DETERMINATION OF SELECTED BIOCHEMICAL COMPOUNDS AND BIOACTIVITY PROPERTIES OF HAQ JUICE

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DEDICATION

This thesis is dedicated to my beloved parents, who always give me motivation, support and encouragement that I can achieve whatever I want to. It is also dedicated to my dearest husband, who always gives me love and trust to fulfil all the tasks I have. Lastly, to my lovely son, you are my biggest strength to keep me stepping forward and finished what I have started.

THIS IS FOR ALL OF YOU.

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ABSTRACT

Health juice is becoming popular among the locals since it can be beneficial to health. However, the safety and efficiency of health juice have been the subject of continuous argument. The present study was conducted to determine the content of bioactive compounds and bioactivities of Haq juice, which is made up of five natural ingredients namely ginger, garlic, honey, lemon and apple cider vinegar to provide value added information for commercialization and to gain consumer's trust. The study found allicin, the end product from garlic was detected using gas chromatography mass spectroscopy (GCMS) and methylglyoxal from honey was detected using high performance liquid chromatography (HPLC). Proteins in Haq juice were identified using Liquid Chromatography-Tandem Mass Spectrometry (LCMS-MS) analysis. In addition, phenolics and flavonoids content in the juice were also analyzed to determine the antioxidant activity of the juice. The juice sample and its ingredient samples (ginger, garlic, honey, lemon and apple cider vinegar) were tested for their antioxidant activity based on 2,2- diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging assay and Ferric Reducing Antioxidant Power (FRAP) assay. Furthermore, antibacterial activities against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa were tested using agar welldiffusion method. As results, the methylglyoxal in Haq juice is 7.98 ppm and proteins identified were 773 proteins, including several proteins such as peroxidase, cytochrome P450 and defensin like protein. The juice samples were comparable to the samples of its control ingredients where, the radical scavenging activity (RSA) for the juice sample was 56.05 % which is the second highest after ginger (64.28 %). While the FRAP value for the juice sample was 2.79 mM of Fe2+ similar across all samples. Plus, Haq juice contained antibacterial activities against all the bacteria tested. It is concluded that this natural health juice has high antioxidant activity and is good for inhibiting bacterial growth.

ABSTRAK

Jus kesihatan kini menjadi semakin terkenal di kalangan rakyat kerana ia memberi faedah kepada kesihatan. Walau bagaimanapun, keselamatan dan keberhasilan jus kesihatan adalah satu kontroversi yang berterusan. Kajian ini telah dijalankan untuk mengenalpasti sebatian bioaktif dan bioaktiviti jus Haq yang mengandungi lima jenis ramuan semula jadi iaitu halia, bawang putih, madu, limau, dan cuka epal untuk mendapatkan informasi yang boleh menambah nilai bagi tujuan pemasaran dan meraih kepercayaan pengguna. Keputusan daripada kajian telah menunjukkan alisin, hasil akhir daripada bawang putih telah ditemui menggunakan Kromatografi Gas Spektroskopi Jisim (GCMS) dan metilglioksal daripada madu telah ditentukan menggunakan Kromatografi Cecair Prestasi Tinggi (HPLC). Protin telah dikenalpasti melalui analisis Kromatografi Cecair Spektrometri Jisim (LCMS-MS). Tambahan pula, kandungan fenolik dan flavonoid dalam jus Haq juga telah dikenalpasti untuk menentukan aktiviti antioksidan jus ini. Aktiviti antioksidan jus Haq dan sampel bahan kandungan jus (halia, bawang putih, madu, limau, dan cuka epal) telah diuji melalui kaedah 2,2-difenil-1-pikrilhidrazil hidrat (DPPH) dan Ferric Reducing Antioxidant Power (FRAP). Tambahan pula, aktiviti antibakteria ke atas Escherichia coli, Staphylococcus aureus dan Pseudomonas aeruginosa telah diuji dengan menggunakan kaedah peresapan agar. Sebagai hasil, jus Haq mengandungi 7.98 ppm metilglioksal dan 773 protin telah dikenalpasti, termasuk protin yang dikenalpasti sebagai peroksidase, sitokrom P450 dan protin seperti defisin. Sampel jus mempunyai aktiviti antioksidan yang setanding dengan sampel ramuan di mana jus Haq menunjukkan aktiviti skaveng radikal bebas (RSA) yang kedua tertinggi (56.05 %) selepas halia (64.28 %). Manakala, nilai FRAP untuk jus Haq adalah 2.79 mM Fe2+, sama rata dalam kalangan sampel lain. Tambahan pula, jus Haq juga telah menunjukkan aktiviti antibakteria ke atas semua bakteria yang diuji. Kesimpulannya, jus kesihatan semula jadi ini mempunyai aktiviti antioksidan yang tinggi dan bagus dalam menghalang pembiakan bakteria.

