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ANTIHYPERTENSIVE EFFECT OF *Raphanus sativus* (RADISH) TAPROOT ETHANOL EXTRACT ON ALBINO *Mus musculus* (MOUSE) WITH INDUCED HYPERTENSION

Teresita E. Chiu, Angelo Miguel Ballesteros, Jude Oliver Aves, Edward Bongalos, Dianne Dente, Mahender Devaram Choudhary, Phrexus Docena, Godan Longakit, Zipporah Keim Ortega, Carolly Tabaloc, Raphaella Kailtim

College of Medicine
Cebu Doctors' University

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Corresponding Author: Teresita E. Chiu, Angelo Miguel Ballesteros, Jude Oliver Aves, Edward Bongalos, Dianne Dente, Mahender Devaram Choudhary, Phrexus Docena, Godan Longakit, Zipporah Keim Ortega, Carolly Tabaloc, Raphaella Kailtim, College of Medicine, Cebu Doctors' University, Mandaue City, Cebu, Philippines

Abstract: This study aims to determine the antihypertensive effect of *Raphanus sativus* (radish) taproot ethanol extract on albino *Mus musculus* (mouse) with induced hypertension. Antihypertensive effect was defined as a decrease of 3% and 4% in systolic blood pressure (SBP) and diastolic blood pressure (DBP), respectively. A randomized controlled trial experimental procedure was conducted with a total of 36 albino mice as subjects, with 12 mice in each treatment group (experimental, positive control, and negative control groups). The Non-invasive Blood Pressure Recorder Series 5800 and Lab Chart ver. 8 software were used to measure and record blood pressure. The baseline blood pressure was recorded in the first week. Hypertension was induced using fructose-NaCl solution as drinking water ad libitum for 7 days, with blood pressure recordings performed thereafter. The powdered plant material underwent soxhlation to extract the *Raphanus sativus* (radish) taproot ethanol extract. Treatment was administered for 7 days via oral gavage, with blood pressure recordings performed thereafter. Post-treatment SBP and DBP of the positive control group were within the normotensive range (Pre-treatment SBP: 202.20±14.71 mmHg, DBP: 154.48±24.47 mmHg; post-treatment SBP: 137.16±7.82 mmHg, DBP: 93.69±18.96 mmHg) while those in the experimental group were above the normotensive range (Pre-treatment SBP: 200.80±13.95 mmHg, DBP: 159.52±22.68 mmHg; post-treatment SBP: 172.79±11.91 mmHg, DBP: 127.28±11.00 mmHg) but there was a significant decrease in their SBP and DBP. Lastly, the negative control showed no decrease in SBP and DBP (Pre-treatment SBP: 206.47±19.66 mmHg, DBP: 162.75±22.23 mmHg; post-treatment SBP: 214.69±18.36 mmHg, DBP: 160.06±30.44 mmHg). *Raphanus sativus* taproot ethanol extract had a potential antihypertensive effect since there was at least a 3% decrease in systolic blood pressure and at least a 4% decrease in the diastolic blood pressure in the albino mice with induced hypertension. Further

exploration and testing of the antihypertensive effect could be done in future studies through increasing the duration of treatment and performance of toxicity testing. Extract purification technique is also recommended to have a greater yield of the active component of the extract and the use of genetically hypertensive mice for a more homogenous baseline value.

Keywords: Antihypertensive effect, radish taproot ethanol extract, *Raphanus sativus taproot*, *Mus musculus with induced hypertension*

I. INTRODUCTION

Hypertension is one of the leading causes of morbidity and mortality worldwide (Haileamlak, 2019). Being a disease itself, hypertension is the leading global risk factor for cardiovascular, renal, neurological, and ophthalmological diseases.

Numerous drugs have been produced and made available to prevent, control, and manage hypertension, but hypertension continues to be a health problem. The Philippine government provides free antihypertensive medications, but the stocks are limited. According to Administrative Order no. 2016-0014, one has to be a member of the DOH Antihypertensive and Diabetes Club and undergo assessment and screening (Department of Health [DOH], 2016). The steps needed to obtain the medications were challenging, especially for hypertensive individuals in low-income and remote communities. These issues, along with strong recommendations from family members, neighbors, and friends, have led many to opt for traditional and alternative medicines (Rahmawati & Bajorek, 2017).

