Evaluation of ginger extract’s yield, using cold extraction method and its antimicrobial activity against pathogens

Bikram Gautam¹ ²*, Richa Nepal¹, Rupa Bhandari¹ and Shishir Gyawali¹

¹Department of Microbiology, St. Xavier’s College, Maitighar, Kathmandu, Nepal
²Research Center for Applied Science and Technology, T.U., Kritipur, Nepal

*Corresponding author: Bikram Gautam, Department of Microbiology, St. Xavier’s College, Maitighar, Kathmandu, Nepal. E-mail: gautambikr@gmail.com

Received: 07 June 2018/Accepted: 18 February 2019/ Published: 31 March 2019

Abstract: Ginger is commonly used herb across the world either in a meal or in herbal products. The chemical constituents of ginger possess antioxidants, can modulate apoptosis, inhibit vascular endothelial growth factor and inhibit inflammatory reactions. The main aim of this study is to assess the antimicrobial activity of the ginger extracts. For this purpose, ginger rhizome (R), packaged ginger powder (P) and ginger leaves (L) were collected from Urlabari, Morang and Kathmandu, Nepal. The plant materials were first minced (except P) and then extracted using cold extraction technique. For assessing the antimicrobial activity of the extracts against the American Type Culture Collection (ATCC) strains, cup well method was preferred. The percentage yield of extracts R, P, L and ginger rhizome’s juice (J) was 10.79 ± 0.03 %, 9.76 ± 0.16 %, 8.17 ± 0.07 % and 16.8 ± 1.98 % respectively. The extract R were found to susceptible against the pathogens Streptococcus pneumoniae, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus and methicillin resistant Staphylococcus aureus (MRSA). The extract P were found to susceptible against the pathogens E. coli, S. aureus and MRSA. The methanol extract of L was found to be susceptible to S. aureus, K. pneumoniae and MRSA. All the extracts (R, P, L) were resistant against Pseudomonas aeruginosa. All 6 bacterial isolates were resistant against the extract J. The extract shows antimicrobial property only when the extract is concentrated. The ginger leaves also have antimicrobial property.

Keywords: ginger; extract; antimicrobial activity; yield

1. Introduction
Ginger (Zingiber officinale) belongs to family Zingerberaceae) is a horizontal, branched, fleshy, aromatic white to yellow colored perennial herb with a leafy stem (Rahmani, 2014) up to 60 cm with narrow leaves (Malu et al., 2009) 20 cm long and 1.5-2cm wide, has long been used in the field of medicine (Chan et al., 2008, Mashhadi et al., 2013). It is rich in phenolic compounds (gingerol, paradol and shogaol), volatile sesquiterpenes (zingiberene and bisabolene) and monoterpenoids (curcumin and citral) (Sadigic et al., 2003; Mashhadi et al., 2013; Rahmani, 2014; Kafeshani, 2015). Since ancient times ginger has been used all over the world as herb (Mashhadi et al., 2013), and even recently it has used for complications like diabetes (Mahluji et al., 2013; Mashhadi et al., 2013; Rahmani, 2014; Shidfar et al., 2015), cancer (Rhode et al., 2007; Chan et al., 2008; Mahluji et al., 2013; Mashhadi et al., 2013; Rahmani, 2014; Riaz et al., 2015; Shidfar et al., 2015) (as its contents like superoxide anion, hydroxyl radicals and gingerol aid in modulating genetic pathways by activating tumor suppressor gene), renal problems (Kafeshani, 2015), modulating apoptosis (Liu et al., 2012) (terpenoids have been found to induce apoptosis in endometrial cancer cells through the activation of p53), inhibit vascular endothelial growth factor (Rhode et al. 2007, Rahmani 2014), inflammatory responses (Mueller et al., 2010; Mashhadi et al., 2013; Rahmani, 2014; Riaz et al., 2015), anti-inflammatory (Tjendraputra et al., 2001; Mueller et al., 2010; Mashhadi et al., 2013; Riaz et al., 2015) (such as inhibition of COX) and inhibition of nuclear
factor kB (Grzanna et al., 2005; Rhode et al., 2007; Mueller et al., 2010; Mashhadi et al., 2013; Rahmani, 2014) properties. Similar to antibiotics, the antimicrobial activity of these compounds depends on type and composition of the spice(s), amount used, type of microorganism, the composition of the food, pH value and temperature of the environment (Sagdic et al., 2003). Herbs contains thermolabile constituents which requires effective extraction techniques with high yield. Several studies relating to extraction exists to date but none with cold extraction method. This study focuses on yield, yield percentage and the extracts (R, P, L and J) antimicrobial activity against pathogens. This data will also shine light on the myth that food containing ginger has surplus amount of antimicrobial compounds. The aim of the study was to analyze the antimicrobial activity of ginger extract against pathogens.

