International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (2): 796-802
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

GCMS Analysis and Zebrafish Studies Reveal Presence of Antilipidemic Phytochemicals in Methanolic Seed Extracts of *Phaseolus vulgaris*

Suma Sarojini and Della Mariya Thomas

Department of Life Sciences, CHRIST (Deemed to be University), Hosur Road, Bangalore, Pin code: 560 029.

Received: 17 Jan 2019 / Accepted: 19 Mar 2019 / Published online: 1 Apr 2019
Corresponding Author Email: suma@christuniversity.in

Abstract

The past few decades saw a rise in cases of metabolic syndrome (MetS) which includes visceral obesity, dyslipidaemia, hyperglycaemia, and hypertension. This has become a major focus of the World Health Organization (WHO) and is now commonly referred to as an "epidemic of obesity and diabetes" ("diabesity"). Multiple factors have contributed to MetS, the most significant being adoption of a diet characterized by excessive consumption of simple sugars and salt, saturated and trans-esterified fatty acids and a sedentary lifestyle. Though severe cases require pharmacologic interventions, borderline cases can be treated using phytochemicals. Scientists world over are in search of potential phytochemicals to reduce the damage of MetS. The current study involves a preliminary screening of methanolic seed extracts of *Phaseolus vulgaris* in reducing cholesterol levels, one of the factors which contributes to MetS. The extracts were analysed by GCMS and preliminary *in vivo* studies conducted using the model organism, zebra fish (*Danio rerio*).

Keywords

Phaselous vulgaris, GCMS, Zebrafish, anti-cholesterol.

*****

INTRODUCTION

Legumes are a good source of proteins, dietary fibres, minerals and starch. Many bioactive compounds have been isolated from different legumes which have the capabilities of drastically reducing several human ailments. The need of the hour is to explore more of such properties of phytochemicals. Kidney beans (*Phaseolus vulgaris*) is native to Mexico and Central America and is rich in protein, mineral, vitamins and energy in diet. Previous studies have explored various medicinal properties of phytochemicals from this legume. Of late, India has become one of the top countries in non-communicable diseases such as obesity and high cholesterol. Cholesterol is a waxy fat like substance found in all cells which are carried by lipoproteins through blood streams. 80% of body's cholesterol is synthesised by liver and rest comes from diet such as poultry, fish, egg, dairy products etc. Though the human body requires cholesterol for its perfect

functioning, any amount of high cholesterol can be detrimental to human physiology. High amounts of serum cholesterol can lead to its deposition in the arteries leading to a condition known as Atherosclerosis which in turn can pave way for heart attacks. The present study was conducted with a view to check the bioactive phytochemicals in *Phaseolus vulgaris* especially ones which can confer antilipidemic properties. A preliminary study was also carried out to check the effect of the seed extract on induced hypercholesteremia in zebra fishes.

MATERIALS AND METHODS

Collection and processing of seeds

The seeds of *Phaseolus vulgaris* were bought from a supermarket, washed and dried. The dried seeds were powdered using a grinder. The Soxhlet apparatus was used to prepare the methanolic extract of the plant material using GC grade methanol. This was then centrifuged and the supernatant was collected for injection into the GCMS system.

GCMS

The GC was equipped with TG 5MS silica Capillary column of 30 m length, 0.25 mm ID and a film thickness of 0.25 µm. The initial oven temperature of 80°C was used for 2 minutes followed by a rate increase of 9°C/min to 200°C and a final hold at 300°C for 5 minutes. Mass spectra was obtained with 70 eV electron impact ionisation and a source temperature of 230°C. The mass spectrometer was continuously scanning from *m/z* 50-550 at a scan speed of 0.2 seconds. Helium was used a s a carrier gas. The data was collected with Xcaliber software.