Radish or "labanos" (*Raphanus sativus* L.), is a root vegetable observed to have antihypertensive properties (Rahman et al., 2020). The distinctive taste of its roots and fruit has led to it being widely cultivated in countries like Japan, the Philippines, and Hawaii (Gupta et al., 2003; Nishio, 2017). In previous studies, extracts of its seeds or leaves had been shown to have an antihypertensive effect in rats (Sham et al., 2013). The positive effect of radish seeds and leaves on blood pressure has been attributed to the presence of compounds such as flavonoids and alkaloids (Gamba et al., 2021). Another part of the radish commonly consumed, the taproot, has been shown to possess the same BP-lowering properties (Gutierrez & Perez, 2004). However, at the time being, no formal studies have been done yet to support its possible antihypertensive effect.

Raphanus sativus contains major active compounds, namely: glucosinolates, alkaloids, brassinosteroids, and flavonoids, which have antihypertensive and anti-cancer properties (Gutierrez & Perez, 2004). A study done in Pakistan in 2013 showed that the root also contains phytochemicals such as flavonoids, tannins, phlorotannins, saponins, anthraquinones, steroids, carbohydrates, phytosterol, amino acids, cardiac glycosides, alkaloids, terpenoids, and chalcones (Janjua, 2013). In a study done in Japan by Kumakura et al. (2017), arginine and linoleic acid were identified as other possible antihypertensive factors.

Choline, a metabolite from alkaloid degradation, might be responsible for activating the NO-NOS system, increasing NO synthesis, and causing vasodilation, thus reducing blood pressure (Sham et al., 2013). *Rutin*, a flavonoid in radish, has an in vitro angiotensin-converting enzyme (ACE) inhibitory effect (Dimaandal FP, n.d.). ACE activity is a fundamental pathogenic feature of hypertension, indirectly increasing blood pressure by vasoconstriction (Klabunde, 2022).

For this study, the researchers utilized a drug with an ACE inhibitory effect, Captopril, as a positive control. It is rapidly absorbed orally with a 75% bioavailability and blood levels peak within an hour with a half-life of 2 hours. It is eliminated in the

urine. Oral dosage ranges from 6.25 mg three times daily to 25 mg twice daily. It is among the most active and potent ACE inhibitors (Hardman et al., 2001).

Generally, this study aims to determine the antihypertensive effect of *Raphanus sativus* (radish) taproot ethanol extract on albino *Mus musculus* with induced hypertension, using Captopril as the positive control, distilled water as the negative control and *Raphanus sativus* taproot ethanol extract as the test substance.

II. METHODOLOGY

The study utilized a randomized controlled trial experimental method with three treatment groups namely experimental group treated with *Raphanus sativus* taproot ethanol extract, positive control group treated with Captopril, and negative control group given with distilled water. Research subjects used in this study were mice obtained from and acclimatized at the Cebu Doctors' University (CDU) Animal Research Laboratory (ARL). Measurement of the blood pressure was conducted in the Physiology Laboratory of Cebu Doctors' University College of Medicine (CDU-CM), Mandaue City, Cebu, Philippines.

The number of research subjects, a total of 36 albino mice with 12 per treatment group, was determined using R software for a two-sample comparison of means designed to detect a difference of 21.92 mmHg from the negative control group with 95% certainty and no more than 5% chance of erroneously concluding that a difference exists. To be eligible for inclusion in the study, mice had to be normotensive (SBP 133-160 mmHg; DBP 102-110 mmHg), healthy (groomed themselves), alert, had good appetite, between 8-10 weeks old, male or female albino *Mus musculus* that weighed between 20-40 grams (Timmermann et al., 1998). Excluded in this study were albino house mice whose blood pressures, weight, and age were not within the acceptable range stated above, or were used previously in other experiments.

Data collection and tabulation utilized a Master Data Sheet. The blood pressure of mice was measured using the Non-Invasive Blood Pressure Recorder Series 5800.