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LIST OF ABBREVIATIONS

1D	-	One dimensional
ACV	-	Apple cider vinegar
AGE	-	Aged garlic extract
$AlCl_3$	-	Aluminium (III) chloride
APS	-	Ammonium persulfate
BSA	-	Bovine serum albumin
CBR-R250	-	Coomassie- brilliant blue- Red 250
CH_3COONa	-	Acetic acid- sodium acetate buffer
DNPH	-	2,4- dinitrophenylhydrazine
DNS	-	2,3- dinitrosalicyclic
DPPH	-	2, 2- diphenyl- 1- picryl- hydrazyl- hydrate
DS	-	Direct sample
DTT	-	Dithiothreitol
$FeSO_4$	-	Ferrous (II) sulphate
FRAP	-	Ferric reducing antioxidant power
GCMS	-	Gas chromatography mass spectroscopy
HDL	-	High density lipoprotein
HPLC	-	High performance liquid chromatography
IAA	-	Iodoacetamide
LC-MS/MS	-	Liquid chromatography-tandem mass spectroscopy
LDL	-	Low density lipoprotein
NA	-	Nutrient agar
Na_2CO_3	-	Sodium carbonate
Na_2SO_4	-	Sodium sulphate
NCBI	-	National Centre for Biotechnology Information
NH_4HCO_3	-	Ammonium bicarbonate
NIST	-	National Institute of Standard & Technology
NPD	-	4-nitro-1,2-phenylene-diamine
PDA	-	Photodiode array

QE	-	Quercetin equivalent
ROS	-	Reactive oxygen species
SDS-PAGE	-	Sodium dodecyl sulphate- polyacrylamide gel electrophoresis
TEMED	-	Tetramethylethylenediamine
TFC	-	Total flavonoid content
TG	-	Triglyceride
TPC	-	Total phenolics content
VLDL	-	Very low-density lipoprotein

LIST OF SYMBOLS

ºC	-	Celcius
%	-	Percent
cm	-	Centimetre
g	-	Gram
kDa	-	Kilodalton
kg	-	Kilogram
L	-	Litre
M	-	Molar
µL	-	Microlitre
µM	-	Micromolar
µm	-	Micrometre
µg	-	Microgram
mL	-	Millilitre
mm	-	Millimetre
mg	-	Milligram
nm	-	Nanometre
rpm	-	Rotation per minute
V	-	Volt
v/v	-	Volume per volume
w/v	-	Weight per volume

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CHAPTER 1

INTRODUCTION

1.1 Research background

The increasing awareness of health management and healthy lifestyles in reducing the risks of various diseases among the locals has led to increasing production of health products. Health products specifically health juices provide a simpler way for individuals to practice a healthier lifestyle. This is because by consuming the juice as a daily supplement, an individual can get enough essential nutrition needed by the human body. Besides, some health juices can also target and manage a number of health issues such as diabetes, high blood pressure and high cholesterol level based on the ingredients used. Currently, among the health juices available in local markets that attract many local people are Pamoga juice, Nusantara juice, Al-Sunnah juice and Penawar juice. All these juices were claimed to give energy and treat various ailments for the consumer.

Haq juice is a locally commercialized herbal mixture solution that is made up from five natural products which are ginger, garlic, honey, lemon, and apple cider vinegar. Currently it is gaining popularity among the people as each of the ingredients used is somehow beneficial to the human health (Garcia-Alvarez et al., 2014; Henning et al., 2017). Most of the natural products used in this juice were found to contain bioactive compounds such as flavonoids, phenolics and vitamins that possess antioxidant, antimicrobial, anti-inflammatory and anticancer properties (Naseem et al., 2016).

In Haq juice, the ingredients used such as ginger contains 6-gingerol that has been reported to treat various ailment because of its antioxidant, antimicrobial and anti-inflammatory properties (Rajsekhar *et al.*, 2012; Mathai *et al.*, 2017; Shukla *et*

al., 2019). Whereas allicin as the main component in garlic has been stated to have strong antimicrobial and antioxidant effects (Al Masaudi & Al Bureikan, 2013; Bayan et al., 2014). Next, the polyphenol in honey has strong antioxidant effect and directly becomes one of the main factors for honey to possess anticancer activity (Abubakar et al., 2012; Ahmed & Othman, 2013). Lemon, another ingredient used in this juice is a citrus fruit that is rich with vitamin C and flavonoids. Its antioxidant activity helps in maintaining good health and preventing diseases (Oikeh et al., 2015). Meanwhile, acetic acid contained in apple cider vinegar (ACV) gives hypolipidemic effect that prevents people from getting coronary heart disease and stroke. Beta-carotene found in ACV also acts as an antioxidant and reduces oxidative stress by being free radical scavenger (Naseem et al., 2016).

Since all the ingredients in the juice consist of bioactive compounds that contribute to various medicinal properties, hence their combination has been claimed to promote various ailments. However, the efficacy and safety of this juice have become an argument and controversy among the locals since there is a dumping of "claim" that health products are not safe to be consumed. Therefore, this study was conducted to quantify the bioactive compounds in the juice and test the bioactivity (antioxidant and antimicrobial) of the juice in order to ensure the effectiveness of the juice.