In vivo studies

Zebrafish maintenance

Zebrafish used for the study was maintained under 12-hour dark and 12-hour light conditions with proper aeration. A group of 21 zebra fishes were taken which was further divided into a group of control (n= 3), 5% cholesterol (n= 9) and 10% cholesterol (n=9) diet. Each group was maintained in a separate tank. High cholesterol diet was fed to experimental groups while normal diet was given to the control group. The body weight of zebra fishes was recorded before and after the experiment.

Cholesterol supplemented diet

High cholesterol diet was prepared by supplementation of cholesterol to normal fish food. 5% and 10% cholesterol supplemented food were prepared separately. Briefly, cholesterol was dissolved in 2-3 drops of dimethyl sulfoxide and dry pellets of fish food were mixed with it and kept overnight. This was followed by addition of few drops of egg yolk. This was dried under sterile condition. Group A fishes were fed with 5% cholesterol supplemented food and group B with 10% cholesterol supplemented food twice a day while control group fishes were fed with normal fish food.

Behavioural analysis of zebra fish

Diet induced and control zebrafishes were weighed weekly during feeding trials. For confirmation of obesity, control and test fishes were kept in T-chamber and were subjected to swimming test. The T chamber was filled with water and connected to power supply. Due to power supply, water currents will be generated. Fishes with high levels of cholesterol will find difficult to move across water current while cholesterol free fishes will swim freely.

Testing with plant sample

Fishes of 5% and 10% cholesterol groups were further divided into groups of control and test. Test groups were treated with working standard of plant extract while control groups were continued to be fed with 5% and 10% cholesterol supplemented diet. This process was continued and weight of zebrafishes were recorded after 2 weeks.

RESULTS AND DISCUSSION

Seeds of *Phaseolus vulgaris* were subjected to methanolic extraction in a Soxhlet apparatus and the extract was subjected to GCMS analysis. Of the several phytochemicals observed in GCMS studies, (1), one compound has been correlated with substantial anti-lipidemic properties. Cholesterol is a lipid required by the human body for cell membrane functions and efficient reproductive system functions. But, high amount of cholesterol can lead to the formation of plaque along the side of arterial wall which causes them to narrow. Obesity is a condition in which an individual is 20% overweight. People with increased levels of cholesterol have high chances of becoming obese too. Lipids are stored in the form of triglycerides in adipocytes. Due to accumulation of excess lipids, the size of adipocyte increases, even though its number does not increase. This in turn will lead to obesity. Using two pool model of calculation, it was shown that cholesterol production is related to body weight and number of fat cells. [1]. With every additional kg of body weight, the cholesterol production was found to increase by 22 mg/ day/ kg [2].

Many factors regulate the level of triglycerides in blood plasma by either lipolysis or secretion. One such factor is the low concentration of High-Density Lipopolysaccharide or HDL. Most of the human HDL deficiencies are associated with

hypertriglyceridemia. The high levels of triglycerides in the blood plasma of experimental mice was associated with defective lipolysis. This was attributed to the deficiency of HDL apoC-II. Cholesterol is converted into fatty acids and triglycerides in vertebrates. These are then released to hepatocytes and β-oxidation of fatty acids occur in mitochondria. Accumulation of triglycerides will occur in liver upon consumption of excess cholesterol. This will lead to formation of fatty liver [3]. Triglycerides are hydrolysed to glycerol and fatty acid by lipase enzyme.