Upon the approval by the research panel and CDU's Institutional Animal Care and Use Committee (IACUC), transmittal letters were sent to the Dean of CDU-CM to request permission to access and use the facilities needed for this study, namely: Physiology Laboratory, Experimental Research Laboratory, and the Animal Research Laboratory.

Radishes were bought from Sergio's Farm in Maluray, Dalaguete, Cebu. These were authenticated by a botanist from the Department of Agriculture Regional Field Office No. 7 located in Maguikay Cabahug St., Mandaue City, Cebu, Philippines.

The 5 kg of radish taproot were finely sliced into strips using a peeler and oven-dried at 50°C (Liu, 2011) until the weight was 91% less than the fresh weight (Imthiyas & Seran, 2017), then shredded and pulverized to get a uniformly sized coarse powder. The powdered plant material was subjected to Soxhlet extraction for 7 hours using 95% ethanol as a solvent at a temperature of 60°C (Janjua, 2013). After filtration, the filtrate was evaporated using a rotary vacuum evaporator for the reduction of the volume of the solvent. Complete removal of the solvent was done in a water bath at a temperature not exceeding 60°C to obtain the crude extract. An amber container was used to store the crude extract. The radish taproot ethanol extract was screened for the following phytochemicals: alkaloids, flavonoids, saponins, glycosides, tannins, steroids, and triterpenoids.

Throughout the entire study, food and water were made available to the research

subjects. Acclimatization of mice was done for three weeks, which included exposure to the mouse restrainer and the researchers.

The baseline systolic and diastolic pressures of mice were recorded. High salt (4% NaCl) with 20% fructose dissolved in distilled water was provided as drinking water ad libitum for 7 days to induce hypertension in the mice (Klein & Kiat, 2015). Twenty-five mL was initially placed in the water feeding bottle and the volume was measured every day to determine the amount consumed by the mice each day. The solution was replaced every day to avoid contamination and sediment formation. The monitoring of mice's systolic and diastolic pressures was done after the induction of hypertension. Hypertension was successfully induced if systolic pressure was greater than 160 mmHg and diastolic was greater than 110 mmHg [normal BP: 110-120/80] (Timmermann et al., 1998).

Raphanus sativus taproot ethanol extract was subjected to solubility testing with distilled water. One gram of extract in 1 mL of distilled water was used as a basis for the concentration of the test solution. The extract was then diluted to the appropriate amount of solvent wherein 19g of the radish taproot ethanol extract was diluted in 19 mL of distilled water, producing 1 g/mL of stock solution. *Raphanus sativus* taproot ethanol extract was subjected to quantification for total phenolic content using the Folin-Ciocalteau micro method (Waterhouse, n.d.).

Twenty-four pieces of 25 mg Captopril tablets were dissolved and diluted in 17.14 mL of distilled water, creating a 35 mg/mL solution. There was no administration of anesthetics on any of the mice. Mice from the experimental group were given a 500 mg/kg mouse body weight dose of radish taproot extract once a day for 1 week via oral gavage using a 20-gauge plastic feeding tube about 1.5-2.0 inches long with a smooth, rounded tip.

Mice from the positive control group were given through oral gavage, a dose of 9.1 mg for every 20 g mouse body weight, which was equivalent to a human dose of 50 mg/kg daily for about 1 week. Mice from the negative control group were given distilled water orally ad libitum for 1 week. Blood pressures were recorded after 1 week of treatment.

A non-invasive blood pressure measurement was done using a tail cuff connected to the Powerlab Chart Reader 8 which detected and analyzed volume changes in the tail vasculature. The mice were restrained with the tail hanging freely. Mice were given for adoption after the experiment. Experimental protocols used were approved by the CDU Institutional Animal Care and Use Committee (IACUC).

Repeated Measures ANOVA was used for the comparison of pre-treatment induced hypertension and post-treatment systolic and diastolic blood pressures within the treatment groups. IBM SPSS version 22 was used for data processing and analysis. A $p < 0.05$ was considered significant. Other tests that were used included the Kolmogorov-Smirnov (KS) test, which was utilized to test the normality distribution assumption of one-way ANOVA or ANCOVA using the residuals. Levene's test was also used to test the equality of the treatment group variances using the said residuals. Results were then presented using tables and graphs.

