ASEAN Journal of Science and Engineering

Journal homepage: http://ejournal.upi.edu/index.php/AJSE/

Hypocholesterolemic Effect of Mature Leaf Extract of Sugarcane, Saccharum Officinarum (Linnaeus, 1753), in Induced Rats

Raamah Rosales[1*]

[1]College of Arts and Sciences, Cebu Technological University-Main Campus, Cebu City 600, the Philippines
Correspondence: E-mail: raamah.rosales@ctu.edu.ph

ABSTRACTS

Hypercholesterolemia is a common health risk factor for various cardiovascular diseases. The use of the medicinal plant as an alternative treatment is gaining much attention due to the reported adverse effects of statins on liver and muscle toxicity. This study aimed to determine the hypocholesterolemic properties of the mature leaf of the sugarcane plant using hypercholesterolemia-induced rats. Thirty-two Sprague Dawley rats were divided into 4 groups and fed with a normal diet and a 4% high cholesterol diet. The dose conversion was based on the human dose to surface area ratio of 0.025 at 1mg/20g rat body weight. The treatment with mature leaf extract of *S. officinarum* significantly lowered the cholesterol levels. The hypocholesterolemic activity and percent reduction of the plant extract were comparable to the effects of a synthetic drug, Fluvastatin, in lowering the cholesterol levels. This study presents the potential of mature leaf extract of *S. officinarum* as an alternative therapeutic strategy for hypercholesterolemia.

ARTICLE INFO

Article History:
Received 09 Aug 2021
Revised 07 Sep 2021
Accepted 09 Sep 2021
Available online 11 Sep 2021

Keyword:
Hypocholesterolemic,
Mature,
Rats.

1. INTRODUCTION

Hypercholesterolemia is a health risk condition that plays a role in developing cardiovascular diseases, including atherosclerosis, high blood pressure, or acute myocardial infarction. Hypercholesterolemia is a lipoprotein metabolic syndrome characterized by elevated serum concentration of low-density lipoprotein (LDL) and blood cholesterol (Yokozawa *et al.,* 2006). Various hypocholesterolemic drugs are known to lower cholesterol levels but may have long-term adverse effects such as muscle and liver damage, renal failure, and myopathy. The usage of herbal medicine may provide a safer strategy in treating human diseases.

Plant medicinal potential largely depends on biological compounds derived from secondary metabolites (Masa *et al.,* 2016). These metabolites vary quantitatively among species, between populations of the same species, and between the developmental stages of plant organs such as leaf (Achakzai *et al*., 2009; Azam *et al.,* 2013; El Sawi *et al.,* 2013; Del Valle *et al.,* 2015). Masa *et al., (2016)* quantified flavonoids and diterpenes in leaves of *Cistus ladanifer* L. at different ages. The result showed varying phenolic contents in young, mature leaves and near senescent ones. Watanabe *et al.,* (2016) explained that leafage, either young or mature, affects the distribution of minerals in a different leaf of vegetables cultivated in an open field.

Sugarcane is an economically important agricultural crop in the Philippines. Annual sugar production produces a massive volume of sugarcane leaves that are not fully utilized for other significant purposes. There are no reports in any published literature in a scientific journal on the hypocholesterolemic effects specific to the mature leaf of the sugarcane plant to date. To address this gap, this study aimed at investigating the hypocholesterolemic effect of the mature leaf of sugarcane plant in induced rats.

2. METHODS

2.1 Plant Material

Mature leaf samples were collected from sugarcane plantations in Northern Cebu Island, Philippines (11.1491° N, 123.9861° E) between March to June 2018 and authenticated by the Department of Biology, University of San Carlos, Cebu Philippines. Mature sugarcane leaf is fully expanded and highly exposed to sunlight (Gianotto *et al.,* 2011) (see **Figure 1**). Samples collected were designated as leaf +2, +3, and +4 following the Kujiper (1915) numbering system for the sugarcane plant (Gianotto *et al.,* 2011; Zhao *et al.,* 2012).

