

Comparison between Antibacterial Effects of Ethanolic and Isopropyl: Hexan (7:3) Extracts of *Zingiber officinale* Rose

Tahereh Naji, and Mahsa Jassemi

Abstract—In this investigation, the antibacterial effects of ethanolic and 7:3 isopropyl –hexane mixture extracts of *Zingiber officinale* were evaluated against three Gram positive bacteria, *B. cereus*, *S. epidermidis*, *S. aureus* and three Gram negative bacteria, *E. coli*, *K. pneumoniae* and *P. aeruginosa*. Utilizing paper disk diffusion and well methods in-vitro, MIC and MBC were determined by macrodilution. The results showed that ethanolic rhizome extract of ginger had significantly active than Isopropyl –hexan extract. Further work needs to be done in these extracts including fractionation to isolate active constituents and subsequent pharmacological evaluation.

Keywords—Antibacterial, Medicinal plant extract, *Zingiber officinale*.

I. INTRODUCTION

MICROBIAL activity is a primary mode of deterioration of many foods and is often responsible for the loss of quality and safety [1]. Concern over pathogenic and spoilage microorganisms in foods is increasing due to the increase in outbreaks of food borne disease [2-3-4].

Currently there is a growing interest to use natural antibacterial compounds, like plant extracts of herb and spices for the preservation of foods, as these possess a characteristic flavor and sometimes show antioxidant activity as well as antimicrobial activity [5].

Strategies for reduction of food –borne illness include methods at preharvest at the processing stage and at the consumer level. At the processing stage, antimicrobial food preservatives may be added to assist in inactivating viable pathogens or preventing growth of contaminating pathogens during subsequent storage of food. Much interest has centered on utilization of plant –derived compounds as antimicrobials in foods [6]

Ginger is a perennial, creeping plant, on thick tuberous rhizome, producing on erect annul stem 60 – 120 cm tall [7]. Ginger (*Zingiber officinale* Rose) has a long history of medicinal use dating back 2500 years in China and India for conditions such as headaches, nausea, rheumatism and colds

Tahereh Naji is with Biology Department, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran (phone:+982122640054; fax:+982122602059; e-mail: tnaji2002@gmail.com).

Mahsa Jassemi is with Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran.

[8]. Ginger is used as a food seasoner, and flavouring material in the food, cosmetics and pharmaceutical industries [9]. Ginger had antibacterial activity against respiratory tract pathogens [10-11].

The objective of this research was to determine the antimicrobial activity from ginger (*Z. officinale*) by measuring the minimum inhibitory against six Gram positive and negative bacteria.

II. MATERIALS AND METHODS

Ginger (*Zingiber officinale*) rhizomes purchased fresh from market in Tehran. They were manually washed to remove dust and adhering sand and spreading them to dry. The moisture content was reduced to use an air oven set at 40°C and were cut into small pieces. They were ground in a grinding machine and extracted with ethanol and an isopropyl: hexan at a ratio of 7:3 mixture as solvents. The solvent was concentrated in a rota–evaporator. Rota evaporation was used to concentrate the smaller quantity of extract, then provided four different concentrations ranging from 0.2 to 0.8 %.

Inhibition of microbial growth were tasted by using the paper disc diffusion and well methods against three Gram positive and three Gram negative bacteria that strains were obtained from Scientific and Technological Research Center, Tehran, Iran. Each bacteria was suspended in Muller Hinton Broth and incubated at 37 °c for 24 h. Muller Hinton Agar was used for testing antibacterial activities.

Steriled filter discs (6mm) were soaked with 20µl in different concentrations of each extracts (0.2, 0.4, 0.6 and 0.8 %) and wells were impregnated with 10 µl of different concentrations of the extract solvents without extracts served as negative control. Ampicillin 10 mcg was used as positive control for Gram positive bacteria and Gentamicin 10 mcg was used as positive control for Gram negative bacteria.

