Comparative analysis of antimicrobial activity of petroleum ether extract with other different extracts of *Syzygium aromaticum* (Linn.) against food borne pathogens

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**Abstract**
The objective of the study was to cure commillimeteron respiratory tract and digestive system diseases. So, by characterizing the antimicrobial estimation of prepared different extracts (aqueous, petroleum ether, chloroform and ethanol) of medicinal plant named as *Syzygium aromaticum* (Linn.), commillimeteronally known as clove which acted against food borne pathogens (*E. coli, K. pneumoniae, S. aureus* and *S. pneumoniae*) by agar diffusion susceptibility test that revealed inhibition zone against microbes growth. Petroleum ether extract showed highest zone of inhibition against *Staphylococcus aureus* with all concentrations that is also verified by previous investigations. From petroleum ether extract of selected plant with less concentration could develop a new drug for inhibiting pathogens growth and combat diseases in small children as well in adults, old people.

**Citation:**

1. Introduction

Next, to the air we breathe and the water we drink, food has been basic to our existence. Food regulates the body process. Thus, food has many physiological functions to play (Alex. V. Ramani, 2009). Microorganisms can be detrimental to foodstuff when they cause food spoilage leading to heavy economic loss in the production phase or in the consumption phase (Vijayaramesh, 2007). Therefore, the demand for plant based therapeutics has increased. Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens (Ahmed e. al., 2001) because plants are the source of energy for animal kingdom. In addition, plants can synthesize a large variety of chemical substances which have their physiological importance (Kretovich 2005). *Syzygium aromaticum* (Linn.) cloves the aromatic dried flower buds of a tree in the family Myrtaceae (Srivastava and Malhotra, 1991 and Chaieb et al., 2007a) cloves are used in Ayurveda, Chinese medicine and western herbalism. In addition, the cloves are antimutagenic (Miyazawa and Hisama, 2003), anti-inflammillimeterary (Kim et al., 1998), antioxidants (Chaieb et al., 2007b), antiulcerogenic (Bae et al., 1998 and Li et al., 2005), antithrombotic (Srivastava and Malhotra, 1991) and antiparasitic (Yang et al., 2003).
2.1 Iqbal Ahmed; Arma Z Beg (2001) studied antimicrobial and phytochemical studies on 45 Indian medicinal plants against human pathogens were demonstrated the active constituents present in ethanolic extract of *Syzygium aromaticum* (Linn.) plant inhibited the pathogens.

2.2 Ram Kumar Pundir et al., 2010 studied antimicrobial activity of *Syzygium aromaticum* (Linn.) against food associated bacteria where the growing concern about food safety has recently led to the development of natural activity against food borne and control spoilage microorganisms.

2.3 Nazrul S.K. et al., 2011 demonstrated antimicrobial activity of *Syzygium aromaticum* (Linn.) extracts including petroleum ether, chloroform and ethanol tested against health hazardous microbes and reported strong inhibition for microbes.

3. Material and Methods

3.1 Objective of Research
The present study was planned to assess the antimicrobial properties of petroleum ether, chloroform, ethanol and aqueous extract of *Syzygium aromaticum* (Linn.) on food borne pathogens. To find out the inhibition of pathogens it is important to start with less concentration. In this investigation Disc Diffusion Method is applied for estimating antimicrobial activity which is easy to employ and could find the measurement of zones for pathogens with the potential of plant extract.

3.2 Collection of sample
Plant material of *Syzygium aromaticum* (Linn.) or Clove buds is used in this study was collected from Provision market, Usman road, T. nagar, Chennai-17, India dated on 25. October.2012 and authenticated by Mrs. Prema sambath and Vice Principal of Plant Biology and Plant Biotechnology Department from Ethiraj college for Women, Egmore, Chennai:

3.3 Extraction
The dried buds of *Syzygium aromaticum* (Linn.) were homogenised to a fine powdered and stored in airtight bottle.

3.4 Preparation of aqueous extract
50g of fine powdered *Syzygium aromaticum* (Linn.) were mixed with 250ml of distilled water and boiled in a low flame for 2 hours. The extract was then filtered and used.

3.5 Preparation of petroleum ether, chloroform and ethanolic extract
20g of powder of *Syzygium aromaticum* (Linn.) were extracted with 250ml of 80% of petroleum ether, 90% of chloroform and 40% of ethanol in a flask of soxhlet apparatus for 3 hours respectively. After that the extract was concentrated in rotator vacumm evaporation with temperature ranging from 30° C -40° C.

3.6 Antimicrobial screening
Screening for antimicrobial activity was done by the agar disc diffusion method.

3.7 Pathogens tested for antimicrobial activity:
**Test strains:**
The strains of food borne pathogens which categorized as gram negative bacteria and gram positive. The lyophilized cultures were cultivated in the Department of Microbiology, Asan Memorial college of Arts and Science (AMCAS), Chennai-100.