1.2 Problem statement

There are claims of many local products being health beneficial in the market but not fully supported by proof of its safety and efficacy (Ismail et al., 2020). This is due to reports claiming consumers having bad side effects after using the products and even worst, a few were reported to be dead due to prolonged use of these products. Besides, Ismail et al. (2020) also stated that there are also a number of health juices being sold without proper verification and little information on its effectiveness. Hence, many people have doubts on whether to risk and consume the local health products as an addition to their food or as a daily supplement. The combination of 5 natural products namely ginger, garlic, honey, lemon and apple

cider vinegar is known to alleviate common ailments such as high blood pressure, indigestion, bloating, high cholesterol, asthma, skin problems, obesity, and stomach ulcer, similarly to the use of prescription drugs but with lesser side effects (Javed et al., 2014; Naseem et al., 2016). In previous studies done by Javed et al., (2014) and Naseem et al., (2016), the combination of these five ingredients have revealed the lipid lowering and cardio-protective effect in experimental animal models of hyperlipidaemia. Besides, it has also found out that the combination of the extracts exerts cardio protective and anti-atherogenic effects. The study was done because the researchers assumed that each of the components have antioxidant effects that contributes to reducing cholesterol level. However, how the antioxidant properties present and work in the mixtures has not been discussed and this research aims to determine the antioxidant activity of this herbal mixture. On the other hand, a similar study was done by Metwali et al., (2014) on antimicrobial activity of natural products combination but they only combined three ingredients which were garlic, ginger, and honey. From the study, the researchers discovered that the combination of garlic, ginger and honey in a non-aqueous vehicle is effective against gram-positive bacteria and gram-negative bacteria. Thus, the five ingredients combination is assumed to have stronger antibacterial activity. However, there is still no published work done on the combination of all these five natural products on bioactive compounds, antioxidant and antibacterial activity. Therefore, this research aims to determine the bioactive compounds, to identify the protein and to determine the antioxidant and antibacterial activities of combination of five natural products.

1.3 Significance of study

The combined medicinal properties of the five natural products in Haq juice provides cheaper and safer alternative compared to pharmaceutical drugs since medicinal plants are believed to have equal effect as pharmaceutical drugs. From this study, the protein content, bioactive compound presence, and bioactivity properties such antioxidant and antimicrobial properties of the juice will be known. Hence, this study will provide information on the properties and activities of Haq juice which will add more value for its commercialization and reap its benefit to mankind.

Besides, it could also be a guideline to increase public awareness on the health products sold in the market and additionally, the results obtained in this study will help in the trustworthiness of the product. Therefore, this study is an alternative to gain the trust and support of the consumer towards local health product specifically the Haq juice.

1.4 Research objectives

The research objectives are:

a. To determine nutritional contents (vitamin C, reducing sugar) and bioactive compounds (total phenolic content, total flavonoid content) in Haq juice.
b. To evaluate the bioactivity of Haq juice by determining the protein content, antioxidant and antibacterial activities.
c. To identify the proteins in Haq juice.

1.5 Scope of work

The level of two bioactive compounds in Haq juice namely allicin (from garlic) and methylglyoxal (from honey) was determined using high performance liquid chromatography (HPLC) and gas chromatography mass spectroscopy (GCMS). The total phenolic content was determined using Folin-Ciocalteu method and total flavonoid content via aluminium (III) chloride ($AlCl_3$) solution method. The reducing sugar was determined by method using 3,5-dinitrosalicylic acid (DNS) reagent and the vitamin C content in the juice was estimated by method using reagent of 5 % metaphosphoric acid- 10 % acetic acid. Then, the antioxidant activity of the juice was studied using ferric reducing power assay (FRAP) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate assay (DPPH) while the antibacterial activity was determined using disc diffusion method on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. Finally, protein content in the juice was identified by conducting protein digestion using in solution digestion method

and the digested protein was sent for Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) analysis and then interpreted through National Center for Biotechnology Information (NCBI), Uniprot and GoMapMan.

REFERENCES

Abubakar, M. B., Abdullah, W. Z., Sulaiman, S. A., and Suen, A. B. (2012) 'A review of molecular mechanisms of anti-leukemic effects of phenolics compounds in honey', *International Journal of Molecular Sciences*, 13(11), 15054-15073.

Ahmed, S. and Othman N. H. (2013) 'Honey as a potential natural anticancer agent: A review of its mechanisms', *Evidence-based Complementary and Alternative Medicine,* 1-7.

Ajayi, O. B., Akomolafe, S. F., and Akinyemi, F. T. (2013) 'Food Value of Two Varieties of Ginger (*Zingiber officinale*) Commonly Consumed in Nigeria. *ISRN nutrition*, 359727.

Alanazi, A. M., Mostafa, G. A. E., and Al-Badr, A. A. (2015) 'Glutathione', *Profiles of Drug Substances, Excipients and Related Methodology*, 43–158.

Ali, B. H., Bluden, G., Tanira, M. O., and Nemmar, A. (2008) 'Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale Roscoe*): A review of recent research', *Food and Chemical Toxicology*, 46, (2), 409–420.

Ali, S. H., Obaid, Q. A. and Awaid, K. G. (2020). 'Lemon juice antioxidant activity against oxidative stress,' *Baghdad Science Journal*, 17(1), 0207.

Al Masaudi, S. B. and AlBureikan, M. O. (2013) 'Antimicrobial activity of garlic juice (*Allium sativum*), honey and garlic-honey mixture on some sensitive and multiresistant microorganisms', *Life Science Journal*, 10(4), 2429-2435.

Ankri, S. and Mirelman, D. (1999) 'Antimicrobial properties of allicin from garlic', *Microbes and infections,* 1, 125-129.

Arnhold, J. (2020) 'Oxidation and Reduction of Biological Material', *Cell and Tissue Destruction,* 55–97.

Aruoma, O. I., B. Landes, D. Ramful-Baboolall, E. Bourdond, V. Neergheen-Bhujune, K. Wagnerf, et al. (2012) 'Functional benefits of citrus fruits in the management of diabetes', *Prev. Med.*, 54, 12–16.

