Assessment of Antimicrobial Activity Of Nanoparticle Ginger Rhizome Water Extract

Norhidayah, A.[*,] , Noriham, A.[**] ;Rusop, M.D.[***]**

[*] Faculty of Hotel and Tourism Management, Universiti Teknologi MARA Puncak Alam, Selangor
[**] Faculty of Applied Science, Universiti Teknologi MARA Shah Alam, Selangor
[***] NANO-SciTech Centre, Institute of Science, Universiti Teknologi MARA Shah Alam, Selangor

Abstract- Study on nanoparticle of herbs on the antimicrobial activity is lacking in the literature, thus this study was conducted with the aimed to evaluate the effectiveness of coarse (CP), fine (FP) and nanostructured ginger rhizome particles (NP) against growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Bacillus subtilis*, *Rhizopus sp*, *Aspergillus nige*, *Candida albicans* by using disc diffusion assay. NP had the strongest inhibitory effect against all tested bacteria (8.3 mm) followed by FP (7.5 mm) while CP (6.7 mm) showed the weakest maximum inhibition zone respectively ($p < 0.05$). However, ginger rhizome water extract at all particle sizes were found not to inhibit yeast and mold. Nanosize particle of ginger rhizome water extract able to inhibit bacteria better than the CP and FP. Hence it is suggested that nanosize particle ginger rhizome water extract has the potential to be used as antibacterial agent in food and pharmaceutical industry.

Index Terms- Antimicrobial, Ginger, Nanoparticle, Nanotechnology Process, Water Extract

I. INTRODUCTION

Ginger (*Zingiber officinale*) which belongs to a tropical and sub-tropical family of Zingiberaceae, originated from South East Asia [1-2] and commercially cultivated not only in South East Asia but also India, China, West Indies, Mexico, Africa, Jamaica, Fiji, Nigeria and Australia and in parts of Japan, Austria, Latin America and Africa [3]. The world's ginger production is 1.4 million metric tonnes per year (mt/year) and India is the major manufacturer (420,000 mt/year or 30% of world production), followed by China (285,000 mt/year) and Indonesia (177,000 mt/year). Furthermore, it has been reported that ginger was among the top selling herbal supplements in the USA, amounting to US $ 1.2 million by the year 2001 [4]. It is due to the great bioactivity possessed in ginger which includes antimicrobial property. The antimicrobial activity of ginger was well documented in the literature. [5] revealed that ginger effectively inhibit the growth of *E.coli*, *L. monocytogene*, *Salmonella* and *S.aureus* load in the treated chicken sausages during the 3 month of frozen storage even at very low concentration (0.5%). Furthermore, dramatically enhanced in the antifungal and antibacterial properties when ginger extract concentration increased into 1% as revealed by the same authors. In addition, ginger also effectively against the growth of bacteria [6] Due to its effectiveness, no doubt ginger being used as antibiotics [7].The presence of its active compounds include gingerol and shagoal [8] reported to contribute to the microbial growth inhibition effectiveness. However, the herbs which contain flavonoids and lignans as their major constituents are difficult to be effectively absorbed due to their poor water solubility, larger particle size and complex chemical structure [9-10]. Thus research being conducted to enhance the herbs bioactivity. Size reduction technology particularly nanotechnology give promising outcome. Nanotechnology produced nanoparticle with particle size in the range of 10 nm to 1000 nm in size [11]. Several researchers have confirmed the bioavailability particularly antimicrobial property enhancement showed in the sample that went through nanotechnology [9, 12-13]. It was documented in the literature that nanochitosan effectively against the growth of bacteria, fungi, and viruses [14].Furthermore, [15] et al. also revealed that nanochitosan significantly inhibited both Gram positive and Gram negative bacteria, in fact the activity found to be better than acetic acid and antibiotics doxycycline. The effect of nanotechnology in improving the biaoactivity and antimicrobial activity are quite established for other material but not on ginger, the common type of herb that is consumed in our daily life. Thus, in this study the effect of nanotechnology on antimicrobial activity of ginger rhizome was explored and thoroughly discussed.