The MIC was considered to be the lowest concentration of the tested sample able to inhibit the growth of bacteria after 24 h and MBC was defined as minimum extract concentration that killed 99 % of bacteria in the initial well.

III. RESULTS

The antimicrobial activities of *Zingiber officinale* of mixture and ethanolic extracts in different concentrations were

assayed against six Gram positive and negative bacteria and antibiotics such as Gentamicin and Ampicillin. results have shown in Tables I-IV and compared with standard

TABLE I
 ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF *ZINGIBER OFFICINALE ROSE*
 BY DISC DIFFUSION METHOD

| Bacterial strain | Concentration (%) | Mean Inhibitory Zone(mm) | MIC ppm | MBC ppm | Gentamicin 10mcg (mm) | Ampicillin 10mcg (mm) |
|-------------------------------------|-------------------|--------------------------|---------|---------|-----------------------|-----------------------|
| <i>S.aureus</i> ATCC(6538) | 0.2 | 7.01 | 0.125 | 0.25 | | |
| | 0.4 | 8.50 | | | | |
| | 0.6 | 9/30 | | | | |
| | 0.8 | 11/0 | | | | |
| <i>S.epidermidis</i> ATCC(12228) | 0.2 | 7/70 | 0.25 | 0/5 | | 12 |
| | 0.4 | 7/80 | | | | |
| | 0.6 | 8/00 | | | | |
| | 0.8 | 10/0 | | | | |
| <i>B.cereus</i> ATCC(11778) | 0.2 | 8/00 | 0.25 | 0/5 | | |
| | 0.4 | 9/67 | | | | |
| | 0.6 | 13/43 | | | | |
| | 0.8 | 14/0 | | | | |
| <i>P.aeruginosa</i> ATCC(9027) | 0.2 | 12/1 | 0/015 | 0/031 | 13 | |
| | 0.4 | 16/33 | | | | |
| | 0.6 | 18/03 | | | | |
| | 0.8 | 25/00 | | | | |
| <i>E.coli</i> ATCC(8739) | 0.2 | 12/00 | 0/031 | 0/062 | 18 | |
| | 0.4 | 15/00 | | | | |
| | 0.6 | 17/21 | | | | |
| | 0.8 | 20/39 | | | | |
| <i>K.pneumoniae</i> ATCC(10031) | 0.2 | 10/88 | 0/125 | 0/25 | 23 | |
| | 0.4 | 13/21 | | | | |
| | 0.6 | 14/00 | | | | |
| | 0.8 | 14/65 | | | | |

TABLE II
ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF *ZINGIBER OFFICINALE ROSE* BY WELL METHOD

| Bacterial strain | Concentration (%) | Mean Inhibitory Zone(mm) | MIC ppm | MBC ppm | Gentamicin 10mcg (mm) | Ampicillin 10mcg (mm) |
|-------------------------------------|-------------------|--------------------------|---------|---------|-----------------------|-----------------------|
| <i>S.aureus</i> ATCC(6538) | 0.2 | 11/18 | 0.125 | 0.25 | | |
| | 0.4 | 1/89 | | | | |
| | 0.6 | 16/78 | | | | |
| | 0.8 | 17/23 | | | | |
| <i>S.epidermidis</i> ATCC(12228) | 0.2 | 10/10 | 0.25 | 0/5 | | 12 |
| | 0.4 | 1/11 | | | | |
| | 0.6 | 15/68 | | | | |
| | 0.8 | 17/17 | | | | |
| <i>B.cereus</i> ATCC(11778) | 0.2 | 8/00 | 0.25 | 0/5 | | |
| | 0.4 | 9/67 | | | | |
| | 0.6 | 13/43 | | | | |
| | 0.8 | 14/00 | | | | |
| <i>P.aeruginosa</i> ATCC(9027) | 0.2 | 12/01 | 0.015 | 0.031 | 13 | |
| | 0.4 | 16/33 | | | | |
| | 0.6 | 18/03 | | | | |
| | 0.8 | 25/00 | | | | |
| <i>E.coli</i> ATCC(8739) | 0.2 | 12/00 | 0.031 | 0.062 | 18 | |
| | 0.4 | 15/00 | | | | |
| | 0.6 | 17/21 | | | | |
| | 0.8 | 20/39 | | | | |
| <i>K.pneumoniae</i> ATCC(10031) | 0.2 | 10/88 | 0/125 | 0/25 | 23 | |
| | 0.4 | 13/21 | | | | |
| | 0.6 | 14/00 | | | | |
| | 0.8 | 14/65 | | | | |