Avcı, A., Atlı, T., Ergüder, İ. B., Varlı, M., Devrim, E., Turgay, S. A. M., and Durak, I**.** (2007) 'Effects of apple consumption on plasma and erythrocyte

antioxidant parameters in elderly subjects', *Experimental aging research*, 33, 429–437.

Azlan, A., Kok, Y. W. and Khoo, H. E. (2018) 'Antioxidant content and and activity of polyphenol-rich mixtures', *Journal of Engineering and Applied Sciences*, 13(9), 6973-6979.

Ball, D.W. (2007) 'The chemical composition of honey', *Journal of chemical education*, 84(10), 1643.

Bayan, L., Koulivand, P. H. and Gorji, A. (2014) 'Garlic: A review of potential therapeutics', *Avicenna Journal of Phytomedicine*, 4(1), 1-14.

Boudries, H., K. Madani, N. Touati, S. Souagui, S. Medouni, and M. Chibane. (2012) 'Pulp antioxidant activities, mineral contents and juice nutritional properties of Algerian Clementine Cultivars and Mandarin', *African Journal Biotechnology*, 11, 4258–4267.

Bradshaw, C.E. (2011) 'An in vitro comparison of the antimicrobial activity of honey, iodine and silver wound dressings', *Bioscience Horizons: The International Journal of Student Research*, 4(1), 61–70.

Calligaris, D., Villard, C. and Lafitte, D. (2011) 'Advances in top-down proteomics for disease biomarker discovery', *Journal of Proteomics*, 74(7), 9209-9234.

Carrasco-Castilla, J., Hernández-Álvarez, A. J., Jiménez-Martínez, C., Jacinto-Hernández, C., Alaiz, M., Girón-Calle, J., … Dávila-Ortiz, G. (2012) 'Antioxidant and metal chelating activities of peptide fractions from phaseolin and bean protein hydrolysates', *Food Chemistry*, 135(3), 1789–1795.

Chakrabarti, S., Jahandideh, F. and Wu, J. (2014) 'Food-derived bioactive peptides on inflammation and oxidative stress,' *BioMed Research International*, (1), 608979-608990.

Chen, S., Shen, X., Cheng, S., Li, P., Chang, Y., and Meng, H. (2013) 'Evaluation of garlic cultivars for polyphenolic content and antioxidant properties', *PLoS ONE*, 8(11), e79730.

Cooper, R. (2014) 'Honey as an effective antimicrobial treatment for chronic wounds: is there a place for it in modern medicine?' *Chronic Wound Care Management and Research*, 1, 15–22.

Costa, E. A., Sousa, P. H. M., Siqueira, A. C. P., Figueiredo, E. A. T., Gouveia, S. T., Figueiredo, R. W., Maia, C. S. C., and Gomes, D. S. (2019) 'Fruit pastes

with organic honey texturized with gellan gum: bioacessibility of antioxidant activity and sensory analysis fruit pastes with gellan and organic honey', *Food Science and Technology*, 39(3), 667-676.

Daka D. (2011) 'Antibacterial effect of garlic (*Allium sativum*) on *Staphyloccus aureus*: An in vitro study', *African Journal of Biotechnology,* 10(4), 666-669.

Da Silva, V. L., Cerqueira, M. R. F., Lowinsohn, D., Matos, M. A. C., and Matos, R. C. (2012) 'Amperometric detection of ascorbic acid in honey using ascorbate oxidase immobilised on amberlite IRA-743', *Food Chemistry,* 133(3), 1050–1054.

Del Campo, G., Berregi, I., Santos, J. I., Dueñas, M. and Irastorza, A. (2008) 'Development of alcoholic and malolactic fermentations in highly acidic and phenolic apple musts', *Bioresource Technoogy.,* 99, 2857–2863.

Denisow, B. and Denisow-Pietrzyk, M. (2016) 'Biological and therapeutic properties of bee pollen: A review', *Journal of the Science of Food and Agriculture*, 96(13), 4303–4309.

De Oliveira Carvalho, A. and Moreira Gomes, V. (2011) 'Plant Defensins and Defensin-Like Peptides - Biological Activities and Biotechnological Applications', *Current Pharmaceutical Design*, 17(38), 4270–4293.

Dimopoulos, M. A., Swern, A. S., Li, J. S., Hussein, M., Weiss, L., Nagarwala, Y., and Baz, R. (2014) 'Efficacy and safety of long-term treatment with lenalidomide and dexamethasone in patients with relapsed/refractory multiple myeloma', *Blood Cancer Journal*, 4(11), e257. doi:10.1038/bcj.2014.77

Dugasani, S., Pichika, M. R., Nadarajah, V. D., Balijepalli, M. K., Tandra, S., and Korlakunta, J. N. (2010) 'Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol', *Journal of Ethnopharmacology,* 127, 515–520.

El-Sharaky, A. S., Newairy, A. A., Kamel, M. A., and Eweda, S. M. (2009) 'Protective effect of ginger extract against bromobenzene-induced hepatotoxicity in male rats', *Food and Chemical Toxicology*, 47, 1584–1590.

Ekawati, E., and Darmanto, W. (2019) 'Lemon (Citrus limon) Juice Has Antibacterial Potential against Diarrhea-Causing Pathogen', *IOP Conference Series: Earth and Environmental Science,* 217, 012023.

