Antimicrobial Efficacy of Cinnamon Oil for the Production of Muffins

Naureen Naeem*, 1, Shoaib Ahmad Siddiqi2, Sayyed Zafar Hussain3, Hina Qaiser2 and Sidra Naseer4

1. Department of Home Economics, Lahore Garrison University, Lahore, Pakistan
2. Department of Biology, Lahore Garrison University, Lahore, Pakistan.
3. Department of Chemistry, Lahore Garrison University, Lahore, Pakistan.
4. Department of Food Science & Human Nutrition, University of Veterinary and Animal Sciences (UVAS), Lahore.
*Corresponding Author: Department of Home Economics, Lahore Garrison University, Lahore, Pakistan.
Email: naureen.naeem@lgu.edu.pk

ABSTRACT: There is a renewed interest in the antimicrobial properties of spice oil. Spice plants have been shown to possess medicinal significance. Cinnamon oil has high antimicrobial activity. Oil could act as preservatives by eliminating food-borne pathogens. Antimicrobial activity of the oil extracted from Cinnamomum verum was tested in vitro against four microbial species by following well diffusion assay. Two gram positive (Bacillus cereus, Staphylococcus aureus) and two gram negative (Escherichia coli, Salmonella sp.) were selected in the first phase of the research project. Minimum Inhibitory Concentration (MIC) ranges were calculated by micro-broth dilution method. During second phase, effect of oil was checked by adding it in different ratio in muffins. Muffins were incorporated with 0.2%, 0.4%, 0.6%, 0.8% and 1% of the oil. Sensory analysis of the muffins was assessed. Storage stability was checked by performing sensory evaluation with the interval of five days upto fifteen days. Data obtained from microbial and sensory analysis was evaluated through two way and one way analysis of variance, respectively at a confidence level of P≤0.05. The separation of means was done by applying Duncan Multiple Range test (DMRT). Stability test indicated superb preservative properties of the incorporated oil on muffins at ambient temperature.

Keywords: Cinnamon oil; Antimicrobial activity; Food spoilage; Natural preservatives

INTRODUCTION

Currently due to the increased susceptibility of fresh food towards the microbial stresses, it has become more important to develop certain techniques that can be effectively used against the fresh food spoilage microorganisms. Traditionally, spices have proved to be potential antimicrobial and antifungal agents. Thus we tried to evaluate the essential oil of different natural agents for its antimicrobial activity. The essential oil of aromatic plants and their components have a wide range of applications in ethnic medicine, preservation, food flavoring, fragrances and perfume industries (Ahmad et al., 2013). Therefore, considerable attention has been focused on the various biological effects of these naturally occurring agents. Among them is the cinnamon essential oil and its constituents which are known to possess various antifungal and antimicrobial activities.
Cinnamon, a spice, is commonly used in sweet and savory and baking. Its oil is commonly used in the food industry because of its aroma (Babu et al., 2011). Of all the available species of cinnamon Cinnamomum verum had been focused in this research. Cinnamon spice has extracts, oils (EOs), resins, cinnamic acid, cinnamate and cinnamonaldehyde. It has been revealed to impart antioxidant and strong antimicrobial effects in food products, in which active substances are phenolic constituents Raventos (2014). General composition of cinnamon bark includes 20.3% fiber, 59.5% carbohydrates, 9.9% moisture, 4.6% protein, 2.1% fat and 3.4% total ash (Ariamuthu et al., 2013). Spice oils have been shown to contain inhibitory activity against L. monocytogenes, C.botulinum, Staphylococcus spp., Micrococcus, Bacillus spp., Enterobacteriaceae, Salmonella and E. coli. They are generally more inhibitory against Gram-positive organisms than Gram-negative. While this is true of some spice oils that they are useful against both Gram positive and Gram negative groups (clove, cinnamon and citral). There are also some non-phenolic components of spice oil which are more efficient (allyl isothiocyanate) or quite effective against Gram-negative bacteria (Daud et al., 2013). Spice oil contains antimicrobial effect that has been screened as possible source of new antibacterial components. Some of these oils show activity against pathogenic organisms like S. aureus. Oils could act as preservatives, eliminating or decreasing the pathogenic bacteria and increasing overall quality of foodstuff (Mishra and Bihal, 2010). Important quality of spice oil and its different components is hydrophobicity, which allows them to make partition with lipids of cell membrane and mitochondria, damaging the cell structure and render them permeable. Escape of important cell constituents and ions due to more permeability leads to the death of the bacteria (Aruna and Baskaran, 2010). An Emerging body of data depicts that there is significant effect for use of spice in dietary stuff, especially application to bakery items, for improving the quality and nutritional value of foodstuff, in addition to their strong antimicrobial properties (Hoque et al., 2008). Oil of spices apply direct or indirect effects to increase the stability and quality of food items (Jauharah et al., 2014, Saranraj and Geetha, 2015). Pure oils are mixtures of more than two hundred components with nominal differences between compounds, contain volatile fraction (alcohols, aldehydes, monoterpenes) and non-volatile portion (hydrocarbons, carotenoids, flavonoids etc.) as well (Asghari et al., 2010; Elumalai et al., 2011).

