EFFECT OF *Lentinus strigosus* ETHANOL EXTRACT ON FOOD INTAKE AND LOCOMOTION OF N2 WILD STRAIN *Caenorhabditis elegans*

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ABSTRACT

AQUINO, YUSELA KRIS D.C., Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, **MAY 2019, EFFECT OF *Lentinus strigosus* ETHANOL EXTRACT ON FOOD INTAKE AND LOCOMOTION OF N2 WILD STRAIN *Caenorhabditis elegans***

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Lentinus strigosus is a wood-rotting edible mushroom that grow mostly in clusters. To determine its potential as fat reducing agent, the fruiting body ethanol extract was tested in *Caenorhabditis elegans*. Nematode lethality test, pharyngeal behavior assay, and locomotion assay were conducted. Lethality test has shown that the extract is slightly toxic. Increasing extract concentration increases toxicity, however, the survival rates are not significantly different. Pharyngeal pumping decreases as extract concentration increases. The lowest pumping rate was recorded at 1000 µg/mL (12.78 pumps per minute (ppm)) and highest at 10 µg/mL (15.64 ppm) at 48 hours post-treatment application while the lowest was recorded at 1000 µg/mL (6.17 ppm) and highest at 10 µg/mL (15.64 ppm) at 120 hours post- treatment application. Locomotion increases as extract concentration increases. Acute locomotion assay shows that the dwelling locomotion of the nematodes was highest at 1000 µg/mL (6.99 reversals per minute) and lowest at 10 µg/mL (3.38 reversals per minute). The roaming locomotion of the nematodes was highest at 1000 µg/mL (48.58 body bends per minute (bbpm)) and lowest at 10 µg/mL (37.53 bbpm). In the chronic locomotion assay, the dwelling locomotion of the nematodes was highest at 1000 µg/mL (4.52 reversals per minute) and lowest at 300 µg/mL (3.43 reversals per minute). Meanwhile, the roaming locomotion of

the nematodes was highest at 1000 µg/mL (30.93) and lowest at 10 µg/mL (23.16 bbpm). These results may be the start of the discovery of a new less- invasive, more cost-effective treatment to obesity.

LITERATURES CITED

Agarwal, S. & Rao, A.V. (2000). Tomato lycopene and its role in human health and chronic diseases. *Canadian Medical Association Journal*, 163,739–44.

Ahn, J., Lee, H., Kima, S., Parka, J., & Taeyoul, H. (2008). The anti-obesity effect of quercetin is mediated by the AMPK and MAPK signaling pathways. *Biochemical and Biophysical Research Community*, 373,545–9.

Anke, H. & Sterner, O. (1997). Nematicidal Metabolites From Higher Fungi. Current Organic Chemistry, 1(2), 361-374.

Ashrafi, K. (2007). Obesity and the regulation of fat metabolism. *WormBook*, 1-20.

Ashrafi, K., Chang, F.Y., Watts, J.L., Fraser, A.G., Kamath, R.S., Ahringer, J., & Ruvkun, G.(2003). Genome-wide RNAi analysis of *Caenorhabditis elegans* fat regulatory genes. *Nature*, 421(6920),268-72.

Astrup, A., Breum, L., & Toubro, S. (1995). Pharmacological and clinical studies of ephedrine and other thermogenic agonists. *Obesity Research*, 3,537S–40S.

Astrup, A., Toubro, S., Christensen, N., & Quade, F. (1992). Pharmacology of thermogenic drugs. *American Journal of Clinical Nutrition*, 55,246S–8S.

Avery, L. & Horvitz, H.R. (1990). Effects of starvation and neuroactive drugs on feeding in *Caenorhabditis elegans*. *J. Exp. Zool.* 253,263–270.

Avery, L. & You. Y.J. (2012). *C. elegans* feeding. *WormBook*. Retrieved from http,//www.wormbook.org.