Garcia-Alvarez, A., Egan, B., de Klein, S., Dima, L., Maggi, F. M., Isoniemi, M., … Serra-Majem, L. (2014) 'Usage of Plant Food Supplements across Six European Countries: Findings from the PlantLIBRA Consumer Survey', *PLoS ONE,* 9(3), e92265. doi:10.1371/journal.pone.0092265

Ghasemzadeh, A., Jaafar, H. Z. E., and Rahmat, A. (2010) 'Identification and concentration of some flavonoid components in Malaysian young ginger (*Zingiber officinale Roscoe*) varieties by a high performance liquid chromatography method', *Molecules,* 15(9), 6231–6243.

Ghosh, S. (2016) 'Peptide therapeutics market:forecast and analysis 2015-2025', *Oligos & Peptides,* 34(2), 5–7.

González-Molina, E., Domínguez-Perles, R., Moreno, D. A., and García-Viguera, C. (2010) 'Natural bioactive compounds of Citrus limon for food and health', *Journal of Pharmaceutical and Biomedical Analysis,* 51(2), 327–345.

Gururani, M. A., Venkatesh, J., Upadhyaya, C. P., Nookaraju, A., Pandey, S. K., and Park, S. W. (2012) 'Plant disease resistance genes: Current status and future directions', *Physiological and Molecular Plant Pathology,* 78, 51–65.

Han, Y., Sun, Z., and Chen, W. (2019) 'Antimicrobial Susceptibility and Antibacterial Mechanism of Limonene against Listeria monocytogenes', *Molecules,* 25(1), 33.

Henning, S. M., Yang, J., Shao, P., Lee, R. P., Huang, J., Ly, A., Hsu, M., Lu, Q. Y., Thames, G., Heber, D., and Li, Z. (2017) 'Health benefit of vegetables/fruit juice-based diet: Role of microbiome', *Scientific Reports,* 7(2167), 1-9.

Ismail, S.F., Azmi, I. M. A., Daud, M., Abd. Jalil, J., and Safuan, S. (2020) '*Regulatory control of herbal and traditional medicines in Malaysia: issues and concerns', International Journal of Business and Society,* 21 (S1), 192-204.

Javed, I., Sarfraz, M., Muhammad, F., Aslam, B., Zia-ur-Rahman, Khan, M.Z., Khaliq, T., Khan, F.H., and Ahmad, M. (2014) 'Lipid lowering effect of a herbal mixture in hyperlipidaemic adult male albino mice', *Pakistan Veterinary Journal, 34,* 489-493.

Jomová, K., Hudecova, L., Lauro, P., Simunkova, M., Alwasel, S. H., Alhazza, I. M., and Valko, M. (2019) 'A Switch between Antioxidant and Prooxidant Properties of the Phenolic Compounds Myricetin, Morin, 3′,4′-Dihydroxyflavone, Taxifolin and 4-Hydroxy-Coumarin in the Presence of

Copper(II) Ions: A Spectroscopic, Absorption Titration and DNA Damage Study', *Molecules*, 24(23), 4335.

Juan-Borrás, M., Soto, J., Gil-Sánchez, L., Pascual-Maté, A., and Escriche, I. (2016) 'Antioxidant activity and physico-chemical parameters for the differentiation of honey using a potentiometric electronic tongue', *Journal of the Science of Food and Agriculture*, 97(7), 2215–2222.

Kabuto, H., Nishizawa, M., Tada, M., Higashio, C., Shishibori, T., and Kohno, M. (2005) 'Zingerone [4-(4-hydroxy-3-methoxyphenyl)-2-butanone] prevents 6-hydroxydopamine-induced dopamine depression in mouse striatum and increases superoxide scavenging activity in serum', *Neurochemical Research.*, 30, 325–332.

Kang, N. J., Jin, H.-S., Lee, S.-E., Kim, H. J., Koh, H., and Lee, D.-W. (2019) 'New approaches towards the discovery and evaluation of bioactive peptides from natural resources', *Critical Reviews in Environmental Science and Technology*, 1–32.

Karimi, E., Oskoueian, E., Hendra, R., Oskoueian, A., and Jaafar, H. Z. E. (2012) 'Phenolic compounds characterization and biological activities of *Citrus aurantium* bloom', *Molecules*, 17, 1203–1218.

Khan, S. U., Anjum, S. I., Rahman, K., Ansari, M. J., Khan, W. U., Kamal, S., Khattak, B., Muhammad, A., and Khan, H. U. (2018) 'Honey: Single food stuff comprises many drugs', *Saudi Journal of Biological Sciences*, 25(2), 320–325.

Kim, B.-R., Kim, H. M., Jin, C. H., Kang, S.-Y., Kim, J.-B., Jeon, Y. G., … Han, A.-R. (2020) 'Composition and Antioxidant Activities of Volatile Organic Compounds in Radiation-Bred Coreopsis Cultivars', *Plants*, 9(6), 717.

Korhonen, H., and Pihlanto, A. (2003) 'Food-derived Bioactive Peptides - Opportunities for Designing Future Foods', *Current Pharmaceutical Design*, 9(16), 1297–1308.

