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The Effect of Coffee Arabica Gayo Leaf Extract (*Coffea arabica* L.) in Increasing Phosphoinositide 3-kinase and Glucose Transporter-4 Expression in the Skeletal Muscle

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Abstract

BACKGROUND: Diabetes mellitus (DM) is a chronic and progressive metabolic disease characterized by hyperglycemia due to impaired insulin secretion associated with a lack of phosphoinositide 3-kinase (PI3K) and glucose transporter-4 (GLUT-4) expression in the skeletal muscle membrane.

AIM: The aim of the study is to understand the effect of coffee Arabica Gayo leaf extract (*Coffea arabica* L.) in increasing PI3K expression and GLUT-4 expression in the skeletal muscle membrane.

METHODS: Thirty-five male Wistar rats with Type 2 DM (T2DM) induced using a combination of a high-fat diet for 5 weeks followed by multiple intraperitoneal injections of low-dose streptozotocin (30 mg/kg). Divided into seven groups as such two groups that did not receive treatment and five groups that received treatment. The dosage administered was 150, 200, and 250 mg/kg/day through the nasogastric tube for 30 days. PI3K and GLUT-4 expression in the skeletal muscle membrane was evaluated by Immunohistochemistry in their gastrocnemius muscles.

RESULTS: The study showed an increased expression of PI3K and GLUT-4 expression. There was a significant difference between coffee Arabica Gayo leaf extract and Metformin in increasing GLUT-4 expression ($p = 0.036$) and PI3K expression between coffee Arabica Gayo dose 250 mg/kg/day and group without treatment ($p = 0.008$).

CONCLUSION: Coffee Arabica Gayo leaf extract (*C. arabica* L) at a dose of 250 mg/kg/day can increase PI3K expression in skeletal muscle and a dose of 200 mg/kg/day and 250 mg/kg/day can increase the expression of GLUT-4 in the skeletal muscle membrane greater than metformin.

Introduction

Diabetes mellitus (DM) is a chronic and progressive metabolic disease characterized by hyperglycemia due to impaired insulin secretion, insulin resistance, or a combination of both. This situation is thought to be the most common cause that plays a role in the pathophysiology of Type 2 DM (T2DM). Currently, there are more than 463 million people in the world who live with DM with 90% of DM cases in the world are T2DM. According to the International Diabetes Federation in 2019, there is a tendency of increasing incidence and prevalence of T2DM in the world. For example, it is predicted that the prevalence of DM throughout the world will increase to 578 million in 2045 [1]. Whereas in Indonesia which in 2019 ranked 7th in the world for the number of adults with T2DM, it is predicted with an increased number of DM by 2–3 times from 8.4 million in 2000 to around 21.3 million in 2030 [2]. Insulin resistance is one of the causes of T2DM. Insulin resistance itself can occur due to interference with signal transduction or non-signal transduction pathways. In the disruption of the signal transduction pathway, interference was found in the formation of IRS-phosphoinositide 3-kinase (PI3K) bonds which resulted in impaired glucose transport and protein or glycogen synthesis. Meanwhile, in glucose transportation, glucose transporter-4 (GLUT-4) is the main GLUT that is distributed in the skeletal muscle tissue, brain, heart, and adipose tissue in both humans and rodents [3], [4].

The currently available oral antidiabetic drugs have limited efficacy/safety and combined with

the emergency of this disease which is becoming a global epidemic, has encouraged the development of alternative diabetes therapies to be more efficient and safer. The provision of antidiabetic drugs derived from plants which are quite a lot of natural resources in various regions is expected to overcome this problem [5]. The utilization of the coffee plant is currently still focused on bean, while coffee leaves have not been widely used either as food products or as natural mixtures for food fortification, let alone medicine. Research by Martina *et al*. (2019) shows that giving coffee Arabica Gayo bean and leaf has the same effectiveness in lowering blood sugar in healthy mice [6]. Therefore, this study is expected to continue Martina's *et al*. research by looking at other parameters at the cellular level such as increasing PI3K expression and GLUT-4 expression in the skeletal muscle membrane as a marker of the improvement in insulin resistance through the effect of coffee Arabica Gayo leaf extract (*Coffea arabica* L.).