The aim of this study is to assess the efficacy and antimicrobial activity of essential oil of cinamomum verum, in different concentrations against the food spoilage microorganism, two gram positive (Bacillus cereus, Staphylococcus aureus) and two gram negative (Escherichia coli, Salmonella sp.). Conclusively selection of best concentration of essential oil of cinamomum verum for the production of best quality muffins so that the results can be further used to make an effective food spoilage resistant spray/preservative.

**MATERIALS AND METHODS**

The present research project was carried out in two phases. During first phase antimicrobial efficacy of cinnamon oil was checked after extracting oil through Soxhlet...
apparatus. During second phase effect of oil was checked by adding it in different ratio in bakery product (muffins). These trials were conducted in the Department of Food Science & Human Nutrition, University of Veterinary and Animal Sciences (UVAS), Lahore.

10ml sterilized nutrient broth was taken in each test tube. Fresh bacterial cultures (E.Coli, Salmonella, B.cereus, S.aureus) were inoculated in nutrient broth and cultures were incubated at 37ºC for 24 hrs. Inoculum was standardized by using 0.5 McFarland solutions. The O.D. value of 0.5 MacFarland was found to be 0.1. Bacterial growth was determined in terms of optical density by taking absorbance at 600 nm (OD 600nm). The OD values of all 24 hours old bacterial cultures were taken and adjusted to 0.1 with the help of sterilized nutrient broth. (Elumalai et al., 2011)

**Agar Well Diffusion Method**

Oil of the spice was screened for its antimicrobial activity against four microorganisms. In order to determine the antimicrobial spectrum, antimicrobial activity was performed by using agar well diffusion assay (CLSI standards).

Sterile cotton swab was dipped in to the prepared inoculums and spread all over the nutrient agar plate by rotating through an angle of 60°. After each swabbing finally, the swab was passed round the edges of the agar surface and left to dry for few minutes at room temperature with lid closed. Then with the help of sterilized cork borer, wells were made in the inoculated plate and labeled as 10μl, 15μl, 20μl, 25μl. Prepared suspension of the spice oil was distributed in the respective wells with the help of the micropipette under sterilized conditions. Then the plates were incubated at 37ºC for 24 hr and zone of Inhibition was determined for different oil concentrations. Diameter of zone of inhibition (DIZ) was observed and shown as clear area in millimeters (Nazia and Parveen, 2006).

Three replications were maintained in each treatment.

**Micro-broth dilution Method**

Minimum Inhibitory Concentration (MIC) was determined for the organisms that were sensitive to the oil using a micro-broth dilution method. Micro-broth dilution method was used to determine the MIC of tested organism with little modifications. It was carried out in 96-well micro titer plates. The tested organisms were inoculated in nutrient broth and 18 hour old culture was selected for further dilutions after standardization with 0.5 McFarland. Stock solution of oil was prepared by adding 10% DMSO (Dimethyl sulfoxide) and 100μl oil. 100μl of nutrient broth was added in micro titer plates from well 1 to 12. 100μl from stock solution was transferred to first well and mixed properly. Then 100μl from first well was shifted to second and so on up to 11th well in order to make two fold dilutions, and 12th well was used as the negative control. Then 100μl standardized inoculums was added to each well from 1 to 12th. DMSO concentration never surpassed 10% (v/v). Micro titer plates were incubated at optimum growth temperature for each bacterial strain. Growth was monitored by calculating absorbance through ELISA reader at 630 nm. (El-Baroty et al., 2010). Three replicates were used for each oil concentration.
Antimicrobial Efficacy of Cinnamon Oil