III. RESULTS AND DISCUSSION

Measurements of the BP levels were done pre-treatment and post-treatment. The baseline SBP and DBP among the three treatment groups were within the normal range as depicted in Table 1. Generally, the

mean baseline SBP and DBP of the three treatment groups were homogenous.

Table 1. **Mean of the Baseline Systolic and Diastolic Blood Pressures Among the Treatment Groups**

Treatment Group	Mean±SD (mmHg)		
	n	Systolic	Diastolic
Captopril	12	139.26±8.10	96.33±14.61
Radish Taproot	12	140.45±8.96	96.47±7.87
Distilled Water	12	144.28±15.42	102.02±20.54

Table 2. **Mean of the Pre-treatment Systolic and Diastolic Blood Pressures Among the Treatment Groups**

Treatment Group	Mean±SD (mmHg)		
	n	Systolic	Diastolic
Captopril	12	202.20±14.71	154.48±24.47
Radish Taproot	12	200.80±13.95	159.52±22.68
Distilled Water	12	206.47±19.66	162.75±22.23

Table 2 shows the pre-treatment systolic and diastolic blood pressure recordings among the treatment groups after hypertension was induced. Monitoring of the mice's SBP and DPD was done after induction of hypertension. Hypertension was successfully induced when SBP was greater than 160 mmHg and diastolic was greater than 110 mmHg (Timmerman et al., 1998).

Table 3. **Mean of the Post-Treatment Systolic and Diastolic Blood Pressures Among the Treatment Groups**

Treatment Group	Mean±SD (mmHg)		
	n	Systolic	Diastolic
Captopril	12	137.16±7.82	93.69±18.96
Radish Taproot	12	172.79±11.91	127.28±11.00
Distilled Water	12	214.69±18.36	160.06±30.44

As shown in Table 3, only the post-treatment SBP and DBP of the group treated with Captopril went back to the normotensive range after administration of a daily dose of 9.1 mg/20 g mouse body weight for 7 days. The mice treated with radish taproot extract had a decrease in their SBP and DBP but this was still within the hypertensive range, 3% for SBP and 4% for DBP while those in the negative control group treated with distilled water were not noted to decrease. Bonferroni test for pre-treatment and post-treatment SBP and DBP for mice given distilled water showed no significant change.

Bonferroni post-hoc test showed that on average after induction of hypertension, the SBP of the albino mice treated with Captopril significantly decreased from pre-treatment to post-treatment by 65.04 mmHg,

while the DBP of the mice also significantly decreased from pre-treatment to post-treatment by 58.15 mmHg. This showed at least a 3% and 4% decrease in SBP and DBP, respectively.

Table 4. Bonferroni Test for Pre-treatment and Post-Treatment Systolic and Diastolic Blood Pressures of Albino Mice Among Treatment Groups

	Mean±SD (mmHg)		p-value		Conclusion	
	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic
Positive Control Group	65.04	60.79	<0.0005	<0.0005	Significant	Significant
Treatment Group	28.01	32.24	<0.0005	0.001	Significant	Significant
Negative Control Group	8.22	2.68	0.116	1.000	Not significant	Not significant

Bonferroni post-hoc test also showed that on average, after induction of hypertension, the SBP of the albino mice treated with radish taproot extract significantly decreased from pre-treatment to post-treatment by 28.01 mmHg while the DBP also significantly decreased from pretreatment to post-treatment by 60.79 mmHg.

The Bonferroni Test also showed that on average, after induction of hypertension, the SBP and DBP of the albino mice treated with distilled water did not have any significant changes from pre-treatment to post-treatment. The test showed that on average, post-treatment SBP and DBP of albino mice treated with distilled water did not return back to their baseline value after treatment.