Methods

Research type and location

This is an experimental type research. The research was conducted at the Pharmacology and Therapeutics Laboratory of the Faculty of Medicine, Universitas Sumatera Utara. Histochemical examination of PI3K levels and GLUT-4 levels in skeletal muscle was carried out at the Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara.

Material

In this study, the ingredients used to Reduce Blood Glucose Level were:

1. Coffee Arabica Gayo Leaf Extract (*C. arabica* L.)
2. Metformin.

Animal

This study used male Wistar rats, aged 8 weeks with a bodyweight of 180–200 g, the rats used were healthy and had never been tried in other studies. Using Federer's formula (1963), all experimental animals used were 35 rats [7].

Induction DM

Rats were acclimatized for 7 days, given food and drink ad libitum. Two rats were placed in a cage in a room with a temperature of 22–250 C with a light-dark cycle of 12/12 h. The rats were given a standard diet consisting of 12% fat, 60% carbohydrates, and 28% protein for 2 days, then were given a high-fat diet consisting of 41% fat, 41% carbohydrates, and 18% protein for 5 weeks. After 5 weeks, the rats fasted for one night, then they were injected with a low dose of streptozotocin (30 mg/kg/BW) in 0.1 citrate buffer, pH 4.5) = intraperitoneally. Injection of streptozotocin dose of 30 mg/kg BW in 0.1 citrate buffer, pH 4.5 was repeated for the following week. After 1 week, streptozotocin was injected for the 2nd time. To ensure insulin resistance and T2DM have occurred in the experiment, we measure fasting blood sugar and insulin resistance value (Homeostatic model assessment-insulin resistance [HOMA-IR]). If fasting blood sugar level is above 200 mg/dl and HOMA-IR value is below normal, it shows T2DM has occurred.

Treatment

Wistar rats were divided into seven groups as follows:

- K1 = normal without treatment group
- K2 = normal + extract dose of 250 mg/kg/day group
- K3 = T2DM without treatment group
- K4 = T2DM + metformin group
- K5 = T2DM + extract dose of 150 mg/kg/day group
- K6 = T2DM + extract dose of 200 mg/kg/day group
- K7 = T2DM + extract dose of 250 mg/kg/day group.

Coffee Arabica Gayo leaf extract (*C. arabica* L) administered to rat's orally with a nasogastric tube for 30 days.

Blood sugar check procedure

Measurement of rat blood sugar levels was done using Auto-check. Blood was drawn from the lateral vein of the rat's tail.

Statistical analysis

To find whether the distribution of samples was normal or not, and due to the number of samples provided (<50), Shapiro-Wilk is used for the test. The results show that data is normally distributed and homogenous, therefore, one way ANOVA is used to compare parameters between control and treatment groups, continued with Bonferroni's *post-hoc* analysis to see the differences in each group. Data were processed and analyzed using SPSS with a significance limit of $p < 0.05$.

Results

Based on the research, there were changes in PI3K and GLUT-4 expression in the skeletal muscle. The results are then analyzed as described in the following table and figures.

As explained in Table 1. The mean PI3K expression in the muscle membrane for the treatment group is higher than the control (K3). The order of mean PI3K after giving coffee Arabica Gayo leaf extract (*C. arabica* L.) from the highest to the lowest are K2 (10.20 ± 2.80) > K1 (9.60 ± 0.60) > K7 (8, 60 ± 1.28) > K4 (7.80 ± 1.20) > K6 (6.80 ± 0.97) > K5 (6.80 ± 0.97) > K3 (2.20 ± 0.20). It is seen that the T2DM group which received coffee Arabica Gayo leaf extract (*C. arabica* L.) at a dose of 250 mg/kg/day (K7) had a higher PI3K score than the group that received metformin (K4). The normal group that received the coffee Arabica Gayo leaf extract (*C. arabica* L.) at a dose of 250 mg/kg/day (K2) had the highest PI3K of all groups. Increase in PI3K expression is directly proportional to the increase of the Coffee Arabica Gayo Leaf Extract (*C. arabica* L.) dosage. However, the difference between the groups was not statistically significant (p > 0.05). This means that coffee Arabica Gayo leaf extract has the same effect as metformin in increasing PI3K expression in muscles.

Table 1: The difference between the mean phosphoinositide 3-kinase expression and the mean glucose transporter-4 expression in skeletal muscle in the control group and the treatment group with the coffee arabica gayo leaf extract (*C. arabica* L.)