The seeds of *Phaseolus vulgaris* have the property of reducing the risk of coronary heart disease, obesity, diabetes and it also lowers serum cholesterol concentration. Their consumption helps in controlling postprandial blood glucose level as they contain low glycaemic index (GI) [4] Among all types of legumes, *Phaseolus vulgaris* have higher amount of complex carbohydrate (50-60%), proteins (20-15%) and they are a better source of minerals, vitamins and poly unsaturated free fatty acids. Red kidney bean starch consists of 90% starch, 8.5% moisture, 1.3% protein, 0.5% fat and 0.7% ash. This indicates that the starch in it is pure [5]. Among different varieties of legumes, kidney beans showed highest amylase inhibitory activity [6]. Compared to other edible plants, Kidney beans are rich in lectin. Lectin has many biological properties like preferential agglutination of malignant cells and mitogenic stimulation of lymphocytes [7]. There are proofs for the seed extracts in reduction of body weight, appetite, lipid accumulation, carbohydrate absorption and metabolism [8]. High quantities of LDL (Low density Lipopolysaccharide) cholesterol and triglycerides are both harmful to heart health. Nonvegetarian food sources are generally high in cholesterol content. Plant sources doesn't contain cholesterol. In fact, many plants have been found to carry phytochemicals which can reduce the cholesterol levels in mammals.

Zebra fish (*Danio rerio*) is native to India and is approximately 3cm long. Males fishes are torpedo shaped and thin with gold colour in fin and belly while females are fat once laden with eggs and have lesser golden colour [9]. All kind of fishes prefer lipids as an energy source more than carbohydrates [10]. Zebrafishes have similar genetic structure to humans and share more than 70% genes with us. This organism has the same organs and tissues as humans. More than 80% of genes known to be associated with human diseases have a zebrafish counterpart. Several studies have shown that zebra fish can be preferred as a model for studies than other models such as monkey, rabbit etc. They show similarity to humans in case of function and genomic structure [11]. The present study was aimed at making an attempt in inducing hypercholesteremia in zebrafish by feeding them with diet rich in cholesterol and then testing the efficacy of the *Phaseolus vulgaris* seed extract in reducing the cholesterol levels. The zebrafishes were grouped into two – the test group were fed with cholesterol rich diet (two cohorts of 5% cholesterol in the feed and 10% cholesterol in the feed) and the control group which were fed the normal diet. The increasing in weight was substantially more for the test group zebrafishes (Fig.1 and 2). These fishes were then fed with plant extract diet while control groups were continued to be fed with normal diet. This process was continued and weight of zebrafishes were recorded after two weeks. The results showed a decline in the weight of fishes fed with plant extract supplemented diet when compared to the control. (Fig.3 and 4). This proves the cholesterol reduction properties of the *Phaseolus vulgaris* seed extract This could be attributed to hexadecanoic acid, methyl ester, a phytochemical found in some other leguminous plants too. This compound has previously been found to possess antibacterial [12], anti-inflammatory [13], antioxidant [14] and larvicidal [15] properties. Apart from the high fat diet, gut bacterial composition of an individual also play an important role in deciding the rate of fat accumulation by affecting biochemical 14]. In the current study, accumulation of fat in zebrafish made them obese (figure 1 and 2) and in turn made it difficult for them to swim against water current. Fish fed with food containing seed extract of *Phaselous vulgaris* performed better in swimming tests. There was also reduction in the weight of the zebrafishes as depicted in figures 3 and 4. The cholesterol levels were indirectly checked using T chamber swimming tests as evidenced by reduction in mobility and swimming rates. The zebrafishes fed with normal diet without plant extracts did not show decrease in weight (Fig. 5), neither they could swim efficiently. Polyphenolic compounds and oligosaccharides in *Phaseolus vulgaris* have previous been shown to have potential health benefits. Earlier studies have shown that dry extract of *Phaseolus vulgaris* have an effect on regulating glucose and lipid metabolism in rats. [16]

Metabolic Syndrome is the name of a group of disorders associated with the risk of high levels of blood glucose, triglycerides and LDL and low levels of HDL, which leads to higher chances of getting diabetes, hypertension, obesity, and [17,18,19].

Regular intake of beans in the food is known to be beneficial for healthy and hypercholesterolemic subjects by decreasing the serum triglycerides and LDL and increase HDL [20]. The present study reiterates the cholesterol reducing properties of the seed extract of *Phaselous vulgaris.* Since zebra fishes and humans have many genes in common, the impact of *Phaseolus vulgaris* seed extracts in humans could be expected to have similarities with those in zebra fish. More detailed study on the phytochemicals responsible for this are to be planned in the future with a comparative analysis of this property in other leguminous plants too. Also, the molecular mechanisms have to be elucidated to get a clearer picture of the antilipidemic property of this plant.