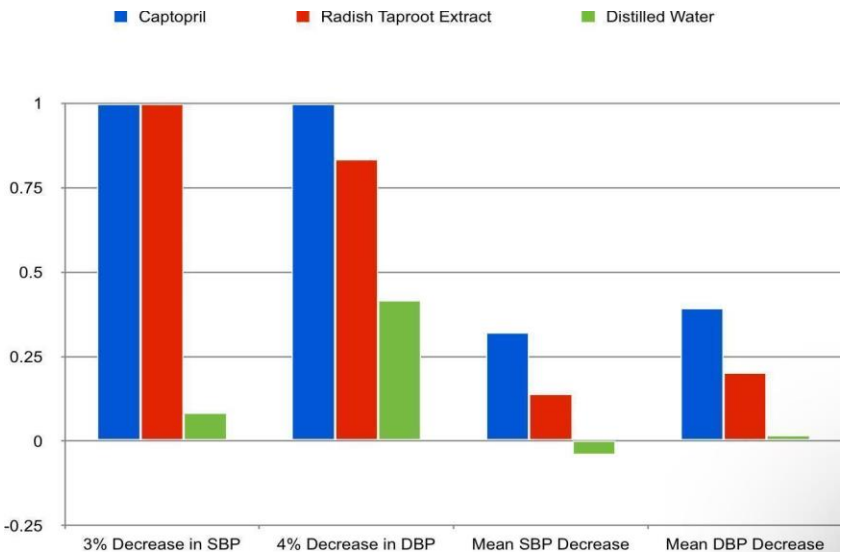

Figure 1. Graphical Representation of the Percentage Decrease of Blood Pressure between Pre-treatment and Post-treatment Reading

Figure 1 shows that all mice treated with Captopril had a decrease of at least 3% in their SBP and at least 4% in their DBP. On the other hand, 100% of the mice given the radish taproot extract had a decline of 3% in their SBP but only 83.33% exhibited at least a 4% decrease in their DBP.

Lastly, only 83.33% and 41.67% of the mice given distilled water exhibited a 3% and 4% decrease in their SBP and DBP, respectively.