Group	K1 (n=5)	K2 (n=5)	K3 (n=5)	K4 (n=5)	K5 (n=5)	K6 (n=5)	K7 (n=5)	p
PI3K Expression in Skeletal Muscle (score)	9,60 ±0,60	10,20 ±1,80	2,20 ±0,20	7,80 ±1,20	6,80 ±0,97	7,40 ±1,03	8,60 ±1,28	0,139
GLUT-4 Expression in Skeletal Muscle (score)	3,20 ±0,73	9,80 ±0,91	2,40 ±0,40	5,60 ±1,69	2,20 ±0,20	8,20 ±1,14	10,20 ±1,20	0,046*

Note: Data shown in mean ± SD form (One-Way Anova Test) *p < 0.05 was statistically significant. K1 = normal without treatment group, K2 = normal + extract dose of 250 mg/kg/day group, K3 = T2DM without treatment group, K4 = T2DM + metformin group, K5 = T2DM + extract dose of 150 mg/kg/day group, K6 = T2DM + extract dose of 200 mg/kg/day group, K7 = T2DM + extract dose of 250 mg/kg/day group.

The mean GLUT-4 for the treatment group was higher than the mean control group. The mean order of GLUT-4 in rat muscle after giving Coffee Arabica Gayo Leaf Extract (*C. arabica* L.) from the highest to the lowest are K7 (10.20 ± 1.20) > K2 (2.40 ± 0.91) > K6 (8.20 ± 1.14) > K4 (65.60 ± 1.69) > K1 (3.20 ± 0.73) > K3 (2.40 ± 0.40) ≥ K5 (2.20 ± 0, 20). There is a significant increase of GLUT-4 levels in the muscles for Coffee Arabica Gayo Leaf Extract (*C. arabica* L.) at a dose of 250 mg/kg/day (K7) and 200 mg/kg/day (K6). Whereas in the 150 mg/kg/day (K5) there was no visible change compared to the untreated T2DM group. Coffee Arabica Gayo Leaf Extract (*C. arabica* L.) at a dose of 250 mg/kg/day (K7) is two times higher than the group that received metformin (K4). The normal group which received Coffee Arabica Gayo Leaf Extract (*C. arabica* L.) at a dose of 250 mg/kg/day (K2) also increased three times compared to the normal control group that was not treated (K1).

Increase in GLUT-4 expression in the skeletal muscle membrane is directly proportional to the increase of the coffee Arabica Gayo leaf extract (*C. arabica* L.) dosage.

Table 2 Presents a follow-up test to determine the differences between groups specifically as well as to find out which of these groups had the most significant change in muscle PI3K scores after the intervention. For this test, it appears that the significance value between groups is if p < 0.05. Based on the output in Table 2, there is a significant difference in the PI3K score in muscle after the intervention at K7 against K3.

Table 2: Results of *post-hoc* bonferroni analysis of phosphoinositide 3-kinase in muscle after intervention

Group	Average Difference	IC 95%		p
		Minimum	Maximum	
K5 vs K4	-1,0	-6,2	4,2	1,000
K6 vs K4	-0,4	-5,6	4,8	1,000
K7 vs K4	0,8	-4,4	6,0	1,000
K5 vs K3	4,6	-0,6	9,8	0,147
K6 vs K3	5,2	-0,0	10,48	0,057
K7 vs K3	6,4*	1,1	11,6	0,008
K5 vs K6	-0,6	-5,8	4,6	1,000
K5 vs K7	-1,8	-7,0	3,4	1,000
K6 vs K7	-1,2	-6,2	4,2	1,000

Note: Data shown in mean ± SD form (*post-hoc* Bonferroni). *p < 0.05 was statistically significant. K1 = normal without treatment group, K2 = normal + extract dose of 250 mg/kg/day group, K3 = T2DM without treatment group, K4 = T2DM + metformin group, K5 = T2DM + extract dose of 150 mg/kg/day group, K6 = T2DM + extract dose of 200 mg/kg/day group, K7 = T2DM + extract dose of 250 mg/kg/day group.