Sl. No.	Apex RT	Area	Identification
1	35.3	3299656.081	Hexadecanoic acid, methyl ester
2	36.06	11722708.94	l-(+)-Ascorbic acid 2,6-dihexadecanoate
3	38.52	2973636.685	8,11-Octadecadienoic acid, methyl ester

Table 1: Table depicting the significant compounds obtained in GCMS data of methanolic seed extract of *Phaseolus vulgaris*.

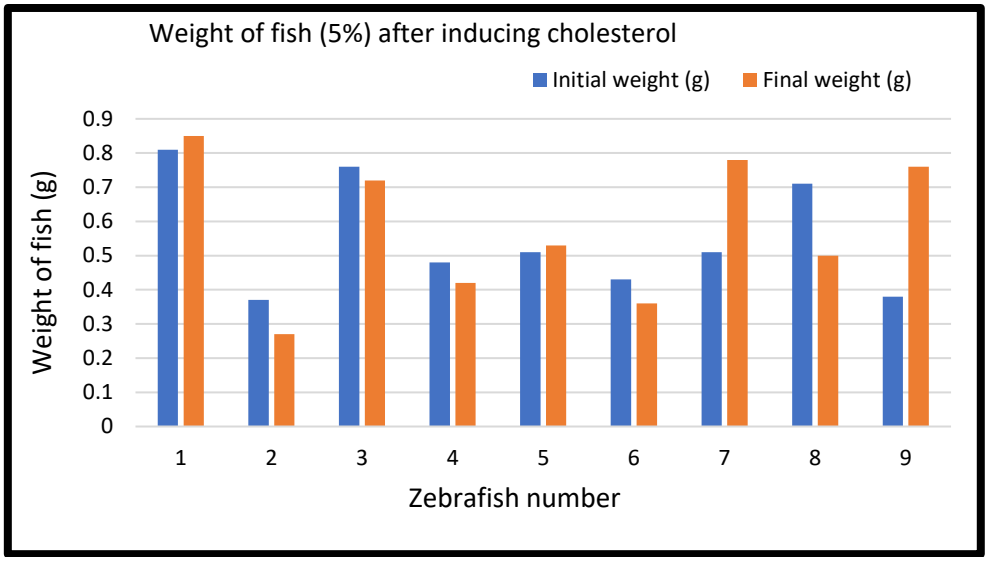

Fig 1: Graph representing weight of fish (after feeding with 5% cholesterol in food)