DOI: http://dx.doi.org/10. 17509/xxxx.xxxx
p- ISSN 2776-6098 e- ISSN 2776-2538

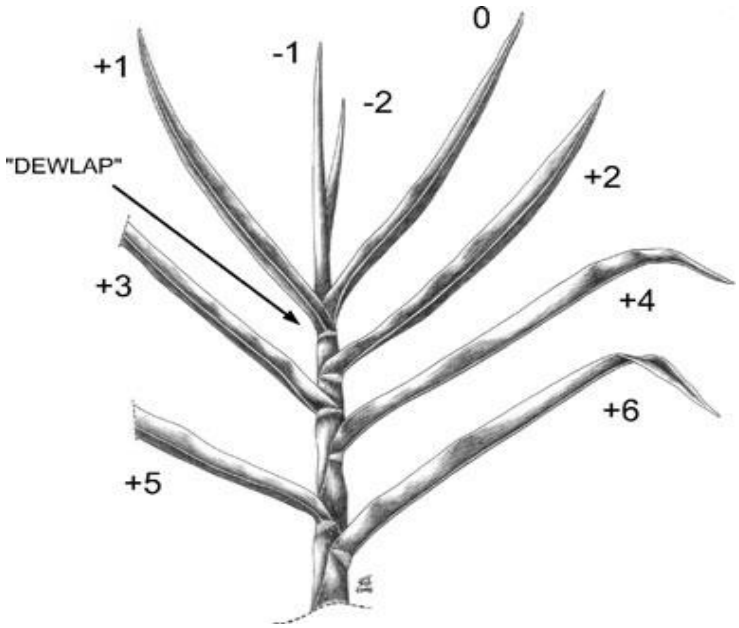

Figure 1. Kujiper's leaf numbering system on sugarcane plant (1915).

2.2 Preparation and Extraction of Plant Samples

A total of 10 kg air-dried and finely chopped leaves macerated with 95 % ethanol were placed in glass containers. The containers were thoroughly shaken and allowed to stand at room temperature with occasional manual agitation using a stirring rod. The mixture was allowed to stand for 72 hr to ensure complete extraction, then strained and the marc discarded. The menstruum was filtered using sterile Whatman no. I filtered paper and concentrated using a rotary evaporator (Heidolph Hei-VAP Platinum 2 Rotary Evaporator, Germany) at 60-65ºC temperature range. The filtrate was placed in a water bath at 75-80ºC temperature range to evaporate to dryness and obtain solvent-free crude extract—a total of 430 g with a percent yield of 4.2 % extracted after the entire concentration process.

2.3 Preparation of Test Solution

Five (5) g of sugarcane leaf extract was dissolved in enough water to make a 10 mL solution. The concentration of this solution reached 50%.

2.4 Preparation of Positive and Negative Control

Forty (40) mg of Fluvastatin sodium tablet was used as the positive control. The tablet was powdered using a mortar and dissolved with a sufficient amount of distilled water to make a 50 mL solution. Distilled water was used for the negative control and administered with the same volume as the positive control.

2.5 Ethical Review

The methods were submitted for ethical review and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of San Carlos-Talamban Campus (USC-TC), Cebu, Philippines, with the approved protocol number: 2018-029-024.

2.6 Preparation of Experimental Animal

Before experiments, male Sprague Dawley rats weighing between 180-200 g of ten (10) days old were obtained from the USC-TC Animal Facility. The rats were acclimated for seven

DOI: http://dx.doi.org/10.17509/xxxx.vxix
p- ISSN NomorISSN e- ISSN NomorISSN

(7) days in an air-conditioned room with an average temperature of 25°C and 55% humidity. The rats were provided free access to food and water on a 12 h light-dark cycle.

Block randomization allocated the test animals through an odd/even scheme. All thirty-two healthy laboratory rats numbered randomly in odd/even. Each of the test groups has an equal number of test animals. The first eight (8) even-numbered rats were assigned to the positive control group, while the first eight (8) odd-numbered rats were assigned to the test group for the crude extract treatment. The same procedure applies to the negative and untreated groups (Conforti *et al.,* 2007).

During the experiments, the rats were in separate cages with eight members big enough for the rats to move around while maintaining the standard conditions to acclimatize them (Kanda *et al*., 2015). The rats fed with the standard animal pellet diet with 225 grams pellets (3.0 grams pellets per rat) and distilled water was given once a day for one week during the entire course of the experiment (Kanda *et al*., 2015). The test animals acclimatize in the laboratory for seven (7) days. The test animals again acclimatize for 10 min before the beginning of testing. The first measurement (T_0) was taken once the acclimation period concluded.