Table 3 above shows further tests to determine the differences between groups specifically as well as to find out which of these groups had the most significant change in muscle GLUT-4 scores after intervention. For this test, it appears that the significance value between groups is if p < 0.05. Based on the output in Table 3, there is a significant difference in the GLUT-4 score in skeletal muscle membrane after intervention at K6 against K3, K7 against K3, K5 against K6, and K5 against K7.

The following Figures 1 and 2 are the results of immunohistochemical staining for examination of PI3K expression in skeletal muscle (gastrocnemius muscle) and GLUT-4 expression in the skeletal muscle membrane (gastrocnemius muscle).

Table 3: Results of *post-hoc* Bonferroni Analysis of glucose transporter-4Scores in Skeletal Muscle Membran After Intervention

Group	Average Difference	IC 95%		P
		Minimum	Maximum	
K5 vs K4	-3,4	-8,1	1,3	0,507
K6 vs K4	2,4	-2,1	7,3	1,000
K7 vs K4	4,6	-0,1	9,3	0,067
K5 vs K3	-0,2	-4,9	4,5	1,000
K6 vs K3	5,8*	1,04	10,5	0,007
K7 vs K3	7,8*	3,0	12,5	0,000
K5 vs K6	-6,0*	-10,7	-1,2	0,005
K5 vs K7	-8,0*	-12,7	-3,2	0,000
K6 vs K7	-2,0	-6,7	2,7	1,000

Note: Data shown in mean ± SD form (*post-hoc* Bonferroni) *p < 0.05 was statistically significant. K1 = normal without treatment group, K2 = normal + extract dose of 250 mg/kg/day group, K3 = T2DM without treatment group, K4 = T2DM + metformin group, K5 = T2DM + extract dose of 150 mg/kg/day group, K6 = T2DM + extract dose of 200 mg/kg/day group, K7 = T2DM + extract dose of 250 mg/kg/day group.

Discussion

Based on the results of this research, there was no difference between the group given metformin and the group given coffee Arabica Gayo leaf extract on increasing PI3K expressions in muscles. This shows that coffee Arabica Gayo leaf extract is as effective as metformin in increasing PI3K expression in muscles. This is possible because coffee Arabica Gayo leaves extract (*C. arabica* L.) contains chlorogenic acid which is a new insulin sensitizer that potentiates the action of insulin similar to metformin [8]. Besides that, the saponin content in Gayo Arabica coffee leaf extract is also believed to increase PI3K expression [9]. Based on a study, saponins showed anti-hyperglycemic activity as a result of increasing insulin through increased PI3K signaling [10]. Likewise, the tannin content contained in the gayo Arabica coffee leaf extract can increase the expression of PI3K. Tannins can increase glucose uptake through insulin signaling pathway mediators, such as PI3K, p38 AMPK, and GLUT-4 translocation [11].

Chlorogenic acid is a polyphenol compound that is an important component of coffee, including the leaves [12]. It was reported that daily consumption of chlorogenic acid significantly reduced the risk of T2DM by 30%. Other clinical trials have also shown that chlorogenic acid can reduce blood glucose in T2DM patients [13]. Chlorogenic acid has been described as a potential antidiabetic agent. Using in vitro studies, Tajik