3.8 Food borne pathogens
(gram negative)
*Escherichia coli*
*Klebsiella pneumonia*
(gram positive)
*Staphylococcus aureus*
*Streptococcus pneumonia*

3.9 Media for test organisms
33.6g of Muller Hinton Agar was added to 90ml of sterile distilled water and autoclaved at 121° C for 15 minutes at 15lbs. 1.0g of dextrose was added to 10ml of sterile distilled water and steam sterilized for 15 minutes. After cooling both the content was mixed and poured into sterile petriplates approximately 4millimeter and allowed to set at ambient temperature and used.

3.10 Inoculum
The microorganisms were inoculated in Nutrient broth and incubated at 37° C for 4 hours and this was used as inoculum.

3.11 Antimicrobial activity by agar disc diffusion method
This method (Kirby Bauer et al., 1966) is suitable for organism that grows rapidly over night at 35° C - 37° C. The antibiotic (specific concentration) impregnated disc absorbs moisture from the agar and antibiotic diffuses into the agar medium. The rate of extraction of the antibiotic from the disc is greater than the rate of diffusion. As the distance from the disc increases there is as logarithmic reduction in the antibiotic concentration. Zone of inhibition
of microbial growth around each disc is measured and the susceptibility measured.

3.12 Procedure
A sterile cotton swab was inserted into the microbial suspension and then rotated and compressed against the wall of the test tube so as to squeeze out the excess fluid. The surface of the agar plate was inoculated with the swab. To ensure that the growth is uniform and confluent (or semi confluent growth) the swab is passed three times over the entire surface. Sterile disc of 5 millimeter in diameter were impregnated with 25µl of different concentration (200mg, 400mg, 600mg, 800mg) of the each extracts were prepared using Dimethyl Sulfoxide: Methanol (1:1) solvent to dissolve the plant extract and then placed on the inoculated agar surface using sterile forceps. A standard disc containing tetracycline 10 mcg/disc were used as reference controls and disc with DMSO: Methanol (1:1) was used as vehicle control. All the petriplates were sealed with sterile laboratory parafilm to avoid eventual evaporation of the test samples. The plates were left for 30 minutes at room temperature to allow the diffusion of extract and then they were incubated at 37° C for 24 hours.

After the incubation period the zone of inhibition was measured.

4. Results

4.1 Effect of petroleum ether extract
Petroleum ether extract of *Syzygium aromaticum* (Linn.) against *Escherichia coli* and *Klebsiella pneumoniae* organisms ranges from 9 millimeter to 20 millimeter, for *Staphylococcus aureus* ranging from 14 millimeter to 22 millimeter at 200, 400, 600, 800 milligram concentrations respectively whereas against *Streptococcus pneumoniae* shows 4 millimeter to 7 millimeter which is lesser than other microbes at same concentration of petroleum ether extract. All extracts were compared with Tetracycline as positive control and solvent dimethyl sulfoxide as negative control.

4.2 Effect of chloroform extract
Chloroform extract of buds of *Syzygium aromaticum* (Linn.) against food borne and respiratory organisms ranges from 4 millimeter to 20 millimeter whereas for *Escherichia coli* showed highest inhibition from 20 millimeter to 23 millimeter diameter of zone of inhibition at 200 mg, 600 mg and 800 mg and effect of chloroform extract against *Streptococcus pneumoniae* shows 7 millimeter, 9 millimeter and 10 millimeter zone of inhibition which is slightly less than other used microorganism.

4.4 Effect of ethanolic extract
The effect of ethanolic extract of *Syzygium aromaticum* (Linn,) significantly inhibited the chosen microbes (*Escherichia coli* and *Klebsiella pneumoniae*) at 200, 400, 600, 800 milligram concentration ranging from 2 millimeter to 10 millimeter respectively. Against *Staphylococcus aureus* and *Streptococcus pneumoniae* showed slightly less degree of inhibition ranging from 2 millimeter to 6 millimeter with the same above mentioned concentration.

4.4 Effect of aqueous extract
The aqueous extract of *Syzygium aromaticum* (linn) against *Staphylococcus aureus* showed the highest inhibition of 11 millimeter with the concentration 200 milligram. Whereas *Streptococcus pneumoniae* ranged from 3.0 to 5.3 millimeters showing the highest inhibition at 800 milligram of plant extract.Antimicrobial activity of aqueous extract of *Syzygium aromaticum* (linn) against food borne organisms *Escherichia coli* ranged from 5 to 10 millimeter which was slightly high compared to *Klebsiella pneumoniae* the chosen other food borne microorganisms

5. Figure and Table for Petroleum ether extract of *Syzygium aromaticum* (linn.) against all pathogens.

**Figure 5.1:** Zone of inhibition formed by petroleum ether extract against *S. aureus*
Table 5.2: Effect of Petroleum ether extract of *Syzygium aromaticum* (Linn.) against pathogens

<table>
<thead>
<tr>
<th>Name of test organisms</th>
<th>Zone of inhibition (mm) Mean ± standard deviation</th>
<th>Positive control vehicle control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200mg</td>
<td>400mg</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>3.6± 0.94</td>
<td>11± 0.0</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>13.3 ± 4.7</td>
<td>13.6± 5.8</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>21.3± 0.44</td>
<td>21 ± 4.24</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>8.3 ± 0.44</td>
<td>9.6± 2.3</td>
</tr>
</tbody>
</table>

6. Discussion

The present study has been undertaken to evaluate the extracts of *Syzygium aromaticum* (Linn.) for its antimicrobial properties.

