staphylococcus saprophyticus* (MTCC 6155) were
obtained from Institute of Microbial Technology (IMTECH), Chandigarh. The stock cultures were then revived and inoculated in Luria-Broth Agar (LBA) plates and further maintained by subculturing. All 4 microorganisms were inoculated into 5ml Luria Broth (LB) each in test tubes and were cultivated at at 37°C in an orbital shaker at 150 rpm for 24 hours.

2.3 Disc Diffusion Assay
Kriby-Bauer Disc diffusion assay was performed to test the antimicrobial property, as it allows preliminary screening of the inhibitory effect of the antimicrobial agents of the sample (8, 9). Glucosamine Hydrochloride was dissolved in 1% Glacial Acetic Acid at a concentration of 1mg/ml and further diluted to test concentrations of 0.75, 0.5 and 0.25mg/ml. 200µL each of overnight grown bacterial cultures was spread on LBA plates. Filter discs were placed on the agar plates using sterile forceps. To each disc on a LBA plate, 30 µl of sample at variable concentration i.e. 1mg/mL, 0.75mg/mL, 0.5mg/mL, and 0.25mg/mL with 1% Glacial Acetic Acid as control was added onto each plate. The plates were then incubated in 37°C incubator for overnight (18 Hrs). The Zone of inhibitory growth (including disc diameter) for each disc on each plate was measured. The scale of measurement is as follows: ≥20mm zone of inhibition is strongly inhibitory; <20-12mm zone of inhibition is moderately and <5mm is no inhibitory. Values are presented as means ± SD of three parallel measurements.

3. Results
3.1 Zone of Inhibition
The diameter of zone of inhibition was measured from the edge of the ring with no bacterial growth was observed using a standardized ruler. Glucosamine like its parent molecule is sparingly soluble in water and was hence dissolved in 1% Glacial Acetic Acid solution. Antimicrobial property of Glacial Acetic acid is well reported (10) and cannot be ignored; being a constituent of the sample preparation.
Figure 1: Disc Diffusion Assay for antimicrobial property activity of Glucosamine Hydrochloride; (a) Zone of Inhibition for *Bacillus subtilis* (MTCC 1427), (b) Zone of Inhibition for *Micrococcus luteus* (MTCC 106), (c) Zone of Inhibition for *Pseudomonas fluorescens* (MTCC 2797), (d) Zone of Inhibition for *Staphylococcus saprophyticus* (MTCC 6155).

Its value was therefore subtracted from all the test component values. The results of the disc diffusion assay showing zones of inhibition for measuring the antimicrobial activity of Glucosamine Hydrochloride is shown in Fig.1 and detailed in Table 1.

Table 1: Antimicrobial activity of Glucosamine Hydrochloride against tested microorganisms using disc diffusion method.

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Control (1% GAA)</th>
<th>Zones of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.25mg/mL</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>5±0.32</td>
<td>6±0.72</td>
</tr>
<tr>
<td><em>Micrococcus luteus</em></td>
<td>8±0.4</td>
<td>10±0.51</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
<td>NI</td>
<td>8±0.43</td>
</tr>
<tr>
<td><em>Staphylococcus saprophyticus</em></td>
<td>NI</td>
<td>7±0.16</td>
</tr>
</tbody>
</table>

*NI means No inhibition.* Values are mean inhibition zone (mm) ± S.D (Standard Deviation) of three replicates. The experimental values have been calculated after deduction from their respective control values.

3.2 Antimicrobial Property
The results obtained showed some degree of potential antimicrobial property of Glucosamine Hydrochloride at all variable concentrations. It also concludes the
probable antimicrobial activity of Glucosamine Hydrochloride even at dilute concentration of 0.25mg/mL. The maximum inhibitory zone i.e. 30±0.71mm was observed against *Staphylococcus saprophyticus* (MTCC 6155) and minimum being against *Micrococcus luteus* (MTCC 106) i.e. 21±0.53mm at 1mg/mL concentration. Glucosamine Hydrochloride is observed to be a strong inhibitory antimicrobial agent at 1mg/mL concentration.

4. Discussion and Conclusion

Glucosamine Hydrochloride is well accepted as a dietary supplement in many countries particularly for individuals suffering from osteoarthritic condition. It is available in various aforesaid form is taken as tablet, capsule, caplets, powder or liquid and is reported to block the progression of cartilage degradation and likely to stimulate production of new cartilage. It is also being well reported for its usage as a nutraceuticals (3, 11).

The present study was able to demonstrate antimicrobial property of food-grade Glucosamine against four common food spoilage microorganisms and was observed to be a strong inhibitor to the microbial growth. Glucosamine being a major component of the parent molecule i.e. chitosan, exhibited antimicrobial properties which needs to be further validated. Possessing antimicrobial activity, this food grade monosaccharide can be further evaluated for its usage in various forms and needs to be further researched upon.

This study confirms that Glucosamine Hydrochloride possesses antibacterial activity. However, for it to be used for this property, issues of safety, toxicity, minimum inhibitory concentration and etc. need to be further addressed.

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References