2.7 Determination of Baseline Cholesterol Level

The rats fasted for twelve hours from 8:00 pm – 8:00 am. The body weights of each rat were determined and recorded using a digital weighing scale. A Beaker of predetermined weight was used to contain the rat to facilitate weighing. Topical lidocaine cream was applied prior to tail clipping to prevent pain. After obtaining the weights of each rat, the tail was swabbed with 95% alcohol and then cut at the tip; a drop of blood was directly placed in the test strips of the cholesterol tester (EasyLife GCU Plus with CE 0197, model: ET-3431, Taiwan) to obtain the baseline output of cholesterol.

2.8 Induction of Hypercholesterolemia

The rats were fed 4% of cholesterol powder technical grade once per day for seven consecutive days.

2.9 Determination of Blood Cholesterol Levels

After seven consecutive days of feeding the rats with 4% of cholesterol powder and distilled water three times a day, the cholesterol level of rats was taken as the hypercholesterolemic value.

2.10 Administration of Test Solution and Controls

The thirty-two rats were divided into four (4) groups: positive control, test group for crude extract, negative control, and untreated. Test animals for positive control were given 0.13mL/20g Fluvastatin Sodium. Test animals for negative control were given distilled water. Test animals for test groups using the crude extract were given 0.8ml/20g rats of Test Solutions. The untreated group was not administered with test drugs for the sole reason of determining the highest possible cholesterol level.

The test solutions and the controls were administered orally to rats three times a day at 8:00 am, 12:00 noon, and 6:00 pm for three consecutive days. Cholesterol levels were recorded twelve hours after the third treatment each day. Oral administration was carried out using oral gavage. Dose conversion was based on the human dose to surface area ratio of rats of 0.025 at 1mg/20g body weight.

DOI: http://dx.doi.org/10. 17509/xxxx.xxxx
p- ISSN 2776-6098 e- ISSN 2776-2538

2.11 Statistical Treatment

Values for activity and reduction were presented as mean ± S.D. Tukey HSD tested the statistical difference between the treatment and the controls to elucidate the variation between test groups.

3. RESULTS AND DISCUSSION

3.1 Hypocholesterolemic Mean Activity and Reduction

The mean percent hypocholesterolemic activity varies among the test groups (**Figure 2**). Positive control has the highest mean value of 100%, while the untreated group and negative control have no hypocholesterolemic activity. The test group for crude extract indicated 80.76 % hypocholesterolemic, relatively close to the mean value of the positive control.

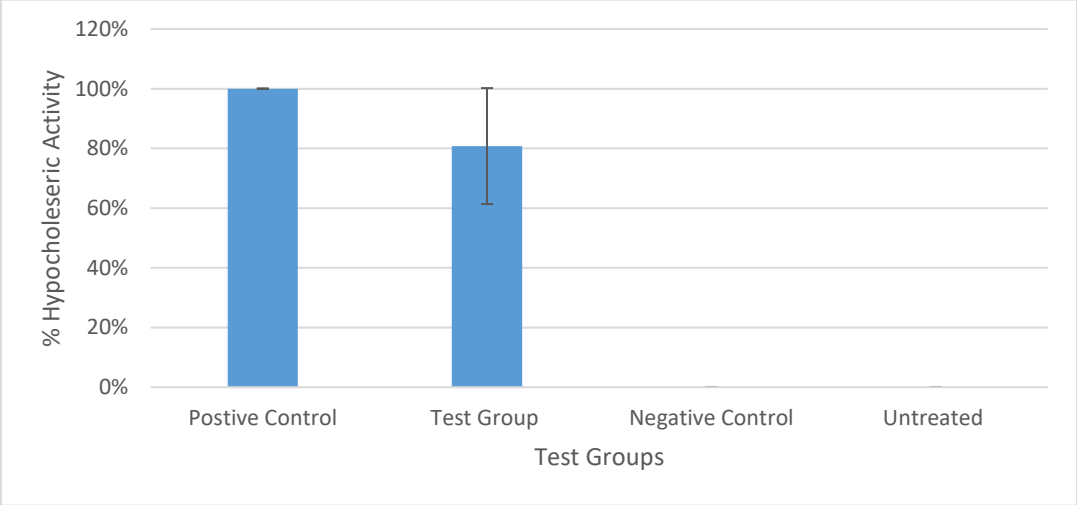

Figure 2. Mean percent hypocholesterolemic activity

Table 1 shows the Tukey HSD test results. The comparison of all groups was significant except between positive control vs. test group for crude extract and negative control vs. untreated group. These groups may have indicated the same % activity and reduction.