6.1 In the present study petroleum ether extracts of part of the plant exhibited strong activity against the selected food borne and respiratory pathogens. The petroleum ether and chloroform extracts of leaf had strong inhibitory effect against all the chosen pathogens than aqueous and ethanol extracts.

The study reported strong antimicrobial activity for all the four extracts in general petroleum ether and chloroform extracts as comparatively strong and slightly less inhibitory effects in ethanol and aqueous extracts against various pathogens.

6.2 Similarly, in another study of clove was found active against food borne, gram positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis* and *Listeria monocytogenes*) gram negative bacteria (*E. coli*, *Yersinia enterocolitica*, *Salmonella choleraesuis* and *P. aerugenosa*) (Lopez et al., 2005).

6.3 It has also been reported that the extract of clove potentially inhibited the growth of *Helicobacter pylori* (Bae et al., 1998; Li et al., 2005). In a study carried out by Betoni et al., (2006) clove extract showed inhibitory effect against *Staphylococcus aureus*.

6.4 In the previous study petroleum ether extract and aqueous extract shows moderate inhibition potential against bacteria suggesting that the plant extracts were bacteriostatic at lower concentration but bactericidal at higher concentration (Maji S et al., 2010).

6.5 Declaration: All these mentioned studies in discussion is verification of petroleum ether and other extract of *Syzygium aromaticum* (Linn.) against similar type of pathogens.

7. Summary

7.1 Food safety
It’s a major focus for human health as well as for animal and environment. Pathogenic bacteria, viruses and toxins produced by microorganisms are all possible contaminants of food.

7.2 Symptoms of food poisoning
It depends upon the specific type of food spoilage, the amount of infectious microorganisms ingested, age, medical history and other factors. Typical symptoms of food poisoning include diarrhea, nausea and vomiting with abdominal pain or abdominal cramps. Other symptoms of food poisoning can include diarrhea, chills, fever, muscle weakness blurred vision, dilated pupils, and . Symptoms in infants can also include a weak cry, lethargy, and weak sucking.

7.3 Importance of selected plant
Therefore, the present study has been undertaken to evaluate the extracts of *Syzygium aromaticum* (Linn.) for its antimicrobial properties. Plant extracts have been used for many thousands of years in pharmaceuticals alternative medicines and natural therapies. In vitro studies in this work showed that all the three extracts of plant inhibited bacterial growth but their effectiveness vary. The antimicrobial activity of many plants extracts have been previously studied and classified as strong, medium and weak. In the present study petroleum ether extracts of part of the plant exhibited strong activity against the selected food borne and respiratory pathogens. The petroleum ether and chloroform extracts of leaf had strong inhibitory effect against all the chosen pathogens than aqueous and ethanol extracts. The study reported strong antimicrobial activity for all the four extracts in general petroleum ether and chloroform extracts as comparatively strong and slightly less inhibitory effects in ethanol and aqueous extracts against various pathogens.
7.4 Comparison of four extracts on pathogens.
Antimicrobial activity of four extracts of buds of Syzygium aromaticum (Linn) against four pathogens (Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae and Streptococcus pneumoniae). The four extracts selected aqueous, ethanolic, chloroform and petroleum ether. Screening against bacteria was done at four different concentrations 200 mg, 400 mg, 600 mg and 800 mg using agar disc diffusion method. Out of the four different concentration of plants extracts activity against four bacterial species. E. coli, K. pneumoniae and S. aureus were inhibited effectively by all the four extracts. Moderate effects were seen in K. pneumoniae for ethanolic and aqueous extracts. Whereas petroleum ether extract showed significant inhibitory effect for these organisms. Comparatively plant extracts were less active against Streptococcus pneumoniae.

Research Highlights
- Use of Natural product, easily available.
- Easy to handle equipments with precautions.
- Could finish within decided time.
- Valid result.
- Trials on living organisms.

Limitations
Syzygium aromaticum (Linn.) plant extract concentrations should be less around 200-400 mg otherwise it may cause gastric problem and with higher concentration it will not show strong effect against food spoiling microbes.

Recommendations
My recommendation on this research is if there is natural way for taking care of health then we could try it without taking any risk of any harmful effect.

Conclusion
From the present study, petroleum ether and chloroform extract have the most potential antimicrobial activity. However ethanolic and aqueous extract was found to be inhibiting Staphylococcus aureus but slightly less inhibition for Escherichia coli. Comparison with tetracycline showed 22 millimeter which is nearer inhibition zone of petroleum ether extract against S. aureus. No inhibition zone formed in solvent that could be evidence to prove the plant extract possesses antimicrobial property not DMSO solvent.

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