Barness, L.A., Opitz, J.M., & Gilbert-Barness, E. (2007). Obesity, genetic, molecular, and environmental aspects. *American Journal of Medical Genetics*. Part A. 143A (24), 3016–34.

Byadgi, S. (2016). Extraction and Phytochemical Analysis of Medicinal Plants.

Colquitt, J.L., Pickett, K., Loveman, E., & Frampton, G.K. (2014). Surgery for weight loss in adults. *Cochrane Database of Systematic Reviews*, S,(8). CD003641.

De Castro, M.A.G. & Dulay, R.M.R. (2015). Toxic and Teratogenic Effects of *Lentinus sajor-caju* and *Pleurotus ostreatus* Ethanolic Extracts in *Danio rerio* Embryo Model. *International Journal of Biology, Pharmacy and Allied Sciences*, 4(4), 2261-2269.

LITERATURES CITED

Agarwal, S. & Rao, A.V. (2000). Tomato lycopene and its role in human health and chronic diseases. *Canadian Medical Association Journal*, 163,739-44.

Ahn, J., Lee, H., Kima, S., Parka, J., & Taeyoul, H. (2008). The anti-obesity effect of quercetin is mediated by the AMPK and MAPK signaling pathways. *Biochemical and Biophysical Research Community*, 373,545–9.

Anke, H. & Sterner, O. (1997). Nematicidal Metabolites From Higher Fungi. Current Organic Chemistry, 1(2), 361-374.

Ashrafi, K. (2007). Obesity and the regulation of fat metabolism. *WormBook*, 1-20.

Ashrafi, K., Chang, F.Y., Watts, J.L., Fraser, A.G., Kamath, R.S., Ahringer, J., & Ruvkun, G.(2003). Genome-wide RNAi analysis of *Caenorhabditis elegans* fat regulatory genes. *Nature*, 421(6920),268-72.

Astrup, A., Breum, L., & Toubro, S. (1995). Pharmacological and clinical studies of ephedrine and other thermogenic agonists. *Obesity Research*, 3,537S–40S.

Astrup, A., Toubro, S., Christensen, N., & Quade, F. (1992). Pharmacology of thermogenic drugs. *American Journal of Clinical Nutrition*, 55,246S–8S.

Avery, L. & Horvitz, H.R. (1990). Effects of starvation and neuroactive drugs on feeding in *Caenorhabditis elegans*. *J. Exp. Zool.* 253,263–270.

Avery, L. & You. Y.J. (2012). *C. elegans* feeding. *WormBook*. Retrieved from http,//www.wormbook.org.

Barness, L.A., Opitz, J.M., & Gilbert-Barness, E. (2007). Obesity, genetic, molecular, and environmental aspects. *American Journal of Medical Genetics*. Part A. 143A (24), 3016–34.

Byadgi, S. (2016). Extraction and Phytochemical Analysis of Medicinal Plants.

Colquitt, J.L., Pickett, K., Loveman, E., & Frampton, G.K. (2014). Surgery for weight loss in adults. *Cochrane Database of Systematic Reviews*, 8,(8), CD003641.

De Castro, M.A.G. & Dulay, R.M.R. (2015). Toxic and Teratogenic Effects of *Lentinus sajor-caju* and *Pleurotus ostreatus* Ethanolic Extracts in *Danio rerio* Embryo Model. *International Journal of Biology, Pharmacy and Allied Sciences*, 4(4), 2261-2269.

Doncaster, C. (1962). Nematode feeding mechanisms. I. Observations on Rhabditis and Pelodera. *Nematologica* 8, 313-320.

Dulay, R.M.R. & Garcia, E.J.B. (2017). Optimization and enriched cultivation of Philippine (CLSU) strain of *Lentinus strigosus* (BIL1324). *Biocatalysis and Agricultural Biotechnology*, 12, 323–328.

Dulay, R.M.R., Kalaw, S.P., Reyes, R.G., & Cabrera, E.C. (2014). Embryo-toxic and Teratogenic Effects of Philippine Strain of *Lentinus tigrinus* (Tiger Sawgill Basidiomycetes) Extract on Zebrafish (*Danio rerio*) Embryos. *Annals of Biological Research*, 5(6),9-14.