Lodish, H., Berk, A., Zipursky, S.L., et al. (2000). *Section 4.4, The Three Roles of RNA in Protein Synthesis. Molecular Cell Biology.* Available from: https://www.ncbi.nlm.nih.gov/books/NBK21603/ (Accessed: 1st September 2021)

Locatelli, D. A., Altamirano, J. C., Luco, J. M., Norlin, R., and Camargo, A. B. (2014) 'Solid phase microextraction coupled to liquid chromatography.

Analysis of organosulphur compounds avoiding artifacts formation', *Food Chemistry*, 157, 199-204.

Lusby, P.E., Coombes, A. B., and Wilkinson, J. M. (2002) 'Honey: a potent agent for wound healing?' *Journal of Wound, Ostomy and Continence Nursing,* 29(6), 295-300.

Lusby, P., Coombes, A. and Wilkinson, J. (2005) 'Bactericidal activity of different honeys against pathogenic bacteria', *Archieves of Medical Research,* 36(5), 464–467.

Mandal, M.D. and Mandal, S. (2011) 'Honey: Its medicinal property and antibacterial activity', *Asian Pacific Journal of Tropical Biomedicine,* 1(2), 154–160.

Manikandan, P. and Nagini, S. (2018) 'Cytochrome P450 Structure, Function and Clinical Significance: A Review', *Current Drug Targets,* 19(1), 38- 54.

Mundo, M.A., Padilla-Zakour, O.I. and Worobo, R.W. (2004) 'Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys', *International Journal of Food Microbiology*, 97(1), 1–8.

Mascolo, N., Jain, R., Jain, S. C., and Capasso, F. (1989) 'Ethnopharmacologic investigation of ginger (Zingiber officinale)', *Journal of Ethnopharmacology,* 27(1), 129-140.

Malu, S. P., Obochi, G. O., Tawo, E. N., and Nyong, B. E. (2009) 'Antibacterial activity and medicinal properties of ginger (*Zingiber officinale*)', *Global Journal of Pure and Applied Sciences,* 15(3), 365-368.

Mashhadi, N. S., Ghiasvand, R., Askari, G., Hariri, M., Darvishi, L., and Mofid, M. R. (2013) 'Anti-oxidative and anti-inflammatory effects of ginger in health and physical activity: A review of current evidence', *Intermational Journal of Prevention Medicine,* 4(1), 36-42.

Mathai, K., Anand, S., Aravind, A., Dinatius, P., Krishnan, A. V., and Mathai, M. (2017) 'Antimicrobial effect of ginger, garlic, honey and lemon extracts on *Streptococcus mutans*', *Journal of Contemporary Dental Practice,* 18(11), 1004-1008.

Maestri, E., Marmiroli, M., and Marmiroli, N. (2016) 'Bioactive peptides in plant-derived foodstuffs', *Journal of Proteomics,* 147, 140–155.

Meena, R.S., Kumar, S., Datta, R., Lal, R., Vijayakumar, V., Brtnicky, M., Sharma, M.P., Yadav, G.S., Jhariya, M.K., Jangir, C.K., Pathan, S.I., Dokulilova, T.,

Pecina, V., and Marfo, T.D. (2020) ‘Impact of Agrochemicals on Soil Microbiota and Management: A Review’, *Land* , *9*, 34.

Metwali, Z., Abdalla, N., and El Barrani, R. (2014) ‘A Natural Alternative to Conventional Antibiotics’, *Research Journal of Pharmaceutical, Biological and Chemical Sciences,* 5(4), 588-599.

Mota, A. C. L. G. and Oliveira, J. D. A. (2014) ‘Antifungal Activity of Apple Cider Vinegar on Candida Species Involved in Denture Stomatitis’, *Journal of Prosthodontics*. 1–7.

Naseem, E., Shamim, M. & Khan, N. I. (2016) ‘Cardioprotective effects of herbal mixture (ginger, garlic, lemon, apple cider vinegar & honey) in experimental animal models of hyperlipidemia’, *International Journal of Biological Research,* 4(1), 28-33.

Nazıroğlu, M., Güler, M., Özgül, C., Saydam, G., Küçükayaz, M., and Sözbir, E. (2014) ‘Apple Cider Vinegar Modulates Serum Lipid Profile, Erythrocyte, Kidney, and Liver Membrane Oxidative Stress in Ovariectomized Mice Fed High Cholesterol’, *The Journal of Membrane Biology*, 247(8), 667–673.

Negrulescu, A., Patrulea, V., Mincea, M. M., Ionascu, C., Vlad-Oros, B. A., and Ostafe, V. (2012) ‘Adapting the reducing sugars method with dinitrosalicylic acid to microtiter plates and microwave heating’, *Journal of the Brazilian Chemical Society,*23(12), 2176–2182.

Niu S, Luo M, Tang J, Zhou H, Zhang Y, et al. (2013) ‘Structural Basis of the Novel S. pneumoniae Virulence Factor, GHIP, a Glycosyl Hydrolase 25 Participating in Host-Cell Invasion’, *PLoS ONE,* 8(7): e68647. doi:10.1371/journal.pone.0068647

Oikeh, E. I., Omoregie, E. S., Oviasogie, F. E and Oriakhi, K. (2015) ‘Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates’, *Food Science & Nutrition,* 4(1), 103–109.

Olaoluwa, O., Moronkola, D., Taiwo, O., and Iganboh, P. (2018) ‘Volatile oil composition, antioxidant and antimicrobial properties of *Boerhavia erecta L*. and *Euphorbia hirta L*’, *Trends in Phytochemical Research*, 2, 171–178.