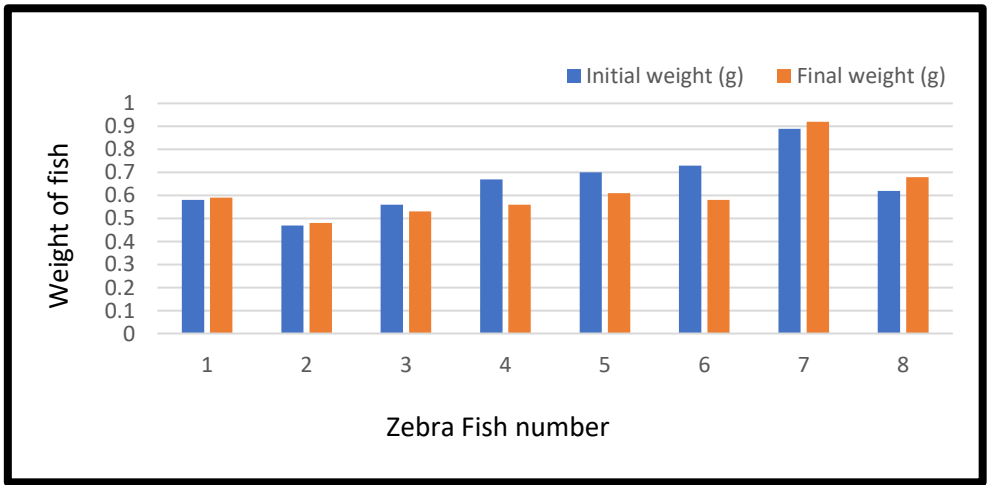

Fig 2: Graph representing weight of fish (after feeding with 10% cholesterol in food)

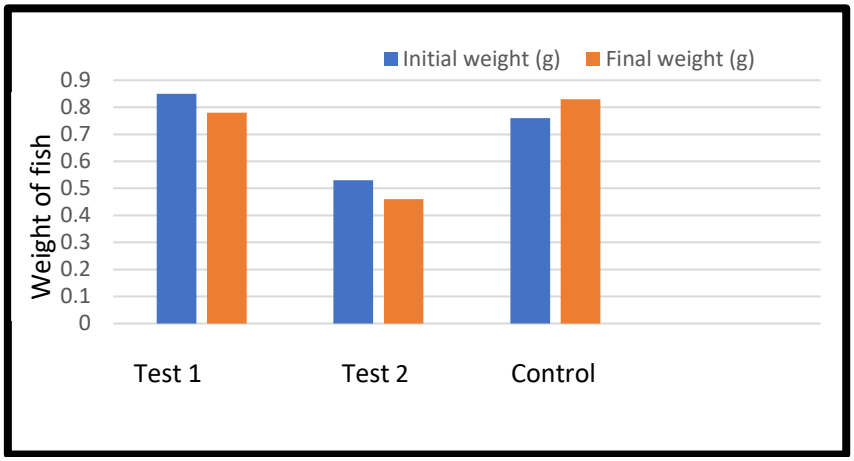

Fig 3: Graph representing weight of fish (fed with 5% cholesterol in food) after treating with different concentration of sample – Test1 with 5 mg/l extract and test 2 with 10 mg/l extract. Control fishes were fed with normal diet.

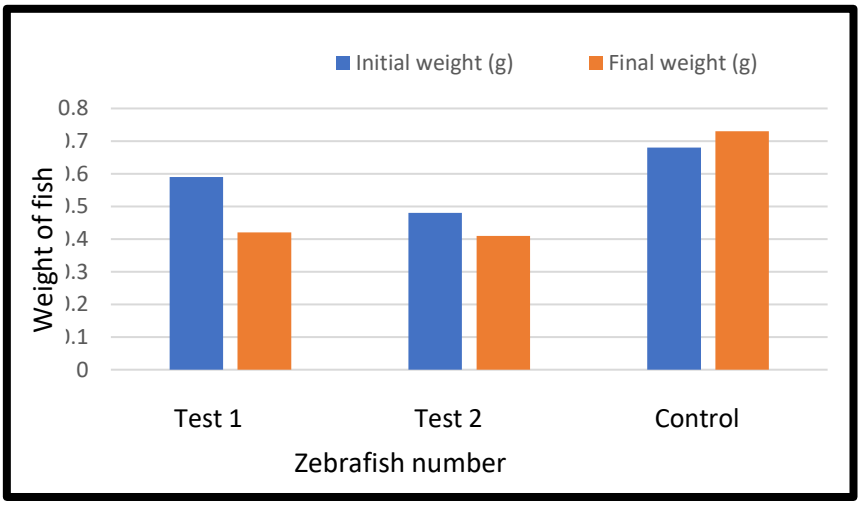

Fig 4: Graph representing weight of fish (fed with10% cholesterol in food) after treating with different concentration of sample – test1 with 5 mg/l extract and test 2 with 10 mg/l extract. Control fishes were fed with normal diet.