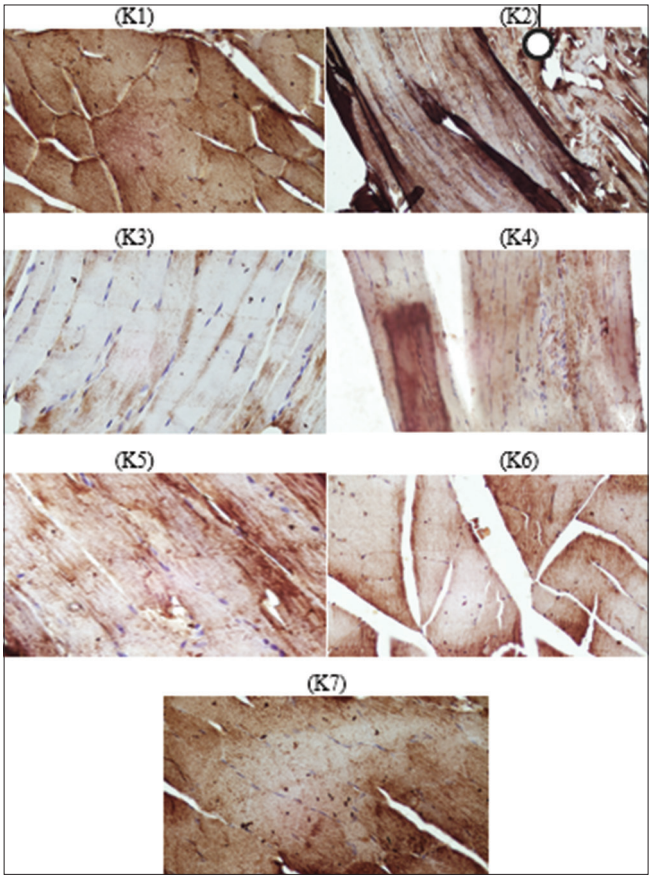

*Figure 1: Immunohistochemical Staining with phosphoinositide 3-kinase Markers, There were differences in the intensity and density of cells exposing phosphoinositide 3-kinase in the normal groups (K1 and K2) and negative control group (K3), positip control group (K4) and the treatment groups (K5–K7). Giving Coffee Arabica Gayo Leaf Extract (C. arabica L.) appeared to increase the number of cells expressing phosphoinositide 3-kinase. *400 times magnification*

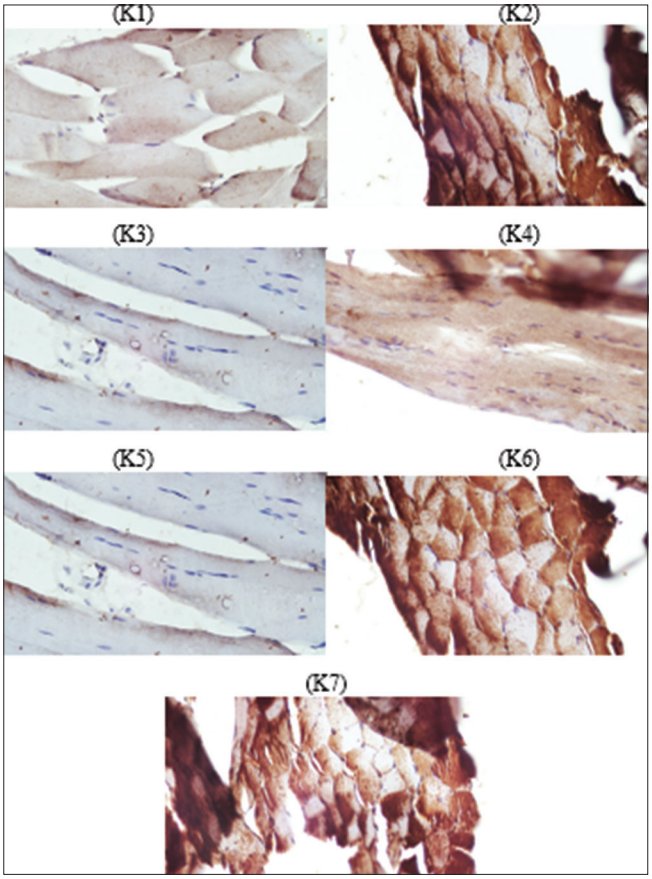

*Figure 2: Immunohistochemical Staining with glucose transporter-4 Marker, There were differences in the intensity and density of cells exposing glucose transporter-4 (GLUT-4) in the normal groups (K1 and K2) and negative control group (K3), positip control group (K4) and the treatment groups (K5-K7). Giving coffee Arabica Gayo leaf extract (C. arabica L.) in the appears to increase the number of cells expressing GLUT-4. *400 times magnification*

et al. (2017) reported that chlorogenic acid increased glucose uptake in L6 muscle cells, an effect observed only in the presence of stimulation of insulin concentrations [14]. Furthermore, it was found that chlorogenic acid stimulates insulin secretion from the insulin-secreting cell lines INS-1E and islets of Langerhans. Clinical trials have also proven that chlorogenic acid can modulate glucose uptake and gastrointestinal hormones and insulin secretion in humans [15].

The intervention of chlorogenic acid over a long period was able to significantly increase PI3K and GLUT-4 expression in the muscle membrane.