Orlich, M. J., Singh, P. N., Sabate´, J., Jaceldo-Siegl, K., Fan, J., Knutsen, S., Beeson, W. L., and Fraser, G.E. (2013) ‘Vegetarian dietary patterns and mortality in Adventist Health Study 2’, *JAMA International Medicine,* 173(13), 1230–1238.

Osés, S. M., Pascual-Maté, A., Fernández-Muiño, M. A., López-Díaz, T. M., and Sancho, M. T. (2016) 'Bioactive properties of honey with propolis', *Food Chemistry*, 196, 1215–1223.

Palaksha, M. N., Ahmed, M. and Das, S. (2010) 'Antibacterial activity of garlic extracts on streptomycin-resistant *Staphylococcus aureus* and *Escherichia coli* solely and in synergism with streptomycin', *Journal of Natural* Science, *Biology and Medicine,*1, 12-15.

Pappalardo, M., Pappalardo, L., and Brooks, P. (2016) 'Rapid and Reliable HPLC Method for the Simultaneous Determination of Dihydroxyacetone, Methylglyoxal and 5-Hydroxymethylfurfural in *Leptospermum* Honeys', *PLOS ONE,* 11(11), e0167006. doi:10.1371/journal.pone.0167006

Pataca, L., Neto, W., Marcucci, M., and Poppi, R. (2007) 'Determination of apparent reducing sugars, moisture and acidity in honey by attenuated total reflectance-Fourier transform infrared spectrometry', *Talanta,* 71(5), 1926–1931.

Patil, S. P., Goswami, A., Kalia, K., ang Kate, A. S. (2019) 'Plant-Derived Bioactive Peptides: A Treatment to Cure Diabetes', *International Journal of Peptide Research and Therapeutics,* 26, 955- 968.

Peterson, J. J., Beecher, G. R., Bhagwat, S. A., Dwyer, J. T., Gebhardt, S. E., Haytowitz, D. B., and Holden, J. M. (2006) 'Flavanones in grapefruit, lemons, and limes: A compilation and review of the data from the analytical literature', *Journal of Food Composition and Analysis,* 19, 74–80.

Pourmouzafar, S., Hajimardloo, A. and Mindare, H. K. (2017) 'Dietary effect of apple cider vinegar and propionic acid on immune related transcriptional response and growth performance in white shrimp', Litopenaeus vannamei. *Fish Shellfsh Immunology*. 60, 60–71.

Prasad, S. and Tyagi, A. K. (2015) 'Ginger and Its Constituents: Role in Prevention and Treatment of Gastrointestinal Cancer', *Gastroenterology Research and Practice,* 1–11.

Pulido, R.., Bravo, L. and Saura-Calixto, F. (2000) 'Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay', *Journal of Agricultural and Food Chemistry*, 48(8), 3396-3402.

Qamar, S., Manrique, Y. J., Parekh, H., and Falconer, J. R. (2019) 'Nuts, cereals, seeds and legumes proteins derived emulsifiers as a source of plant protein beverages: A review', *Critical Reviews in Food Science and Nutrition,* 1–21.

Rajsekhar, S., Khuldeep, B., Chandaker, A., and Upmanyu, N. (2012) 'Spices as antimicrobial agent: A review', *International Research Journal of Pharmacy,* 3(2), 4-9.

Rizzello, C. G., Tagliazucchi, D., Babini, E., Rutella, G. S., Saa, D. L. T. and Gianotti, A. (2016) 'Bioactive peptides from vegetable food matrices: Research trends and novel technologies for synthesis and recovery', *Journal of Functional Foods,* 27(1), 549-569.

Rekha, C., Poornima, G., Manasa, M., Abhipsa, V., Pavithra Devi, J., Vijay Kumar, H. T., et al. (2012) 'Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits,' *Chem. Sci. Trans,* 1, 303–310.

Rivlin, R. S. (2001) 'Historical perspective on the use of garlic', *Journal of Nutrition,* 131, 951–954.

Rodriguez-Casado, A. (2014) 'The Health Potential of Fruits and Vegetables Phytochemicals: Notable Examples,' *Critical Reviews in Food Science and Nutrition,* 56(7), 1097–1107.

Samarghandian, S., Farkhondeh, T. and Samini, F. (2017) 'Honey and health: A review of recent clinical research', *Pharmacogn. Res.*, 9(2), 121.

Sanchez, M. C., Larrauri, J. A. and Saura, C. F. (1998) 'A procedure to measure the antiradical efficiency of polyphenols', *Journal of the Science of Food and Agriculture,* 76(1), 270-276.

Santhosha, S.G., Jamuna, P. and Prabhavathi, S.N. (2013) 'Bioactive Components of Garlic and Their Physiological Role in Health Maintainance: A Review', *Food Bioscience,* 3, 59-74.

Semiz, G., Semiz, A., and Mercan-Doğan, N. (2018) 'Essential oil composition, total phenolic content, antioxidant and antibiofilm activities of four Origanum species from southeastern Turkey', *International Journal of Food Properties,* 21(1), 194–204.

Shahrajabian, M. H., Sun, W., and Cheng, Q. (2019) 'Clinical aspects and health benefits of ginger (*Zingiber officinale*) in both traditional Chinese medicine

and modern industry', *Acta Agriculturae Scandinavica*, Section B — Soil & Plant Science, 1–11.