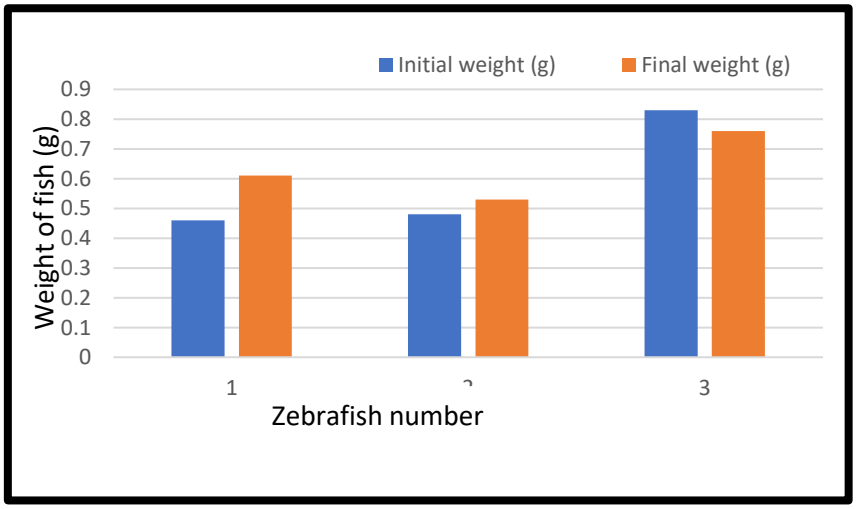

Fig.5: Graph representing weight of control fish - fed without plant extracts

CONCLUSION

In the present study, *Phaseolus vulgaris* seed methanolic extract have been found to show cholesterol lowering effect in zebra fishes. If this beans are included in every day meals, it has high chances of lowering the cholesterol levels of a person and thus decreasing the chances of obesity. This could be attributed to a number of phytochemicals in the legume seed extracts, the prominent being, hexadecanoic acid, methyl ester. Further studies are planned to look at other bioactive phytochemicals in legumes which might have a positive antilipidemic effect. This can in turn reduce the burden of metabolic syndrome which is on the rise in the world, especially in the developed countries. Phytochemicals are indeed a good option for treating MetS, especially in the early stages of detection, the added advantage being less side effects. More *in vivo* studies are planned to get a clearer picture of the antilipidemic property of *Phaseolus vulgaris* seed extracts.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the facilities provided by Durga Femto Technology & Research, Bangalore for the zebrafish studies and the guidance of late Dr Guru Prasad R, DFTR. The authors also kindly acknowledge the Department of Life Sciences, CHRIST (Deemed to be University) for providing the infrastructure for the research work.