Five treatments (T1-T5) were prepared in which different ratio of Cinnamon oil (0.2, 0.4, 0.6, 0.8 and 1%) were added to the recipe of muffins. Muffins were prepared from different treatments of flour and oil along with control according to the method (Yaseen et al., 2012). A batter was prepared in a bowl manually by mixing dry ingredients with the wet ingredients. Batter was poured in muffins tray grease with oil. Oven was pre-heated at 210 °C. Muffins were baked at 180 °C for 15-20 min. Samples were transported and stored at room temperature.

**Organoleptic Evaluation:** On the basis of concentration of oil, organoleptic evaluation and sensory acceptability one best treatment was selected along with the control and its storage stability was checked for a period of fifteen days with the interval of 5 days. Effect of oil on storage stability was examined by doing sensory evaluation after each five days. Data thus obtained from microbial analysis was evaluated through two way analysis of variance at a confidence level of P≤0.05. Data obtained from sensory analysis was evaluated through one way analysis of variance at a confidence level of P≤0.05. The separation of means or significant difference comparison was done by using DMR test (Steel et al., 1997).

**Results and Discussion**

The antimicrobial activity of *Cinnamomum verum*, was assessed on four food spoilage and water borne bacteria, *Staphylococcus aureus, Salmonella, Escherichia coli* and *Bacillus cereus*. Analysis of the effect of cinnamon oil at different concentrations was done after an incubation period of 24 hour. The zone of inhibition was measured for *Cinnamon verum* oil for the gram negative- *Salmonella, Escherichia coli* and gram positive *Bacillus cereus* and *Staphylococcus aureus* bacteria was calculated at different concentrations (Table1) .

Results showed maximum values for zone of inhibition by *Bacillus cereus* followed by *Staphylococcus aureus*. Comparing all the four results, *C. verum* was found to have a better antimicrobial activity at effective concentration of 0.6% and 0.8% (Table 1) clearly shows that among the four bacteria *Bacillus* was found to be most susceptible against the action of cinnamon oil while *Salmonella* to be least.

The results of this work were found to be consistent with the work done by (Ates and Erdogrul, 2003; Shan et al., 2007) who showed different effective concentration of essential oil of cinnamon against *Staphylococcus aureus* in another study (Nazia and Parveen, 2006) it was found that cinnamon oil was effective against *E. coli* (Dobre et al., 2011), Gupta and Garg (2008), who showed that the essential oil of cinnamon inhibit the growth of *Staphylococcus aureus* (Magetsari 2012). These findings are also quite similar with the results of Witkowska et al (2013) who reported that cinnamon bark oil completely inhibited the growth of some gram positive and gram negative bacteria, fungi and yeasts (Gende et al., 2008) and (Saraf et al., 2011). As the main component, cinnamaldehyde has proven to be particularly effective against some species of gram positive and gram negative bacteria (Friedman et al., 2000). It has been proposed that cinnamaldehyde and eugenol inhibit production of an essential enzyme by the bacteria and/or cause damage to the cell wall.
of the bacteria (Tajkarimi et al., 2010; Revati et al., 2013). Therefore, the high antimicrobial activity of cinnamon oil is due to the presence of the high amount of cinnamaldehyde and due to high antibacterial activity of C. verum ascertained by this study. Mean values for sensory evaluation of muffins by the addition of different concentration of cinnamon oil is presented in Table 2. Mean value of color was seen highest in 0.22% i.e. 13.22±1.49 and lowest in 0.8% and 0% 12.07±1.56 and 12.07±1.49 respectively. 0.2% and 0.4% were significant for texture parameter with each other. Similarly for texture 0.2% illustrated the highest value i.e. 13.22±1.04. However for taste, highest value was shown in 0.4% and 0.6%. Mean values for flavor and overall acceptability were seen highest in 0.2% i.e., 13.42±0.76 and 13.17±0.84. Similarly data for storage stability of muffins depicted highest score for 0.2% and 0.4% respectively for storage interval of 10 days (Table 3).