Shi, J., Xu, C., Xiang, L., Chen, J., and Cai, Z. (2020) 'Tris(2,4-di-tert-butylphenyl) Phosphate: An Unexpected Abundant Toxic Pollutant Found in $PM_{2.5}$. *Environmental Science & Technology*. doi:10.1021/acs.est.0c03709

Shukla, A., Goud, V. V., and Das, C. (2019) 'Antioxidant properties and nutritional composition of selected ginger varieties found in Northeast India,' *Industrial Crops & Products*, 128, 167-176.

Shukla, Y. and Singh, M. (2007) 'Cancer preventive properties of ginger: A brief review', *Food and Chemical Toxicology*, 45(5), 683–690.

Sicari, V., Pellicanò, T. M., Giuffrè, A. M., Zappia, C., and Capocasale, M. (2016) 'Bioactive compounds and antioxidant activity of citrus juices produced from varieties cultivated in Calabria', *Journal of Food Measurement and Characterization*, 10(4), 773–780.

So, F. V., Guthrie, N., Chambers, A. F., Moussa, M., and Carroll, K. K. (1996) 'Inhibition of human breast cancer cell proliferation and delay of mammary tumorigenesis by flavonoids and citrus juices', *Nutrition and Cancer*, 26(2), 167–181.

Sonawane, S.K. and Arya, S.S. (2018) 'Plant Seed Proteins: Chemistry, Technology and Applications', *Current Research in Nutrition and Food Science Journal, 6*, 461-469.

Switzar, L., Giera, M. and Niessen, W. M. A. (2013) 'Protein digestion: An Overview of the available techniques and recent developments', *Journal of Proteome Research*, 12(3), 1067-1077.

Terio, V., Bozzo, G., Ceci, E., Savarino, A.E., Barrasso, R., Di Pinto, A., Mottola, A., Marchetti, P., Tantillo, G., and Bonerba, E. (2021) 'Methylglyoxal (MGO) in Italian Honey', *Applied Sciences*; 11(2), 831.

Tocmo, R., Wu, Y., Liang, D., Fogliano, V., and Huang, D. (2016) 'Data on the effect of boiling on the organosulfides and the hydrogen sulfide-releasing activity of garlic', *Data in brief*, 10, 221–226.

Uhlig, T., Kyprianou, T., Martinelli, F. G., Oppici, C. A., Heiligers, D., Hills, D., et al. (2014) 'The emergence of peptides in the pharmaceutical business: From exploration to exploitation', *EuPA Open Proteomics*, 4, 58–69.

Verma, S., Nizam, S., and Verma, P. K. (2013) 'Biotic and Abiotic Stress Signaling in Plants', *Stress Signaling in Plants: Genomics and Proteomics Perspective*, 1, 25–49.

Vlasova, I. I. (2018) 'Peroxidase Activity of Human Hemoproteins: Keeping the Fire under Control', *Molecules*, 23(10), 2561

Wabaidur, S. M., Obbed, M. S., AlOthman, Z. A., AlFaris, N. A, Badjah-Hadj-Ahmed, A. Y., Siddiqui, M. R., AlTamimi, J. Z., and AlDayel, T. S. (2020) 'Total phenolic acids and flavonoid contents determination in Yemeni honey of various floral sources: Folin-Ciocalteu and spectrophotometric approach', *Food Science and Technology*, 40(2), 647-652.

Wang, S., Meckling, K. A., Marcone, M. F., Kakuda, Y., and Tsao, R. (2011) Synergistic, Additive, and Antagonistic Effects of Food Mixtures on Total Antioxidant Capacities. *Journal of Agricultural and Food Chemistry*, 59(3), 960–968.

Yagnik, D., Serafin, V. and Shah, A. J. (2018) 'Antimicrobial activity of apple cider vinegar against *Escherichia coli, Staphylococcus aureus* and *Candida albicans;* downregulating cytokine and microbial protein expression', *Nature: Scientific Reports*, 8(1732), 1-12.

Yoshida, K., Kaothien, P., Matsui, T., Kawaoka, A. and Shinmyo, A. (2003) 'Molecular biology and application of plant peroxidase', *Applied Micobiology and Biotechnology*, 60(6), 665-670.

Yusha'u, M., Garba, L. and Shamsuddeen, U. (2008) 'In vitro inhibitory activity of garlic and ginger extracts on some respiratory tract isolates of gram-negative organisms', *Journal of Biochemistry, Microbiology and Biotechnology*, 4(2), 57-59.

Zheng, W. and Wang, S. Y. (2001) 'Antioxidant activity and phenolic compounds in selected herbs', *Journal of Agriculture Food Chemistry*, 49, 5165–5170.

LIST OF PUBLICATIONS

Non-Indexed Conference Proceedings

1. Wan, N. A. W. M. & Zaidah, R. (2019). Antioxidant activity of local commercially homemade juice. In *AFOB Malaysia Chapter International Symposium.* AFOBMCIS 2019.

2. Wan, N. A. W. M. & Zaidah, R. (2019). Bioactive constituents of natural infused juice. In *2nd International Conference on Biosciences & Medical Engineering* (pp. 46). ICBME2019.

3. Wan, N. A. W. M. & Zaidah, R. (2021). Antioxidant activity of local commercially homemade juice. In *3rd International Conference on Biosciences & Medical Engineering*. ICBME2020