Dulay R.M.R., Kalaw, S.P., Reyes, R.G., Cabrera, E.C., & Alfonso, N.F. (2012). *Philippine Agricultural Scientist*, 95(3), 278–285.

Dulay, R.M.R. & Pamiloza, D.G. (2018). Proximate Composition and Bioactivities of Hairy Sawgill Mushroom, *Lentinus strigosus* (BIL 1324) From the Philippines. *International Journal of Biology, Pharmacy and Allied Sciences*, 7(3), 361-369.

Ells, L.J. (2018). Diet, genes, and obesity. BMJ, 360, k7.

Elston, T., Wang, H., & Oster, G. Energy transduction in ATP synthase. *Nature*, 391, 510-3.

Finkelstein, E., Trogdon, J., Cohen, J., & Dietz, W. (2009). Annual medical spending attributable to obesity: payer-and service-specific estimates. *Health Affairs*, 28, 822-831.

Fujiwara, M., Sengupta, P., & McIntire, S. L. (2002). Regulation of body size and behavioral state of *C. elegans* by sensory perception and the EGL-4 cGMP-dependent protein kinase. *Neuron*, 36, 1091-1102.

Greer, E.L., Dowlatshahi, D., Banko, M.R., Villen, J., Hoang, K., Blanchard, D., Gygi, S.P., & Brunet A. (2007). An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in *C. elegans*. *Current Biology*, 17, 1646–1656.

Gundersen, H.J., Bagger, P., Bendtsen, T.F.,Evans, S.M., Korbo, L., Marcussen, N., Møller, A., Nielsen, K., Nyengaard, J.R., & Pakkenberg, B. (1988). The new stereological tools: disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *Acta pathologica, microbiologica, et immunologica Scandinavica*, 96, 857–881.

Guri, A.J., Hontecillas, R., Si, H., & Liu, D. (2007). Dietary abscisic acid ameliorates glucose tolerance and obesity-related inflammation in db/db mice fed high-fat diets. *Journal of Clinical Nutrition*, 26, 107–16.

Hashmi, S., Wang, Y., Parhar, R.S., Collison, K.S., Conca, W., Al-Mohanna, F., & Gaugler, R. (2013). *Nutrition & Metabolism*, 10, 31.

Hasla, D.W. & James, W.P. (2005). Obesity. *The Lancet*, 366(9492), 1197–209.

Hill, J. (1998). Environmental contributions to the obesity epidemic. *Science*, 280, 1371–1374.

Jensen, E.X., Fusch, C., Jaeger, P., Peheim, E., & Horber, F.F. (1995). Impact of chronic cigarette smoking on body composition and fuel metabolism. *Journal of Clinical Endocrinology and Metabolism*, 80, 2181–5.

Kalaw, S.P., Alfonso, D.O., Dulay, R.M.R., De Leon, A.M., Undan, J.Q., Undan, J.R. & Reyes, R.G. (2016). Optimization of culture conditions for secondary mycelial growth of wild macrofungi from selected areas in Central Luzon, Philippines. *Current Research in Environmental & Applied Mycology*, 6(4), 277–287.

Kang, J.H., Tsuyoshi, G., Han, I.S., Kawada, T., Kim, Y.M., & Yu, R. (2010). Dietary capsaicin reduces obesity-induced insulin resistance and hepatic steatosis in obese mice fed a high-fat diet. Obesity, 18, 780–7.

Kaufinan, P., Cseke, L., Warber, S., Duke, J., Brielmann, H. (1999). Natural products from plants. Boston, CRC Press.

Kim, H.M, Do, C.H., and Lee, D.H. (2009). Characterization of taurine as anti-obesity agent in *C. elegans*. *Journal of Biomedical Science*, 17(Suppl 1), S33.