2. Materials and Methods

2.1. Collection of plant materials, microorganisms and investigation

Local ginger (along with leaves) and packaged ginger powder were purchased from Morang and Kathmandu respectively. American Type Culture Collection (ATCC) stocks of the bacterial isolates were acquired from Institute of Medicine (IOM), Maharajgunj and National Public Health Laboratory (NPHL), Teku; Kathmandu (Table 1). The study was conducted at Microbiology laboratory, Department of Microbiology, St. Xavier’s College, Maitighar, Kathmandu, Nepal during the period of November to December, 2016.

Table 1. Ginger extracts and microorganisms used in the study.

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Extract</th>
<th>Bacteria Gram positive</th>
<th>Bacteria Gram negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R</td>
<td>S. pneumoniae ATCC 6303, S. aureus ATCC</td>
<td>P. aeruginosa ATCC 27853, E. coli ATCC</td>
</tr>
<tr>
<td>2</td>
<td>P</td>
<td>25923, MRSA (obtained from NPHL)</td>
<td>25922, K. pneumoniae ATCC 700603</td>
</tr>
<tr>
<td>3</td>
<td>L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>J</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.2. Extraction of plant material
The plant materials (R and L) were washed with clean water and allowed to air dry. The outer covering of tumor was manually peeled off. Both the R and L were sliced into small pieces. The materials were placed in a hot air oven for drying at a temperature of 65°C for 48 hours. Then the dried samples were minced in a grinder.

2.3. Extraction
Weighed 10g of R, P and L were placed in separate sterilized conical flask and 250ml of absolute methanol. All the conical flasks were placed in the shaker incubator at 30°C at 160 rounds per minute for four days for cold extraction. After four days of cold extraction, the contents of the flasks were vigorously stirred with a glass rod and the mixture was filtered using Whatmann No. 1 filter paper followed by 0.45µm cellulose membrane filtration. The precipitates were discarded and the filtrate was collected for evaporation. The filtrate was poured in the crucibles, placed at 37°C water bath for the evaporation dryness and was monitored for 3 days. Fresh juice extract (J) was also acquired from the ginger using sterile mortar and pestle. These recovered extract of R, P, L and J were stored in a refrigerator at 4°C. The yields % was calculated using the formula:

\[
\text{Yield} \% = \frac{\text{weight of extract (g)} \times 100}{10 \text{ (g)}}
\]

2.4. Sterilization of materials
The extracts were exposed to ultraviolet rays for 24 hours. The sterility was checked by streaking the extracts on a nutrient agar plate and incubated at 37°C for 24 hours (Gautam et al., 2017a; Gautam et al., 2017b; Gautam et al., 2018).

2.5. Screening the extracts for antibacterial activity
The antimicrobial activity of different extracts were determined by cup well diffusion method (Gautam et al., 2017a). The bacterial suspension in nutrient broth was tallied with McFarland standard 0.5 and lawn culture of the test organisms were made on the Mueller Hinton agar plates using a sterile cotton swab. The plates were dried for 15 minutes and wells were made using 4mm sterile borer (Gautam et al., 2017a). Using sterile micropipette 10µl extract (R, P, J and L) were introduced into each of the wells along with control (sterile
distilled water). The plates were incubated at 37°C for 24 hours (Gautam et al., 2017a; Gautam et al., 2017b; Gautam and Adhikari, 2018a; Gautam and Adhikari, 2018b; Gautam et al., 2018; Gautam et al., 2019). The antimicrobial activity was evaluated by measuring the diameter of zones of inhibition (mm).

2.6. Quality control
A sample was triplicated and was repeated 4 times in an interval of a week. Purity plating was performed for the media plates and equipment were calibrated. The sterility was checked by streaking the extracts on a nutrient agar plate. ATCC stocks were confirmed using biochemical tests and antibiotic susceptibility tests as per Clinical & Laboratory Standards Institute (CLSI) guidelines. Statistical analysis was done using SPSS version 19.

3. Results
The 3 materials (R, P, L and J) were subjected to cold extraction using methanol. The yield of extract of R, P, L and J were 1.079 ± 0.003 g, 0.976 ± 0.016 g, 0.817 ± 0.007 g and 1.68 ± 0.199 g respectively. The percentage yield of R, P, L and J were 10.79 ± 0.03 %, 9.76 ± 0.16 %, 8.17 ± 0.07 % and 16.8 ± 1.98 % respectively. This data is presented in Table 2.

Table 2. Solvent used for extraction along with yield and yield percentage.

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Initial weight (g)</th>
<th>Extract</th>
<th>Solvent used</th>
<th>Yield (g)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>R</td>
<td>Methanol</td>
<td>1.079 ± 0.003</td>
<td>10.79 ± 0.03</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>P</td>
<td></td>
<td>0.976 ± 0.016</td>
<td>9.76 ± 0.16</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>L</td>
<td></td>
<td>0.817 ± 0.007</td>
<td>8.17 ± 0.07</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>J</td>
<td></td>
<td>1.68 ± 0.199</td>
<td>16.8 ± 1.98</td>
</tr>
</tbody>
</table>

The methanol extract of R was found to be susceptible towards the 5 bacterial isolates except for *P. aeruginosa*. The methanol extract of P was found to be susceptible to *E. coli, S. aureus* and MRSA. The methanol extract of L was found to be susceptible to *K. pneumonia, S. aureus* and MRSA. All 6 bacterial isolates were found to be resistant towards J. The data is presented in Table 3 and Figure 1 and 2.