II. RESEARCH ELABORATIONS

A. Raw Materials and Sample Preparation

Fresh *Z.officinale* (ginger) rhizome variety Bentong was purchased from local market in Shah Alam, Selangor, Malaysia and deposited at the Herbarium Institute of Bioscience in University Putra Malaysia (SK 2049/12). The coarse ginger rhizome powder (CP) was obtained by grinding the dried ginger rhizome using a food processor (MX-898, Panasonic) for 5 min while the fine ginger rhizome (FP) were prepared by grinding using a hammer mill (IKA@Werile, MF10 basic) at 3000 rpm attached with a 250µm siever. Meanwhile, nanostructured ginger rhizome (NP) was prepared by using the fine ginger rhizome powder previously obtained as the

starting material and milled using a planetary ball mill (Retsch PM 200, Germany) at 550 rpm for 4 hours. All samples were kept in an airtight container prior to further analysis.

B. Microorganisms

Microorganisms stock cultures used in this study were obtained from Microbiological Laboratory, Faculty of Applied Sciences. Universiti Teknologi MARA (UiTM), Malaysia. Nine foodborne pathogens which are *Escherichia coli* (ATCC 11229), *Pseudomonas aeruginosa* (ATCC 10145), *Salmonella typhimurium* (ATCC 27592), *Streptococcus pyogenes* (ATCC 19615), *Staphylococcus aureus* (ATCC 43300), *Bacillus subtilis* (ATCC 6633), *Rhizopus sp* (ATCC 6380), *Aspergillus niger* (ATCC 16404) and *Candida albicans* (ATCC 10231) were used in this study while Streptomycin (10 µg/disc) and sodium hydrochlorite were used as positive control for bacteria and fungi inhibition activity respectively. On the other hand, distilled water (dH_2O) with no plant extract was used as a negative control for both bacteria and fungi inhibitory activity.

C. Particle Size Determination

The mean particle size of CP and FP were identified using Mastersizer 2000 (Malvern Instrument, United Kingdom). Meanwhile, the particle size of NP was determined by using ZetaSizer S (Malvern Instrument, United Kingdom) based on light diffraction at 25°C and the reading was taken at 173° scattering angle.

D. Surface Morphology

The surface morphology characteristics of CP, FP and NP were identified using the Field Emission Scanning Electron Microscope (FESEM), (Carl Zeiss SMT- SUPRA 40VP, Germany) at 5.00 KV accelerated voltage with 5.0K magnification.

E. Determination of Antimicrobial Activity Using Disc Diffusion Assay

The antimicrobial activity of different particle sizes of ginger rhizome extracts was identified using Kirby Bauer Disc Diffusion Method [16]. The test was performed using nutrient agar (NA) for bacteria and potato dextrose agar (PDA) for fungi. Initially, 20 ml of NA and PDA were transferred aseptically into sterilized petri plate and left for 30 min to let the agar to become solid. Then, accurately 1 ml of bacterial cultures was evenly swabbed on agar plates using a sterile non-toxic cotton swab and let to dry. Twenty microliter (20 µL) of crude extracts (at concentrations of 15.63, 31.50, 61.50 and 125 mg/ml or 0.31, 0.63, 1.25, 2.50 and 5.00 mg/disc, respectively) were loaded onto each sterile paper disc (6 mm) and incubated for 24 h at 37^{o}C and 72 h at room temperature for bacteria and fungi respectively.

III. RESULTS AND DISCUSSION

Particle size of CP, FP and NP are presented in Table I.