Kirk, S.F.L., Penney, T.L., McHugh, T.L.F., and Sharma, A.M. (2011). Effective weight management practice, a review of the lifestyle intervention evidence. *International Journal of Obesity*.

Lau, D.C., Douketis, J.D., Morrison, K.M., Hramiak, I.M., Sharma, A.M., and Ur, E. (2007). "2006 Canadian clinical practice guidelines on the management and prevention of obesity in adults and children [summary]". *Canadian Medical Association Journal (Practice Guideline, Review)*, 176 (8), S1–13.

Leung, K.F., Baron, R., & Seabra, M.C. (2006). Thematic review series: Lipid posttranslational modifications. *Journal of Lipid Research*, 47, 467-475.

Lindequist, U., Niedermeyer, THJ., & Julich, W.D. (2005). The pharmacological potentials of mushrooms. *Evidence Based Complementary and Alternative Medicine* (eCAM), 2, 285–299.

Liu, L.X., Spoerke, J.M., Mulligan, E.L., Chen, J., Reardon, B., Westlund, B., Sun, L., Abel, K., Armstrong, B., Hardiman, G.,King, J., McCague, L., Basson, M., Clover,

R., & Johnson, C.D. (1999). High-Throughput Isolation of *Caenorhabditis elegans Deletion Mutants. Genome Research*, 9, 859–867.

Lutz, T.A. & Woods, S.C. (2012). Overview of Animal Models of Obesity. *Current Protocols in Pharmacology*.

Mak,Y., Nelson, L.S., Basson, M., Johnson, C.D., & Ruvkun, G. (2006). Polygenic control of *Caenorhabditis elegans* fat storage. *Nature Genetics*, 38, 363–368.

McKay, R.M., McKay, J.P., Avery, L., & Graff, J.M. (2003). *C. elegans*: a model for exploring the genetics of fat storage. *Developmental Cell*, 4(1),131-42.

Misra, A. & Khurana, L. (2008). Obesity and the metabolic syndrome in developing countries. *The Journal of Clinical Endocrinology and Metabolism*, 93(11 Suppl1), S9.

Moores, D. (2018). Obesity. Healthline Newsletter. Retrieved from https://www.healthline.com/health/obesity

Must, A., Spadano, J., Coakley, E.H., Field, A.E., Colditz, G., & Dietz, W.H.(1999). The disease burden associated with overweight and obesity. *The Journal of the American Medical Association*, 282(16), 1523–1529.

Mohamed, G.A., Ibrahim, S.R.M., Elkhayat, E.S., & El Dine, R.S. (2014). Natural anti-obesity agents. *Bulletin of Faculty of Pharmacy*, Cairo University.

Mokdad, A.H., Marks, J.S., Stroup, D.F., & Gerberding, J.L. (2004). Actual causes of death in the United States, 2000. *The Journal of the American Medical Association*, 291 (10), 1238–45.

Ness-Abramof, R. & Apovian, CM. (2006). Diet modification for treatment and prevention of obesity. *Endocrine*, 29(1), 5-9.

Ofei, F. (2005). Obesity – A Preventable Disease. *Ghana Medical Journal*, 39(3).

Pascua, M.S., Kalaw, S.P., & De Leon, A.M. (2016). Proximate Composition, Mycochemical Analysis and Antibacterial Activity of *Lentinus squarrosulus* (Mont.) Singer. *Advances in Environmental Biology*, 10(3), 58-68.

Patel, Y., Naraian, R., & Singh, V.K. (2012). Medicinal Properties of Pleurotus Species (Oyster Mushroom), A Review. *World Journal of Fungal and Plant Biology*, 3(1), 1-12.

Peeters, A., Barendregt, J.J., Willekens, F., Mackenbach, J.P., Al Mamun, A., & Bonneux, L. Obesity in adulthood and its consequences for life expectancy: a life-table analysis. *Annals of Internal Medicine*, 138, 24-32.