TABLE III
ANTIBACTERIAL ACTIVITY OF ISOPROPYL: HEXAN (7:3) EXTRACT OF *ZINGIBER OFFICINALE ROSE* BY DISC DIFFUSION METHOD

| Bacterial strain | Concentration (%) | Mean Inhibitory Zone(mm) | MIC ppm | MBC ppm | Gentamicin 10mcg (mm) | Ampicillin 10mcg (mm) |
|-------------------------------------|-------------------|--------------------------|---------|---------|-----------------------|-----------------------|
| <i>S.aureus</i> ATCC(6538) | 0.2 | 7.01 | 0.25 | 0.5 | | |
| | 0.4 | 8.24 | | | | |
| | 0.6 | 8.96 | | | | |
| | 0.8 | 9.34 | | | | |
| <i>S.epidermidis</i> ATCC(12228) | 0.2 | 6.53 | 0.5 | 1 | | 12 |
| | 0.4 | 7.81 | | | | |
| | 0.6 | 7.82 | | | | |
| | 0.8 | 8.00 | | | | |
| <i>B.cereus</i> ATCC(11778) | 0.2 | 5.88 | 0.5 | 1 | | |
| | 0.4 | 6.66 | | | | |
| | 0.6 | 7.55 | | | | |
| | 0.8 | 7.55 | | | | |
| <i>P.aeruginosa</i> ATCC(9027) | 0.2 | 9.21 | 0.25 | 0.5 | 13 | |
| | 0.4 | 11.28 | | | | |
| | 0.6 | 11.29 | | | | |
| | 0.8 | 12.60 | | | | |
| <i>E.coli</i> ATCC(98739) | 0.2 | 11.10 | 0.125 | 0.25 | 18 | |
| | 0.4 | 12.89 | | | | |
| | 0.6 | 14.00 | | | | |
| | 0.8 | 16.21 | | | | |
| <i>K.pneumoniae</i> ATCC(10031) | 0.2 | 5.61 | 1 | 2 | 23 | |
| | 0.4 | 6.11 | | | | |
| | 0.6 | 7.01 | | | | |
| | 0.8 | 7.01 | | | | |

TABLE IV
ANTIBACTERIAL ACTIVITY OF ISOPROPYL: HEXAN (7:3) EXTRACT OF *ZINGIBER OFFICINALE ROSE* BY WELL METHOD

| Bacterial strain | Concentration (%) | Mean Inhibitory Zone(mm) | MIC ppm | MBC ppm | Gentamicin 10mcg (mm) | Ampicillin 10mcg (mm) |
|-------------------------------------|-------------------|--------------------------|---------|---------|-----------------------|-----------------------|
| <i>S.aureus</i> ATCC(6538) | 0.2 | 7.81 | 0.25 | 0.5 | | |
| | 0.4 | 8.14 | | | | |
| | 0.6 | 9.19 | | | | |
| | 0.8 | 9.62 | | | | |
| <i>S.epidermidis</i> ATCC(12228) | 0.2 | 7.00 | 0.5 | 1 | | 12 |
| | 0.4 | 8.00 | | | | |
| | 0.6 | 7.82 | | | | |
| | 0.8 | 8.00 | | | | |
| <i>B.cereus</i> ATCC(11778) | 0.2 | 5.87 | 0.5 | 1 | | |
| | 0.4 | 6.73 | | | | |
| | 0.6 | 7.63 | | | | |
| | 0.8 | 7.84 | | | | |
| <i>P.aeruginosa</i> ATCC(9027) | 0.2 | 9.70 | 0.25 | 0.5 | 13 | |
| | 0.4 | 11.50 | | | | |
| | 0.6 | 11.30 | | | | |
| | 0.8 | 13.60 | | | | |
| <i>E.coli</i> ATCC(8739) | 0.2 | 11.87 | 0.125 | 0.25 | 18 | |
| | 0.4 | 13.51 | | | | |
| | 0.6 | 14.00 | | | | |
| | 0.8 | 16.81 | | | | |
| <i>K.pneumoniae</i> ATCC(10031) | 0.2 | 5.73 | 1 | 2 | 23 | |
| | 0.4 | 6.22 | | | | |
| | 0.6 | 7.88 | | | | |
| | 0.8 | 8.02 | | | | |