Conclusion

In this study, it was found that coffee Arabica Gayo leaf extract (*C. arabica* L) at a dose of 250 mg/kg/day can increase PI3K expression in skeletal muscle and a dose of 200 mg/kg/day and 250 mg/kg/day can increase the expression of GLUT-4 in the skeletal muscle membrane greater than metformin.

Limitation

Examining HOMA-IR values which is the parameter that shows whether insulin resistance is occurring in this research was done at the end of the study because it had to take a considerable amount of blood samples. While at the beginning of the study there were only four rats used as a representation.

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References

1. International Diabetes Federation. IDF Diabetes Atlas. 7th ed. Brussels, Belgium: In International Diabetes Federation; 2019. Available form: https://www.idf.org/e-library/epidemiology-research/diabetes-atlas.html. [Last accessed on 2020 Dec 20].
2. PERKENI Perkumpulan Endokrinologi Indonesia. Konsensus Pengelolaan dan Pencegahan Diabetes Mellitus Tipe 2 di Indonesia. Diunduh dari; 2015. Available form: https://www.pbperkeni.or.id. [Last accessed on 2020 Dec 20].
3. Cheeseman C, Long W. Structure of and functional insight into the GLUT family of membrane transporters. Cell Health Cytoskeleton. 2015;7:167-83. https://doi.org/10.2147/CHC.S60484.
4. Khorami SA, Movahendi A, Huzwah K, Sokhini AM. P13k/Akt pathway in modulating glucose homeostasis and its alteration in diabetes. Ann Med Biomed Sci. 2015;1(2):46-55.
5. Hussain SA, Marouf BH. Flavonoids as alternatives in treatment of Type 2 diabetes mellitus. Acad J Med Plants. 2013;1(2):31-6. http://doi.org/10.15413/ajmp.2012.0111
6. Martina SJ, Govindan PA, Wahyuni AS. The difference in effect of Arabica coffee Gayo beans and leaf (*Coffea arabica* L. Gayo) extract on decreasing blood sugar levels in healthy mice. Maced J Med Sci. 2019;7(20):3363-65. http://doi.org/10.3889/oamjms.2019.423
7. Federer WT. Experimental design: Theory and application. In: Hanafiah KA. Rancangan Percobaan-teori Dan Aplikasi. Vol. 3. Indonesia: Raja Grafindo Persada; 1995. p. 9-10.
8. McCarty MF. A chlorogenic acid induce increase in GLP-1 Production may mediate the impact of heavy coffee consumption on diabetes risk. Med Hypothesis 2005;64(4):848-53.
PMid:15694706
9. Hu X, Wang S, Xu J, Wang DB, Chen Y, Yang GZ. Triterpenoid saponins from Stauntonia chinensis ameliorate insulin resistance via the AMP-activated protein kinase and IR/IRS-1/PI3K/Akt pathways in insulin-resistant HepG2 cells. Int J Mol Sci. 2014;15(6):10446-58. https://doi.org/10.3390/ijms150610446.
PMid:24918297
10. Olusola O, Kamde JP. Saponins as a adipokines modulator: A possible therapeutic intervention for Type 2 diabetes. World J Diabetes. 2017;8(7):337-45.
PMid:28751956
11. Sieniawska E. Activities of tannins-from *in vitro* studies to clinical trials. Nat Prod Commun. 2015;10(11):1877-84.
PMid:26749816
12. Yan YX, Xiao HB, Wang SS, Zhao J, He Y, Wang W, *et al*. Investigation of the relationship between chronic stress and insulin resistance in a chinese population. J Epidemiol. 2016;26(7):355-60. http://doi.org/10.2188/jea.JE20150183
PMid:26830350
13. Ahren B. Avoiding hypoglycemia: A key to success for glucose-lowering therapy in Type 2 diabetes. Vasc Health and Risk Manag. 2013;9:155-63. http://doi.org/10.2147/VHRM.S33934
PMid:23637538
14. Tajik N, Tajik M, Mack I, Enck P. The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: A comprehensive review of the literature. Eur J Nutr. 2017;56(7):2215-44. http://doi.org/10.1007/s00394-017-1379-1
PMid:28391515
15. Marques V, Farah A. Chlorogenic acids and related compounds in medicinal plants and infusions. Food Chem. 2009;113(4):1370-6. https://doi.org/10.1016/j.foodchem.2008.08.08.