Poulain, M., Doucet, M., Major, G.C., Drapeau, V., Sériès, F., Boulet, L.P., Tremblay, A., & Maltais, F. (2006). The effect of obesity on chronic respiratory diseases: pathophysiology and therapeutic strategies. *Canadian Medical Association Journal*, 174(9), 1293-9.

Qiao, Y., Zhao, Y., Wu, Q., Sun, L., Ruan, Q., Chen, Y., Wang, M.,Duan, J., & Wang, F. (2014). Full Toxicity Assessment of Genkwa Flos and the Underlying Mechanism in Nematode *Caenorhabditis elegans*. *Public Library of Science One*, 9(3), e91825.

Raizen, D., Song, B., Trojanowski, N., & You, Y.J. (2005). Methods for measuring pharyngeal behaviors. *WormBook: The Online Review of C. elegans Biology*.

Rayalam, S., Della- Fera, M.A., & Baile, C.A. (2008). Phytochemicals and regulation of adipocyte life cycle. *Journal of Nutritional Biochemistry*, 19, 717-26.

Reyes, R.G., Kalaw, S.P., Dulay, R.M.R., Yoshimoto, H., Miyazawa, N., Seyama, T. & Eguchi, F. (2013). Philippine Native and Exotic Species of Edible Mushrooms Grown on Rice-Straw-Based Formulation Exhibit Nutraceutical Properties. *The Philippine Agricultural Scientist*, 96 (2), 198–204.

Reyes-Escogido, M.L., Gonzalez-Mondragon, E.G., & Vazquez-Tzompantzi, E. (2011). Chemical and pharmacological aspects of capsaicin. *Molecules*,16,1253–70.

Santos, A.P., Rogero, M.M., & Bastos, D.H. (2010). Edible plants, their secondary metabolites, and antiobesogenic potential. *Recent Patents on Food, Nutrition and Agriculture*, 2,195-212.

Segalat, L. (2007). Invertebrate animal models of diseases as screening tools in drug discovery. *American Chemical Society Chemical Biology*, 2, 231–236.

Seymour, M., Wright, K., & Doncaster, C. (1983). The action of the anterior feeding apparatus of *Caenorhabditis elegans* (Nematoda: Rhabditida). *Journal of Zoology Society of. London*, 201, 527-539.

Shick, S.M., Wing, R.R., Klem, M.L., McGuire, M.T., Hill, J.O., & Seagle, H. (1998). Persons successful at long-term weight loss and maintenance continue to consume a low-energy, low-fat diet. *Journal of the American Dietetic Association*, 98(4), 408–13.

Shimoda, H., Seki, E., & Aitani, M. Inhibitory effect of green coffee bean extract on fat accumulation and body weight gain in mice. *BMC Complementary Alternative Medicine*, 6,9-13.

Son, M.J., Rico, C.W., Nam, S.H., & Kang, M.Y. (2010). Influence of oryzanol and ferulic acid on the lipid metabolism and antioxidative status in high fat-fed mice. *Journal of Clinical Biochemistry and Nutrition*, 46, 150–6.

Srinivasan, S., Sadegh, L., Elle, I.C., Christensen, A.G.L., Faergeman, N.J., & Ashrafi, K. (2008). Serotonin regulates *C. elegans* fat and feeding through independent molecular mechanisms. *Cell Metabolism*, 7, 533–544.

Stiernagle, T. (2006). Maintenance of *C. elegans*, WormBook, ed. The *C. elegans* Research Community, *WormBook*.

Suh, J.M., Zeve, D., McKay, R., Seo, J., Salo, Z., Li, R., Wang, M., & Graff, J.M. (2007). Adipose is a conserved dosage-sensitive antiobesity gene. *Cell Metabolism*, 6, 195-207.

Tate, D.F., Jeffery, R.W., Sherwood, N.E., & Wing, R.R. (2007). "Long-term weight losses associated with prescription of higher physical activity goals. Are higher levels of physical activity protective against weight regain?". *The American Journal of Clinical Nutrition (Randomized Controlled Trial*, 85 (4), 954–9.