IV. CONCLUSION

The isopropyl: hexan extract had antibacterial effects and also in concentration of 0.8 % had the maximum antibacterial activity. The study showed that this extract had the maximum antibacterial effects against *E. coli* and had less effect against *K. pneumonia* and *B. cereus*, but the results showed that ethanolic extract had the best effect against *P. areuginosa* and *E.coli*.

Ultimately, minimum inhibitory concentration (MIC) were reported 0.075 -0.5 ppm on test organism for ginger ethanol extract and 0.125-1 for isopropyl & hexan (7:3). This research confirmed the fact that ethanolic extract of ginger had a considerable antibacterial activity and extract of isopropyl & hexan (7:3) had a less antibacterial activity. These results were similar to those obtained by Thongson et al at 2004 [11].

According to the results from this study there are hopes that ethanolic extract of ginger may be used for treatment of some resistant types of bacteria such as *Pseudomonas areuginosa* and *Escherchia coli*. Also it can be used as preserving material for food stuff in food industries.

ACKNOWLEDGMENT

The authors are grateful to Dr. Hakemi (Biotechnology Department, Pasteur Institute) and Ms. Bagheri for helpful assistance during the experiment.

REFERENCES

- [1] G. K. Jayaprakasha, et al. "Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts Food" Research International, vol. 36, pp. 117-122. 2003.
- [2] C.H. Collins, P.M. Lyne, Microbiological Methods (3rd ed.) University Park Press, Baltimore, 1970.
- [3] A., G. Mensah, "Ghana herbal pharmacopoeia", Advent press Ltd, 1992.
- [4] R.V. Tauxe, "Emerging foodborne disease: an evolving public health challenge". Dairy, food Environmental Sanitation, vol. 17. pp. 788-795, 1997.
- [5] E. J. Smid, L.G.M. Gorris. "Natural antimicrobials for food preservation." In: M.Shafiur Rahman (Ed.) Handbook of food preservation, NewYork: Marcel Dekker, pp.285-308, 1999.
- [6] P. M. Davidson, A. S. Naidu, Phyto-phenols. In Natural food Antimicrobial systems ed.Naidu, A.S, pp.265- 294. LLC, Boca Raton, FL: CRC Press, 2000.
- [7] M. Stuart, "The colour dictionary of herbs and herbalism". London:Orbis. Ltd. 1982.
- [8] K. L. Grant, 'et al'. Am J Health Syst. pharm, vol. 57, pp.945-947, 2000.
- [9] G. E. C., Akomas, E., oti, "Developing a technology for the Processing of Nigerian ginger (*Zingiber officinale*)" / umudike, Nigeria , pp . 93-100. 1988.
- [10] J.F., Akoachere, 'et al'. Antibacterial effect of *Zingiber officinale* and *Garcinia kola* on respiratory tract pathogen. East Afr. Med J., vol.79.no.11, pp.588-920.2002.
- [11] C., Thangson 'et al'. "Antimicrobial activity of ultrasound -assisted solvent extracted spices". Letters in Applied Microbiology vol. 24, no. 39, pp. 401-406. 2004.