TABLE I. PARTICLE SIZE OF COARSE PARTICLE (CP), FINE PARTICLE (FP) AND NANOSTRUCTURED GINGER RHIZOME (NP)

Sample	Particle Size (Nm)	Polydispersity Index (PdI)
CP	19538.33 ± 588.99^{a}	0.52 ± 0.04^{a}
FP	4115.67 ± 675.42^{b}	0.5 ± 0.03^{a}
NP	223.8 ± 17.76^{c}	0.25 ± 0.01^{b}

The particle size used in the study was 19538 nm, 4115 nm and 223 nm for CP, FP and NP respectively. The results revealed that almost 10 fold smaller of particle size demonstrated in the NP than the CP as a result of nanotechnology process. Narrow and homogenize particle size distribution demonstrated in NP depicted by significantly lower in the polydispersity index (PdI) as compared to CP and FP ($p < 0.05$). PdI value more than 0.3 indicates the broader particle size distribution while uniform particle size distribution portrayed by PdI values in the range of 0.2 to 0.3 [17].

The results revealed that planetary ball milling for 4 hours exposed the particles to mechanical impact and consequently forced the particles into breaking point. When the breaking point reached, breakage of particles into small fragments occurred [18]. Additionally, the mechanical impact generated through nanotechnology process caused the shape of ginger particle shifted into irregular flake like shape which previously was solid spherical shape in CP and FP as exhibited in Fig 1. The force absorbed during milling process lead to development of flat and irregular flakes like small fragments [19].

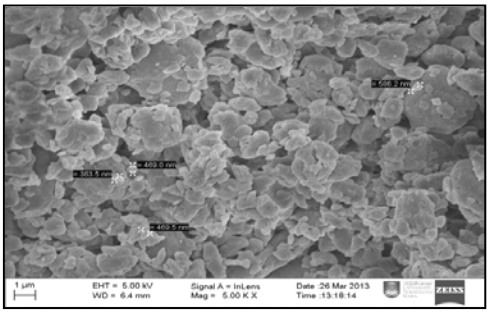

Fig. 1 Surface Morphology of Nanostructured Ginger (NP)

The antimicrobial activity of CP, FP and NP ginger rhizome powder was presented in Table 2. From the results obtained, it can be seen that CP was effectively inhibited the Gram negative bacteria than Gram positive bacteria. In contrast, FP found to be equally sensitive to both, meanwhile NP inhibited almost all the Gram positive and Gram negative bacteria excluding *B. subtilis.* In addition, NP showed the strongest inhibition activity ($p < 0.05$) for almost all microbial tested followed by fine and coarse particle ($p > 0.05$) which proved that nanotechnology improved the antimicrobial activity of ginger rhizome. However, the inhibition zone was relatively much lower than the positive control streptomycin ($p < 0.05$).

TABLE II. ANTIMICROBIAL PROPERTIES OF GINGER WATER EXTRACT AGAINST VARIOUS MICROORGANISMS USING AGAR DISC DIFFUSION METHOD

Microorganisms	**Zone of growth inhibition (mm)**				
	Control (+ ve)	Control (-ve)	CP	FP	NP
Gram positive					
Staphylococcus aureus	11.50 ± 0.71a	n.d	6.40 ± 0.07c	6.6 ± 0.64c	8.50 ± 0.71b
Streptococcus pyogenes	13.5 ± 0.71a	n.d	n.d	n.d	6.50 ± 0.71b
Salmonella typhimurium	10.10 ± 0.14a	n.d	n.d	6.2 ± 0.14c	8.00 ± 1.41b
Gram negative					
Bacillus subtilis	12.5 ± 0.71a	n.d	n.d	7.5 ± 0.71b	n.d
Pseudomonas aeruginosa	9.50 ± 0.71a	n.d	6.70 ± 0.14b	n.d	6.50 ± 0.71b
Escherichia coli	15.50 ± 0.71a	n.d	6.30 ± 0.07d	6.5 ± 0.06c	7.30 ± 0.35b
Rhizopus sp	14.00 ± 1.41 a	n.d	n.d	n.d	n.d
Aspergillus niger	15.50 ± 0.71 a	n.d	n.d	n.d	n.d
Candida albicans	13.9 ±0.14 a	n.d	n.d	n.d	n.d