The *C. elegans* Sequencing Consortium. (1998). Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science*; 282, 2012–2018.

Tsai, A.G., Wadden, T.A., Volger, S., Sarwer, D.B., Vetter, M.,Kumanyika, S., Berkowitz, R.I., Diewald, L., Perez, J., Lavenberg, J., Panigrahi, E.R., & Glick, H.A. (2013). Cost-effectiveness of a Primary Care Intervention to Treat Obesity. *International Journal of Obesity*, 37(1), S31–S37.

Tucci, S.A. (2010). Phytochemicals in the control of human appetite and body weight. *Pharmaceuticals*, 3, 748–63.

Turner, M., Jannah, N., Kahan, S., Gallagher, C., & Dietz, W. (2018). Current Knowledge of Obesity Treatment Guidelines by Health Care Professionals. *Obesity*, 2, 665-671.

Uemura, T., Goto, T., Kang, M.S., Mizoguchi, N., Hirai, S., Lee, J.Y., Nakano, Y., Shono, J., Hoshinm S., Taketani, K., Tsuge, N., Narukami, T., Makishima, M., Takahashi, N., & Kawada, T. (2011). Diosgenin, the main aglycon of fenugreek, inhibits LXRa activity in HepG2 cells and decreases plasma and hepatic triglycerides in obese diabetic mice. *Journal of Nutrition*, 141, 17–23.

Vivekananthan, D.P., Penn, M.S., Sapp, S.K., & Hsu, A. (2003). Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomized trials. *The Lancet*, 361, 2017–23.

Wagne, G.P. (2007). The developmental genetics of homology. *Nature Reviews Genetics*, 8, 473-479.

Watts, J.L. & Browse, J. (2002). Genetic dissection of polyunsaturated fatty acid synthesis in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 5854-5859.

Weintraub, K. (2014). New allies in war on weight. *The Boston Globe*. Retrieved 11 August 2018.

Wicks, S.R., Yeh, R.T., Gish, W.R., Waterston, R.H., & Plasterk, R.H. (2001).Rapid gene mapping in *Caenorhabditis elegans* using a high density polymorphism map. *Nature Genetics*, 28,160–164.

Wood W. (1998). Introduction to *C. elegans* Biology. *Cold Spring Harbor Monograph Archive*, 1–16.

World Health Organization. (2007). Global strategy on diet, physical activity and health. *WHO: Geneva, Switzerland*. Retrieved 11 August 2018.

World Health Organization. (2008). Obesity and overweight. Retrieved 11 August 2018.

World Health Organization. (2009). WHO | Physical Inactivity: A Global Public Health Problem.

Yazdi, F.T., Clee, S.M. & Meyre, D. (2015). Obesity genetics in mouse and human: back and forth, and back again. *Journal of Life and Environmental Sciences*, 3, e856.

Yen, K., Le, T.T., Bansal, A., Narasimhan, S.D., Cheng, K., & Tissenbaum, H.A. (2010). A Comparative Study of Fat Storage Quantitation in Nematode *Caenorhabditis elegans* Using Label and Label-Free Methods. *Public Library of Science One*, 5(9), e12810.

Zheng, J.,Vasselli, J.R., King, J.F., King, M.L., We, W., Fitzpatrick, Z., Johnson, W.D., Finley, J.W., Martin, R.J., Keenan, M.J., Enright, F.M., & Greenway, F.L. (2014). Using *Caenorhabditis elegans* as a Model for Obesity Pharmacology Development. *American Journal of Therapeutics*, 23(6), e1363-e1370.

Zied, D.C., Pardo, J.E., Tomaz, R.S., Miasaki, C.T., & Pardo-Gimenez A. (2017). Mycochemical Characterization of *Agaricus subrufescens* considering Their Morphological and Physiological Stage of Maturity on the Traceability Process. *BioMed Research International*, 27(1), 37-42.