REFERENCES

1. Nestel PJ, Whyte HM and Goodman DS., Distribution and Turnover of Cholesterol in Humans. Journal of Clinical Investigation, *48* (6): 982-991, (1969).
2. Miettinen T, Cholesterol Production in Obesity. Circulation, *44*(5): 842-850 (1971).
3. Justin David C, Rajapriya R, Veena V and Kumaresan G, High Cholesterol Diet Induces Obesity in Zebrafish. Int. J. Adv. Sci. Eng, *2*(4): 188-192, (2016)
4. Madhujith T, Naczk M and Shahidl F., Antioxidant Activity of Common Beans (*Phaselous Vulgaris* L.). Journal of Agricultural Food Chem, 50 (24): 6975–6980, (2004).
5. Reddy CK, Suriya M and Haripriya M., Physico-chemical and functional properties of Resistant starch prepared from red kidney beans (*Phaseolus vulgaris*.L) starch by enzymatic method. Carbohydrate polymers, *95*(1): 220-226, (2013).
6. Powers JR and Whitaker JR.,Purification and some physical and chemical properties of red kidney bean (*Phaselous Vulgaris*) α-amylase inhibitor. Food biochemistry, 1 (3): 217-238, (1977)
7. Zhang J, Shi J, Ilic S, Xue SJ and Kakuda Y., Biological Properties and Characterization of Lectin from Red Kidney Bean (*Phaseolus Vulgaris*). Food reviews international, *25*(1): 12-27, (2009).
8. Carai, M., Fantini, N., Loi, B., Colombo, G., Gessa, G., Riva, A., Bombardelli, E. and Morazzoni, P, Multiple cycles of repeated treatments with a *Phaseolus vulgaris* dry extract reduce food intake and body weight in obese rats. British Journal of Nutrition, 106(05): 762-768, (2011).
9. Wixon, J, *Danio rerio*, the Zebrafish. Yeast, 1(3): 225-231, (2000).
10. Stoletov, K., Fang, L., Choi, S., Hartvigsen, K., Hansen, L., Hall, C., Pattison, J., Juliano, J., Miller, E., Almazan, F., Crosier, P., Witztum, J., Klemke, R. and Miller, Y, Vascular Lipid Accumulation, Lipoprotein Oxidation, and Macrophage Lipid Uptake in Hypercholesterolemic Zebrafish. Circulation Research, *104*(8): 952-960 (2009)
11. Meguro, S., Hasumura, T. and Hase, T, Body Fat Accumulation in Zebrafish Is Induced by a Diet Rich in Fat and Reduced by Supplementation with Green Tea Extract. PLoS One, 10(3): 1-12, (2015).
12. Awa EP, Ibrahim S and Ameh DA., GC/MS analysis and antimicrobial activity of diethyl ether fraction of methanolic extract from the stem bark of *Annona senegalensis* Pers. Int. J. Pharm. Sci. Res. 3, 4213–4218, (2012).
13. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C and Haridas M., Anti-inflammatory property of n-hexadecanoic acid: Structural evidence and kinetic assessment. Chem. Biol. Drug Des., 80, 434–439, (2012).
14. Kumar PP, Kumaravel S and Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. Afr. J. Biochem. Res., 4, 191–195, (2010).
15. Rahuman AA, Gopalakrishnan G, Ghouse BS, Arumugam S and Himalayan B. Effect of *Feronia limonia* on mosquito larvae. Fitoterapia, 71, 553–555, (2010).
16. Louise Anin Anin Atchibri, Lenifere Chantal Soro, Jean Jacques Diagou, Dago Gnakri, Effect of Aqueous Extract of Phaseolus Vulgaris on the Regulation of Glucose and Lipid Metabolism in Diabetic Rats. Journal of Biotechnology and Biochemistry, *3*(1): 43-49 (2017).
17. Baudry J, Lelong H, Adriouch S, Julia C, Allès B, Hercberg S, Touvier M, Lairon D, Galan P and Kesse-Guyot E., Association between organic food consumption and metabolic syndrome: cross-sectional results from the NutriNet-Sante study. Eur J Nutr, 57 (7): 2477-2488, (2017.)
18. Leite PB, Dâmaso AR, Poli, VS Sanches RB, Silva SGA, Fidalgo JPN, Nascimento MA, de Oliveira CAM and Caranti, DA., Long-term interdisciplinary therapy decreases symptoms of binge eating disorder and prevalence of metabolic syndrome in adults with obesity. Nutr. Res, 40: 57-64, (2017).
19. M. Yimam, P. Jiao, M. Hong, L. Brownell, Y.C. Lee, H.J. Kim, J.B. Nam, M.R. Kim, Jia, A botanical composition from *Morus alba, Ilex paraguariensis*, and *Rosmarinus*

officinalis for body weight management. Journal of Medicinal Food, 20 (11): 1100-1112 (2017).

20. Luhovyy BL, Mollard RC, Panahi S, Nunez MF, Cho F and Anderson GH., Canned navy bean consumption reduces metabolic risk factors associated with obesity. Can. J. Diet Pract. Res, 76 (1): 33-37, (2015).