Values for zone of growth inhibition are presented as mean ± standard deviation. Values marked by the different superscript letters from a to c between a column denote statistically significant differences ($p<0.05$)

Similar antimicrobial enhancement as a result of nanotechnology process was also assessed in the nanoparticles chitosan [15], tea tree nanoparticle [20], curcumin nanoparticle [21] andiroba oil nanoparticle as well as copaiba oil nanoparticle [22]. In fact the activity of nanoparticles were better than that of coarse particle. Hence they made a conclusion that nanotechnology improved the antimicrobial activity. Increased in the material solubility and dispersion in water due to nanotechnology may become a possible reason for greater in the NP antimicrobial activity as compared to CP and FP found in current work [23]. The degree of particles dispersion in water play an important role in antimicrobial effectiveness since greater dispersion rate lead to more efficient transport of ginger extract to the bacteria cell wall thus enhanced the antimicrobial activity [21]. In addition, the smaller in the particle size possessed by nanoparticle allow the nanoparticles to attached tightly to the surface of bacterial hence inhibited the growth [24]. This is due to the increased in the ratio of surface area to volume which increased the positive charge density as results stronger binding of particle to the bacteria's cell walls and membranes occurred. This lead to completely breakage of cell membrane and causing leakage of intracellular substances that lead to cell death [15, 21]. In addition, ability of nanoparticle to successfully penetrate into the cell and disrupt the cell organelles structure further enhanced the antimicrobial activity [25]. Furthermore, greater surface area [26] demonstrated in the nanoparticle caused more active compounds exposed to the surrounding therefore react better than the coarse and fine particle hence showing greater antimicrobial activity.

However, none of the particle size of ginger rhizome inhibit the *Rhizopus species, Aspergillus niger* and *Candida albicans* growth. The result obtained was relatively in opposite to the previous study where it was documented that ginger ethanolic extract had a strong inhibitory activity (20 mm) against *Candida albican* after exposed at room temperature for 24 h. While in other work, methanolic and n-hexane ginger extract significantly inhibited against the growth of bacteria and fungi [27]. The weak antimicrobial activity towards

yeast and mold observed in the current work possibly due to the used of water as solvent extraction. It was found that organic extract of dried ginger had a greater inhibitory effect against microbial growth while, the inhibitory activity was not observed in the ginger aqueous extract [28] which supported our finding.

IV. CONCLUSION

The antimicrobial activity of ginger rhizome extract as affected by nanotechnology process. Results revealed that NP showed better antimicrobial activity towards Gram positive and Gram negative bacteria as compared to its coarse and fine particle counterpart. However ginger water extract at all particle sizes did not yeast abd mold growth. Hence it can be concluded that nanosize particle ginger rhizome was more effective in bacteria inhibitory better than the larger size particles.

ACKNOWLEDGMENT

The authors are grateful to the Universiti Teknologi MARA (UiTM) for the facilities, Ministry of Higher Education Malaysia for the scholarship and Ministry of Science, Technology and Innovation (MOSTI) Malaysia for the e-science grant (06-01-01-SF0390).

REFERENCES

[1] I. Sasidharan and A. N. Menon, "Comparative chemical composition and antimicrobial activity fresh & dry ginger oils (Zingiber Officinale Roscoe)," *International Journal of Current Pharmaceutical Research*, vol. 2, 2010.

[2] H. Mohamad, Y. Andriani, M. A. W. Effendy, and T. M. T. Sifzizul, "Antibacterial, radical- scavenging activities and cytotoxicity properties of Phaleria macrocarpa (scheff.) boerl. leaves in hepg2 cell lines," *International Journal of Pharmaceutical Sciences and Research*, vol. 2 pp. 1700-1706, 2011.

[3] U. Bhandari, R. kanojia, and K. K. Pillai, "Effect of ethanolic extract of Zingiber officinale on dyslipidaemia in diabetic rats," *Journal of Ethnopharmacology*, vol. 97, pp. 227-230, 2005

[4] AOAC, *Determination of peroxide content. 965.33. In Official methods of analysis* 17 ed. Gaithersburg, Maryland: Association of Official Analytical Chemists, 2002.