### Table 1: Zone of inhibition of different microflora at different concentrations

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Concentration of oil (%) v/v</th>
<th>Zone of Inhibition (mm)</th>
<th>Zone of Inhibition (mm)</th>
<th>Zone of Inhibition (mm)</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2.</td>
<td>0.2</td>
<td>23</td>
<td>28</td>
<td>24.33</td>
<td>29.67</td>
</tr>
<tr>
<td>3.</td>
<td>0.4</td>
<td>24.33</td>
<td>28.67</td>
<td>31.67</td>
<td>25.33</td>
</tr>
<tr>
<td>4.</td>
<td>0.6</td>
<td>27.33</td>
<td>28.33</td>
<td>35.67</td>
<td>25.67</td>
</tr>
<tr>
<td>5.</td>
<td>0.8</td>
<td>28.33</td>
<td>28.33</td>
<td>26.33</td>
<td>36.67</td>
</tr>
<tr>
<td>6.</td>
<td>1</td>
<td>27.38</td>
<td>28.69</td>
<td>24.37</td>
<td>29.65</td>
</tr>
</tbody>
</table>

### CONCLUSION

By the present study it can thus be concluded that *Cinnamomum verum* can be very successfully be used against the food spoilage bacteria *E.coli*. *Staphylococcus* bacteria are easily encountered in during food handling and treatment hence *C.verum* can also be employed for limiting the spread of these bacteria through handling or reducing their concentration at minimal damaging limit. The minimum concentration required for *C. verum* to act upon these spoilage bacteria was found to be 0.06 % v/v. Such a small concentration can be easily imparted in food products like apple juice (spoiled by *E.coli*), flavored milk (spoiled by *Pseudomonas aeruginosa*) and bakery products (cakes and muffins) to inhibit the spoilage.
Table 2: Sensory evaluation scores of cinnamon oil added muffins

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Color</th>
<th>Texture</th>
<th>Taste</th>
<th>Aroma</th>
<th>Flavor</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>12.07±1.49</td>
<td>12.68±1.02</td>
<td>11.83±1.16</td>
<td>11.58±1.03</td>
<td>12.13±1.13</td>
<td>12.42±1.29</td>
</tr>
<tr>
<td>0.2%</td>
<td>13.22±1.04</td>
<td>13.48±0.60</td>
<td>12.9±1.05</td>
<td>12.8±1.02</td>
<td>13.42±0.76</td>
<td>13.17±0.84</td>
</tr>
<tr>
<td>0.4%</td>
<td>13.05±0.90</td>
<td>12.18±0.88</td>
<td>12.18±1.16</td>
<td>11.77±1.05</td>
<td>12.07±1.25</td>
<td>12.66±1.36</td>
</tr>
<tr>
<td>0.6%</td>
<td>12.87±1.33</td>
<td>12.5±0.98</td>
<td>12.18±1.07</td>
<td>11.56±1.09</td>
<td>12.05±1.17</td>
<td>12.47±1.49</td>
</tr>
<tr>
<td>0.8%</td>
<td>12.07±1.56</td>
<td>12.39±1.22</td>
<td>11.37±0.95</td>
<td>11.33±1.37</td>
<td>11.68±1.28</td>
<td>11.82±1.29</td>
</tr>
<tr>
<td>1%</td>
<td>12.05±1.56</td>
<td>12.49±1.22</td>
<td>11.45±0.95</td>
<td>11.4±1.37</td>
<td>11.38±1.28</td>
<td>11.42±1.29</td>
</tr>
</tbody>
</table>

Table 3: Effect of different time intervals and concentrations of Cinnamon oil on sensory attributes of muffins

<table>
<thead>
<tr>
<th>Sensory Attributes</th>
<th>TIME INTERVAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Day</td>
</tr>
<tr>
<td></td>
<td>T1</td>
</tr>
</tbody>
</table>

* T1:0.0%,  T2:0.2%,  T3:0.4%,  T4:0.6%,  T5:0.8%,  T6:1%
REFERENCES