Table 3. Antimicrobial effect of the extracts against the pathogens.

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Isolates</th>
<th>Zone of inhibition (ZOI) of extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P</td>
</tr>
<tr>
<td>1</td>
<td><em>S. pneumoniae</em></td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td><em>P. aeruginosa</em></td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td><em>K. pneumoniae</em></td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td><em>E. coli</em></td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td><em>S. aureus</em></td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>MRSA</td>
<td>+</td>
</tr>
</tbody>
</table>

+++ = ZOI greater than 20 mm, ++ = ZOI 10-20 mm, + = ZOI less than 10 mm, - = resistant

![Figure 1. Antimicrobial effect with ZOI of the extracts (R, P and L) and J against pathogens.](image-url)
Figure 2. Antimicrobial activity screening against MRSA.
Ginger extracts R, P, L were potent against MRSA while MRSA is resistant towards J.

Figure 3. Antimicrobial activity screening against S. aureus.
Ginger extracts R, P, L were potent against S. aureus while S. aureus is resistant towards J.

4. Discussion
The yield of an extract of R, P, L and J were 1.079 ± 0.003 g, 0.976 ± 0.016 g, 0.817 ± 0.007 g and 1.68 ± 0.199 g respectively. The percentage yield of T, P, L and J were 10.79 ± 0.03 %, 9.76 ± 0.16 %, 8.17 ± 0.07 % and 16.8 ± 1.98 % respectively (Table 2). The methanol extract of R was found to be susceptible towards the 5 bacterial isolates except for P. aeruginosa (Figures 1-3). The methanol extract of P was found to be susceptible to E. coli, S. aureus and MRSA (Figures 1-3). The methanol extract of L was found to be susceptible to K. pneumonia, S. aureus and MRSA (Figures 1-3). All 6 bacterial isolates were found to be resistant towards J (Table 3 and Figures 1-3). The results (Table 3 and Figures 1-3) indicated that extracts (R, P and L) of ginger have antibacterial activity with a variable degree of susceptibility against the isolates. Ginger extracts (R, P, L and J) did not show response towards P. aeruginosa. The antibacterial activities of the extracts might be due to the compounds like flavonoids, volatile oil, dissolved organic solvents like sesquiterpenes (Malu et al., 2009; Rahmani, 2014; Riaz et al., 2015). The results of this study harmonize with the results of Malu et al. (2008) which also reached the conclusion, where aqueous extract didn’t exhibit the antimicrobial effect. The study of this result coincides with the study of Riaz et al. (2015) where methanol extract was sensitive towards S. aureus and E. coli. Fresh ginger juice did not show its activity towards the selected microorganisms. This might be due to the high content of water and scanty concentration of antimicrobial compounds in the extract. Phytochemical with the antimicrobial property is more concentrated in the root than in the leaves as the nutrients might get accumulated there (Pandotra et al., 2015). The composition of a ginger or other herbal products varies according to nutrients available (Pandotra et al., 2015; Gautam et al., 2018), soil composition (Pandotra et al., 2015), stage of development of the plant material (Pandotra et al., 2015), climate and the state of rhizomes either dry or fresh (Elvin-Lewis, 2001). The component of the extract may bind to the other components of the glassware or equipment and even prevent biofilm formation (Quave et al., 2008; Sandasi et al., 2010). Based on Table 3 and Figure 1-3, it is clear that ginger used in meals, ginger ale, tea etc. contains a low concentration of the antimicrobial compounds but when cooked with alcoholic beverages like wine, rum, beer etc. the ethanol contained in them may dissolve these antimicrobial compounds (Auta et al., 2011). Using appropriate techniques the phytochemicals can be extracted from the ginger and can be used in herbal products like medicines (Sloand and Vessey, 2001; El-Ghorab et al., 2010) (toothpaste, allopathic etc.). Extract of herbal medicines are subject to degradation and decomposition on storage due to the volatile nature of the active ingredients (El-Mahmood and Doughari, 2008) and can even require preservatives (Gautam et al., 2017a; Gautam et al., 2017b) to increase the longer shelf-life.

5. Conclusions
Thermolabile components of the ginger can be extracted through this technique. The ginger leaves also have antimicrobial compounds. The extracts show antimicrobial property only when the extracts are concentrated (T, P, L).

Acknowledgements
The authors are thankful to Mr. Sudhakar Pant, HOD, Department of Microbiology, St. Xavier’s College for providing the laboratory facilities. The authors are also thankful to Ms. Ganga Shrestha and Mr. Prakash Manandhar.
None to declare.

References


Riaz H, A Begum, SA Raza, ZMUD Khan, H Yousaf and A Tariq, 2015. Antimicrobial property and phytochemical study of ginger found in local area of Punjab, Pakistan.