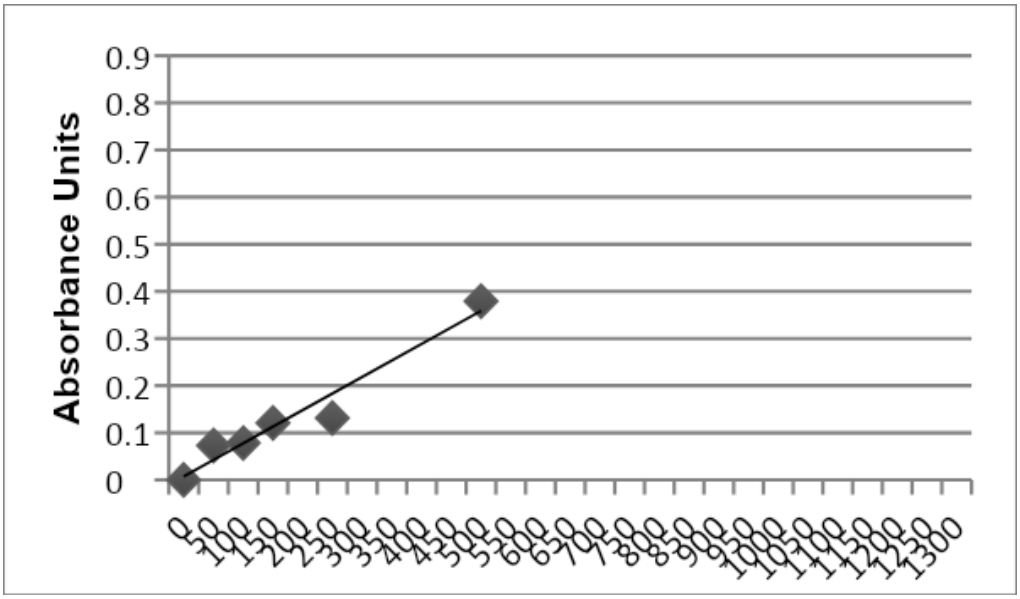

Figure 2. Folin-Ciocalteau Gallic Acid Standard Curve

The Folin-Ciocalteau Gallic Acid Standard (Aryal, 2019) was used to calculate the total phenolic content of the radish taproot extract. The total phenolic content (TPC) was determined by substituting the mean absorbance of the radish taproot extract at 0.735 with y on the formula: y = 0.000 7x + 0.0074, resulting in 1039.43 mg/L Gallic Acid Equivalent (GAE) which was then multiplied by 10, which is the dilution of the sample, yielding 10.39 g/L GAE. These phenolic compounds quantitated in the radish taproot extract include flavonoids and non-flavonoid compounds. These compounds have been associated with a reduced risk of cardiovascular diseases through various mechanisms like lowering platelet aggregation, a decrease in LDL oxidation, and an increase in endothelial Nitric Oxide (NO) (Sharifi-Rad et al., 2021). NO activates the guanylate cyclase located in the smooth muscle cells of blood vessels - which eventually results in the activation of cGMP-dependent protein kinase (PKG) (Pirahanchi & Dimri, 2022). As a result, PKG causes the blood vessels to relax, leading to a subsequent decrease in the pressure exerted against the vessels.

IV. CONCLUSION

The *Raphanus sativus* (radish) taproot ethanol extract was able to produce a 3% and 4% decrease in the systolic and diastolic pressures, respectively, in the albino mice with induced hypertension, however, the post-treatment blood pressures did not go back to baseline values unlike those of the positive control (Captopril) group. Possible reasons for this difference include: degradation of potential antihypertensive substances by the extraction process, low bioavailability of

phenolic compounds once administered to the subjects, the onset of antihypertensive action could have been delayed, or there was tachyphylaxis. The covariates: age of mice (in weeks) and pretreatment SBP and DBP, also influenced the post-treatment blood pressures. Another possibility was that the number of radishes needed to produce the antihypertensive effect may not be adequate if used as monotherapy but could be beneficial if used as a supplement to conventional therapies.

Further exploration and testing of the antihypertensive effect could be done in future studies, which could utilize at least 3 different concentrations of the extract, and an increase in the duration of treatment to 28 days to be able to estimate the effective dose, and to do toxicity studies. Spectrophotometric studies could also be done for verification and comparison of the effect of the test and control substances as well as the different concentrations of the extract substance. In addition, some substances in the plant extract could be labile, so a more efficient preparation or extraction method may be utilized. Acclimatization of the test subjects should be optimized with the use of multiple restrainers in a quiet and warm area while also increasing the duration and shortening the time between the acclimatization period and the administration of the test substances. Finally, genetically hypertensive mice could be used instead of inducing hypertension using high salt and fructose water.

References

Aryal S., Baniya, M.K., Danekhu, K., Kunwar P., Gurung, R., & Koirala, N. (2019) Total phenolic content flavonoid content and antioxidant potential of wild vegetables from western Nepal. *Plants,* *8*(4), 96. https://doi.org/10.3390/plants8040096

Department of Health (2016, May 23). *Implementing guidelines on the organization of health clubs for patients with hypertension and diabetes in health facilities*. https://dmas.doh.gov.ph:8083/Rest/GetFile?id=337083

Gamba, M., Asllanaj, E., Raguindin, P.F., Glisic, M., Franco, O., Minder, B., Bussler, W., Metzger, B., Kern, H., & Taulant, M. (2021). Nutritional and phytochemical characterization of radish (Raphanus sativus): A systematic review. *Trends in Food Science & Technology, 113* 205-218. https://doi.org/10.1016/j.tifs.2021.04.045

Gupta, K., Talwar, G., Jain, V., Dhawan, K., & Jain, S. (2003). Salad crops: Root, bulb, and tuber crops. In B. Caballero (Ed.), *Encyclopedia of food sciences and nutrition* (2nd ed., pp. 5060-5073). Academic Press.

Gutierrez, R.M., & Perez, R.L. (2014.) Raphanus sativus (Radish): Their chemistry and biology. *The Scientific World Journal, 4*, 811-837.