Table 1. Post-hoc analysis between each test group for hypocholesterolemic activity.

Tukey HSD	Description	Mean Difference	Std. Error	Sig.	99% Confidence Interval	
					Lower Bound	Upper Bound
(+) Control	Test Group	19.24	4.33	0.020	-38.5378	0.0538
	(-) Control	110.63	14.34	0.000	-129.9307	-91.3391
	Untreated	116.94	15.17	0.000	-136.2347	-97.6431
Test Group	(+) Control	91.39	12.39	0.000	-110.6887	-72.0971
	Untreated	97.69	13.19	0.000	-116.9926	-78.4010
(-) Control	Untreated	6.30	2.13	0.145	-25.5998	12.9910

This study presents the hypocholesterolemic effect of the mature leaf extract of *S. officinarum* in hypercholesterolemia-induced Sprague Dawley rats. Fluvastatin was used as a positive control because it is widely used as a synthetic drug in lowering blood cholesterol

DOI: http://dx.doi.org/10.17509/xxxx.vxix
p- ISSN NomorISSN e- ISSN NomorISSN

levels. The result showed that positive control posted the highest mean % hypocholesterolemic activity value of 100 %, while the test group for crude extract indicated 80.76 % hypocholesterolemic activity. Interestingly, the % hypocholesterolemic activity of the crude extract was relatively close to the synthetic drug. The crude extract has a mean percent reduction of 95.26%, comparable to the 103.94% of the positive control. At a given dose, the result of the crude extract's mean percent reduction and activity showed its potential in lowering blood cholesterol levels. The hypocholesterolemic properties of *S. officinarum* may be due to either the individual or the synergistic action of the phenolic components of the mature plant leaf (Chuerfa & Allen, 2015). Physiological mechanisms of different tissues, such as hydrolytic control of specific lipoproteins and their selective uptake and metabolism, can influence lipidemic profile (Rouhi-Boroujeni *et al.,* 2015).

Sugarcane wax contains phenolic components, including flavonoids, known to possess hypocholesterolemic properties (Singh *et al.,* 2015). Flavonoids can regulate blood lipids by increasing the activity of lecithin acyltransferase (LCAT) (You *et al.,* 2008). LCAT is responsible for the inclusion of free cholesterol in the high-density lipoprotein and its transfer back to very-low-density lipoprotein, which is returned later in liver cells (Dobiásová & Frohlich, 1999).

4. CONCLUSION

In conclusion, this study demonstrates the hypocholesterolemic potential of the mature leaf of *S. officinarum*. It showed hypocholesterolemic activity and reduction comparable to the synthetic drug used in this study. The result explicitly represents the hypocholesterolemic effect of the mature leaf of the sugarcane plant. Future studies may be conducted to confirm these results further.

5. ACKNOWLEDGMENT

The author would like to thank the Department of Science and Technology – Scholarships Education Institute (DOST-SEI) for providing funds for this study. The author would also like to thank Glen Mark Piañar for his assistance in the statistical analysis of the data.

6. AUTHORS' NOTE

The author declares no conflict of interest and the article paper is free of plagiarism.

7. REFERENCES

Achakzai, A.K.K., Achakzai, P., Masood, A., Alikayani, S., and Tareen, R.B. (2009). Response of plants and age on the distribution of secondary metabolites on plants found in Quetta. *Pakistan Journal of Botany.* 41, 2129-2135.

Azam, M., Zhang, B., Jiang, Q., and Chen, K. (2013). Citrus leaf volatiles as affected by developmental stage and genetic type. *International Journal of Molecular Sciences, 14*, 17744-17766.

Kanda, I. (2015). Exotic Animal Formulary. *The Canadian Veterinary Journal, 56*(7), 736.

Chuerfa, M., and Allem, R. (2015). Study of hypocholesterolemic activity of Algerian Pistacia lentiscus leaves extracts in vivo. *Revista Brasileira Farmacognosia, 25*, 142-144.