[5] L. El Sediek, W. M. M. Abozeid, D. H. Alkhalifah, and S. E. A. Farag, "Efficacy of Ginger Extract (Zingiber Officinale) and Gamma Irradiation for Quality and Shelf-Stability of Processed Frozen Beef Sausage," *Life Science Journal* vol. 9, pp. 448 - 461, 2012.

[6] Y. F. M. Kishk and H. E. Elsheshetawy, "Effect of ginger powder on the mayonnaise oxidative stability, rheological measurements, and sensory characteristics," *Annals of Agricultural Science*, vol. 58, pp. 213-220, 2013.

[7] A. Sebiomo, A. D. Awofodu, A. O. Awosanya, F. E. Awotona, and A. J. Ajayi, "Comparative studies of antibacterial effect of some antibiotics and ginger (Zingiber officinale) on two pathogenic bacteria. ," *Journal of Microbiology Antimicrob*, vol. 3, pp. 18-22, 2011.

[8] Z. Atai, M. Atapour, and M. Mohseni, "Inhibitory effect of Ginger Extract on Candida Albicans. ," *American Journal of Applied Science* vol. 6, pp. 1067-1069, 2009

[9] P. Y. Ma, Z. Y. Fu, Y. L. Su, J. Y. Zhang, W. M. Wang, H. Wang, Y. C. Wang, and Q. J. Zhang, "Modification of physicochemical and medicinal characterization of Liuwei Dihuang particles by ultrafine grinding," *Powder Technology*, vol. 191, pp. 194-199, 2009.

[10] R. Sonada, M. Horibe, T. Oshima, T. Iwasaki, and S. Watano, "Improvement of Dissolution Property of Poorly Water-Soluble Drug by Novel Dry Coating Method Using Planetary Ball Mill," *Chem. Pharm. Bull.* , vol. 56(9), pp. 1243-1247, 2008.

[11] D. V. Ratnam, D. D. Ankola, V. Bhardwaj, D. K. Sahana, and M. N. Kumar, "Role of antioxidants in prophylaxis and therapy: a pharmaceutical perspective. ," *Journal of Controlled Release*, vol. 113, pp. 189-207, 2006

[12] J.-R. Liu, G.-F. Chen, H.-N. Shih, and P.-C. Kuo, "Enhanced antioxidant bioactivity of Salvia miltiorrhiza (Danshen) products prepared using nanotechnology," *Phytomedicine*, vol. 15, pp. 23-30, 2008

[13] L. Gao, G. Liu, X. Wang, F. Liu, Y. Xu, and J. Ma, "Preparation of a chemically stable quercetin formulation using nanosuspension technology," *International Journal of Pharmaceutics*, vol. 404, pp. 231-237, 2011.

[14] A.J. Huh and Y.J. Kwon, ""Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era " *Journal of Control Release* vol. 156, pp. 128-145, 2011.

[15] K. Blecher, A. Nasir, and A. Friedman, "The growing role of nanotechnology in combating infectious disease," *Virulence* vol. 2 pp. 395-401, 2011.

[16] M. M. Mackeen, A. M. Ali, N. H. Lajis, K. Kawazu, Z. Hassan, M. Amran, M. Habsah, L. Y. Mooi, and S. M. Mohamed, "Antimicrobial, antioxidant, antitumour-promoting andncytotoxic activities of different plant part extracts ofnGarcinia atro6iridis Griff. ex T. Anders.," *Journal of Ethnopharmacology* vol. 72, pp. 395-402, 2000.

[17] M. P. Alves, A. L. Scarrone, M. Santos, A. R. Pohlmann, and S. S. Guterres, "Human skin penetration and distribution of nimesulide from hydrophilic gels containing nanocarriers.," *International Journa of Pharmaceutical*, vol. 341, pp. 215-220, 2007.