Haileamlak, A. (2019). Hypertension: high and rising burden but getting less attention. *African Journals Online*, *29* (4). http://dx.doi.org/10.4314/ejhs.v29i4.1

Hardman, J. G., Limbird, L. E., Gilman, A. G., Gilman, A., & Goodman, L. S. (2001). Goodman & Gilman's The pharmacological basis of therapeutics. https://doi.org/10.1021/jm020026w

Imthiyas, M., & H. Seran, T. (2017). Marketable tuber yield of radish (Raphanus sativus L.) as influenced by compost and NPK fertilizers. *Research Journal of Agriculture and Forestry Sciences*, *5*(11), 1-4. https://www.researchgate.net/publication/321869289_Marketable_tuber_yield_of_radish_Raphanus_sativus_L_as_influenced_by_compost_and_NPK_fertilizers

Janjua, S. (2013). Phytochemical analysis and in vitro antibacterial activity of root peel extract of Raphanus sativus L. var niger. *Advancement in Medicinal Plant Research*, *1(*1*)*, 1-7

Khonsary, S. (n.d.). Guyton and Hall: Textbook of Medical Physiology. Surgical Neurology International, 8(1), 275.

Klabunde, R. (2022, December 13). *Cardiovascular physiology concept.* https://www.cvphysiology.com/Blood%20Pressure/BP015

Klein, A. V., & Kiat, H. (2015). The mechanisms underlying fructose-induced hypertension: A review. *Journal of Hypertension, 33*(5), 912. https://doi.org/10.1097/HJH.0000000000000551

Kumakura, K., Kato, R., Kobayashi, T., Kimura, N., Takahashi, H., Takahashi, A., & Matsuoka, H. (2017). Salted radish takuan-zuke shows antihypertension effects in spontaneously hypertensive rats. *Food Function*, (10), 3397-3816. https://doi.org/10.1039/C7FO00890B

Liu, W. J. H. (2011). *Traditional herbal medicine research methods: Identification, analysis, bioassay, and pharmaceutical and clinical studies*. https://www.wiley.com/en-sg/Traditional+Herbal+Medicine+Research+Methods:+Identification,+Analysis,+Bioassay,+and+Pharmaceutical+and+Clinical+Studies-p-9780470149362

Nishio, T. (2017). Economic and academic importance of radish. *The radish genome*, Springer. https://link.springer.com/chapter/10.1007/978-3-319-59253-4_1

Pirahanchi Y, & Dimri M. Biochemistry, Guanylate Cyclase. StatPearls. https://www.ncbi.nlm.nih.gov/books/NBK537151/

Rahman, H. S., Bayz, K. A., Hussein, R. H., Abdalla, A.I., Othman, H.H., Amin, K.M., & Abdullah, R. (2020). Phytochemical analysis and hepatoprotective activity of Raphanus sativus var. sativus in Sprage-Dawley rats. *Tropical Journal of Pharmaceutical Research, 19*(8). http://dx.doi.org/10.4314/tjpr.v19i8.25

Rahmawati, R., & Bajorek, B. V. (2017). Self-medication among people living with hypertension: *A review. Family Practice, 34*(2), 147–153. https://doi.org/10.1093/fampra/cmw137

Sham, T.-T., Yuen, A. C.-Y., Ng, Y.-F., Chan, C.-O., Mok, D. K.-W., & Chan, S.-W. (2013). A review of the Phytochemistry and pharmacological activities of raphani semen. *Evidence-Based Complementary and Alternative Medicine*, *2013*, 1–

16. https://doi.org/10.1155/2013/636194

Sharifi-Rad, J., Quispe, C., Zam, W., Kumar, M., Cardoso, S. M., Pereira, O. R., Ademiluyi, A. O., Adeleke, O., Moreira, A. C., Živković, J., Noriega, F., Ayatollahi, S. A., Kobarfard, F., Faizi, M., Martorell, M., Cruz-Martins, N., Butnariu, M., Bagiu, I. C., Bagiu, R. V., Alshehri, M. M., … Cho, W. C. (2021). Phenolic bioactives as antiplatelet aggregation factors: The pivotal ingredients in maintaining cardiovascular health. *Oxidative Medicine and Cellular Longevity*, *2021 (1-19)* https://doi.org/10.1155/2021/2195902

Timmermann, W., Gassel, H.-J., Ulrichs, K., Zhong, R., & Thiede, A. (Eds.). (1998). *Organtransplantation in rats and mice: Microsurgical techniques and immunological principles.* https://www.springer.com/gp/book/9783642721427

Waterhouse, A. (n.d.). *Folin-Ciocalteau micro method for total phenol in wine.* https://waterhouse.ucdavis.edu/folin-ciocalteau-micro-method-total-phenol-wine