DOI: http://dx.doi.org/10. 17509/xxxx.xxxx
p- ISSN 2776-6098 e- ISSN 2776-2538

Conforti, A., Bellavite, P., Bertani, S., Chiarotti, F., Menniti-Ippolito, F., and Raschetti, R. (2007). Rat models of acute inflammation: a randomized controlled study on the effects of homeopathic remedies. *Business Model Canvas Complementary and Alternative Medicine, 7*(1), 1-10.

Del Valle, J. C., Buide, M. L., Casimiro-Soriguer, I., Whittall, J. B., and Narbona, E. (2015). On flavonoid accumulation in different plant parts: Variation patterns among individuals and populations in the shore campion (Silene littorea). *Frontiers in Plant Science, 6*, 939.

Dobiásová, M., and Frohlich, J. (1999). Advances in the understanding of the role of lecithin cholesterol acyltransferase (LCAT) in cholesterol transport. *Clinical Chimica Acta, 286*, 257-271.

El Sawi, S.A.M., Mahmoud, D.M.M., and El Alfy, T.S.M.A. (2013). Phytochemical screening and qualitative analysis of the polysaccharide contents of Chorisia insignis. *Journal of Herbs, Spices, and Medicinal Plants, 19*, 313-320.

Gerhardt, A.L., and Gallo, N.N. (1998). Full-fat rice and oat bran similarly reduce hypercholesterolemia in humans. *The Journal of Nutrition*, *128*, 865-869.

Gianotto, A.C., Bespalhok, J., Arruda, P., and Burnquist, W. (2011). Sugarcane (*Saccharum X officinarum*): A reference study for the regulation of genetically modified cultivars in *Brazilian Tropical Plant Biology. Tropical Plant Biology, 4*, 62-89.

Kujiper, J. (1915). Degroei van bladschijf, bladscheede em stengel van het suikerriet. *Arch Suikerind Ned Indi*, *23*, 528–556.

Masa, C. V., Sosa Díaz, T., Alías Gallego, J. C., and Chaves Lobón, N. (2016). Quantitative variation of flavonoids and diterpenes in leaves and stems of Cistus ladanifer L. at different ages. *Molecules, 21*(3), 275.

Nair, A.B., and Jacob, S. (2016). A simple guide for dose conversion between animals and humans. *Journal of Basic Clinical Pharmacy*, *7*, 27-31.

Rouhi-Boroujeni, H., Heidarian, E., Mohammadizzadeh, E., and Rafieian-Kopaei, M. (2015). Herbs with anti-lipid effects and their interactions with a statin as a chemical anti-hyperlipidemia group drugs: A systematic review. *ARYA Atheroscler, 11*, 244-251.

Singh, A., Lal, U.R., Mukhtar, M., Mukhtar, H.M., Singh, P.S., and Shah, R.K. (2015). Phytochemical profile of sugarcane and its potential aspects. *Pharmacognosy Reviews*, *9*, 45-54.

Watanabe, T., Maejima, E., Urayama, M., Owado, M., and Yamauchi, A. (2016). Distribution of minerals in young and mature leaves of different leaf vegetable crops cultivated in a field. *Open Journal of Plant Science, 1*, 005-009.

Yokozawa, T., Cho, E.J., Satoh, A., Okamoto, T., and Sei, Y. (2006). The protective role of Chinese prescription Kangen-karyu extracts on diet-induced hypercholesterolemia in rats. *Biological and Pharmaceutical Bulletin*, *29*, 760-765.

You, C.L., Su, P.Q., and Zhou, X.X. (2008). Study on effect and mechanism of Scutellaria baicalensis stem-leaf total flavonoids in regulating lipid metabolism. *Zhongguo Zhong Yao ZA Zhi*, *33*, 1064-1066.

DOI: http://dx.doi.org/10.17509/xxxx.xxxx
p- ISSN 2775-6793 e- ISSN 2775-6815

Zhao, D., Glynn, N.C., Glaz, B., Comstock, J.C., and Johnson, R.M. (2012). Development of leaf spectral models for evaluating large numbers of sugarcane genotypes. *Crop Sci*ence, *52*, 1837-1847.

DOI: http://dx.doi.org/10. 17509/xxxx.xxxx
p- ISSN 2776-6098 e- ISSN 2776-2538