[18] G.-y. Ren, D. Li, L.-j. Wang, N. Özkan, and Z.-h. Mao, "Morphological properties and thermoanalysis of micronized cassava starch," *Carbohydrate Polymers*, vol. 79, pp. 101-105, 2010

[19] X. Zhao, Z. Yang, G. Gai, and Y. Yang, "Effect of superfine grinding on properties of ginger powder," *Journal of Food Engineering* vol. 91, pp. 217-222, 2009.

[20] R. C. V. Santos, L. L. Q. S., A. F. S. Alves, V. P. Fausto, K. Pizzutti, V. Barboza, M. E. Souza, R. P. Raffin, P. Gomes, D. Takamatsu, Y. Morinaga, A. A. Boligon, M. L. Athayde, C. C. Felippi, and R. Vaucher, "Antimicrobial activity of tea tree oil nanoparticles against American and European foulbrood diseases agents," *Journal of Asia-Pacific Entomology* vol. 17, pp. 343- 347, 2014.

[21] R. K. Basniwal, H. S. Buttar, V. K. Jain, and N. Jain, "Curcumin nanoparticles: preparation, characterization, and antimicrobial studies," *Journal of Agricultural Food Chemistry*, vol. 59, pp. 2056-2061, 2011.

[22] R. C. Santos, C. F. S. Alves, T. Schneider, L. Q. Lopes, C. Aurich, J. L. Giongo, A. Brandelli, and R. A. Vaucher, "Antimicrobial activity of Amazonian oils against Paenibacillus species.," *Journal of Invertebrata Pathology* vol. 109, pp. 265-268, 2012

[23] Y.I. Su, Z.Y. Fu, C.J. Quan, and W.M. Wang, "Fabrication of nano Rhizama Chuanxiong particles and determination of tetramethylpyrazine," *Transactions of Nonferrous Metals Society of China*, vol. 16, pp. s393-s397, 2006.

[24] M. Moritz and M. M. Geszke, "The newest achievements in synthesis, immobilization and practical applications of antibacterial nanoparticles," *Chemical Engineering Journal*, vol. 228, pp. 596-613, 2013.

[25] A. J. Friedman, J. Phan, D.O. Schairer, J. Champer, M. Qin, A. Pirouz, K. Blecher-Paz, A.Oren, P. T. Liu, R.L. Modlin, and J. Kim, "Antimicrobial and anti-inflammatory activity of chitosan-alginate nanoparticles: a targeted therapy for cutaneous pathogens,," *Journal Investigation Dermatologies* vol. 133 pp. 1231-1239, (2013).

[26] R. Y. Pelgrift and F. A.J., "Nanotechnology as a therapeutic tool to combat microbial resistance☆," *Advanced Drug Delivery Reviews* vol. 65 pp. 1803-1815, 2013.

[27] H. A. Hasan, A. M. Rasheed Raauf, B. M. Abd Razik, and B. A. Rasool Hassan, "Chemical Composition and Antimicrobial Activity of the Crude Extracts Isolated from Zingiber Officinale by Different Solvents," *Pharmaceutica Analytica Acta* vol. 3, p. 184, 2012.

[28] D. Gao and Y. Zhang, "Comparative antibacterial activities of extracts of dried ginger and processed ginger," *Pharmacognosy Journal*, vol. 2, pp. 41-44, 2010.

AUTHORS

First Author –Norhidayah, A., MSc in Food Science, Universiti Teknologi MARA, norhidayah79@gmail.com.
Second Author – Noriham, A. PhD, Universiti Teknologi MARA, noriham985@salam.uitm.edu.my.
Third Author – Rusop, M.D, PhD, NANO-SciTech Centre, Institute of Science, Universiti Teknologi MARA, nano@gmail.com.

Correspondence Author – Norhidayah, A, norhidayah79@gmail.com, norhi813@salam.uitm.edu.my, 6016.2287027.