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Treatment of Diabetes with a Medicinal Plant "*Citrullus colocynthis*" in Wistar Rats

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ABSTRACT

Background: *Citrullus colocynthis* is a plant of the Cucurbitaceous family, native to arid soils. Coloquine is common in humid and moderately dry tropical regions in Algeria. The present study aims to evaluate the *in vitro* anti-free radical activity of *C. colocynthis* fruit extract on diabetic rats by the evaluation of the impact of these extracts on eight (08) blood parameters.

Methods: Following a daily intraperitoneal injection of 1 ml of Alloxan to healthy Wistar Albinos rats (diabetes induction), intraperitoneal injection of 20 mg/kgp.c of cucurbitacin glycosides was carried out. The treatment effect was monitored by assessment of eight blood parameters.

Result: Intraperitoneal injection of 20 mg/kg of cucurbitacins glycosides of *C. colocynthis* exhibited significant antidiabetic, anti-hyperureimia, anti-proteinemia, anti-hyperlipidemia and anti-hypercholesterolemia activity on Alloxan induced diabetic Wistar laboratory rats. These extracts also showed improvement in lipid, protein, creatinine and urea profile. In perspective more thorough investigations are required to unravel the process behind direct control of type II diabetes.

Key words: Alloxan, *Citrullus colocynthis*, Diabetes, Phytotherapy, Rats.

INTRODUCTION

The diabetes epidemic generates high financial costs for individuals and society due to the consequence of the disease and especially its serious complications. This has meant that populations in the Third World (such as Algeria) and even those in developed countries still use medicinal plants as a therapeutic alternative, particularly for type II diabetes Azzi *et al*. 2012; Grimaldi 2009).

Antidiabetic phytotherapy is currently experiencing considerable growth due to discovering a large number of antidiabetic plant extracts. More than 800 plants have been identified and studied as a potential treatment for type II diabetes. The main active ingredients isolated and identified as antidiabetic are flavonoids, mucilages, glycans, triterpenoids, alkaloids, saponosides and tannins (Perez *et al*. 1998; Wang and Ng 1999).

C. colocynthis belongs to the family of cucurbits that was recorded in several ethnobotanical surveys (Abo 2008; Allali *et al*. 2008; Eddouks and Lemhadri 2007; Ziyyat 1997). The antidiabetic effects of crude extracts and active ingredients present in the seeds of this plant have been reported in many studies (Abdel-Hassan 2000; Azzi *et al*. 2012; Huseini *et al*. 2009). In this context, the present study aims to assess the anti-hyperglycemic, anti-hyperureimia, anti-proteinemia, anti-hyperlipidemia and anti-hypercholesterolemia effect of cucurbitacin glycosides of colony seed (*C. colocynthis*) in diabetic rats by the evaluation of the impact of these extracts on eight (08) blood parameters.

MATERIALS AND METHODS

Plant

The fruits [*C. colocynthis (L.)* Schard], family Cucurbitaceous. It grows in the south of Algeria, the seeds were collected from the fruit and dried in a dark room in the laboratory. The dried seeds were then ground into a fine powder using an electric grinder. To remove fats and other lipophilic substances that can disrupt the subsequent extraction process, particularly by inducing emulsion, preliminary delipidation (degreasing) of the crushed seeds was carried out by percolation using a Soxhlet. Finally, the extraction and study of cucurbitacin glycosides from seeds were carried out according to the method of Hatam *et al*. (1989).

Animals

21 Wistar Albinos rats, purchased from the Pasteur Laboratory of Algeria and with an average weight of 200 g, have been used during our experiment. They have been separated into specialized cages with free access to water and rodent chow. The litter lining the cages was changed every two days and the rats were sheltered under the following climatic conditions: temperature and humidity (spring) and a natural photoperiod (one cycle of 12 h day/night).

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Experimental design

The rats were randomly assigned to 3 groups of seven subjects (C: Control, D: Rats rendered Diabetics, TD: Treated diabetic rats). The animals of all the lots were marked using permanent markers. After induction of diabetes in D and TD groups, the animals have received the plant treatment and blood glucose, triglycerides, HDL, cholesterol, total lipids and total proteins have been evaluated after ten days of treatment.

Induction of diabetes

Before diabetes induction, blood glucose was measured in all groups. After that, animals of D and TD groups have received daily intraperitoneal injections of 1 ml of Alloxan to induce diabetes. Blood glucose levels were then measured to confirm the installation of diabetes.

Preparation and administration of the plant

The treatment of diabetic rats was initiated by intraperitoneal injection of 20 mg/kg of cucurbitacins glycosides of the plant. This dose was chosen after the fitting of several doses on other batches of rats.

Blood glucose determination

The blood glucose rate of the three lots was measured daily for ten days and at fixed hours using meter strips Battery CHEK Active. The blood was taken from the caudal vein of the rats after making a small incision in the distal part of the tail. After each regular operation, the animal's tail was disinfected using cotton soaked in povidone-iodine. The last blood glucose measurement was carried out on the 11th day by colourimetric dosage after sacrificing the animals.

Sacrifice and preparation of the samples

In the morning and at the same time chosen to measure blood glucose, all the rats of all the lots were decapitated using blades sterile scalpel. After each decapitation, the blood was taken into Heparinized tubes and immediately centrifuged at 5000 rpm for 25 min. The plasma harvested was then separated into Eppendorf tubes and stored at -18°C for dosing the biochemical parameters.

All plasma parameters were measured using the OLYMPUS plc At 400. Glucose, triglycerides, lipoproteins: HDL, total cholesterol, total protein, urea, creatinine and total lipids of all animals have been assessed. The following formula calculated Total lipids Metais (1999):

Total lipids= Total cholesterol (2.5)+Triglycerides

Statistical analysis

Statistical analyses were performed using Kruskal-Wallis non-parametric test generating a Benjamini Hochberg false discovery rate (FDR) corrected P-value, followed by Dunn's Post hoc pair wise comparison to determine the source of significant difference between the groups. The result was considered statistically significant when $p<0.05$. Besides, in between groups, error bar charts were plotted for each of the eight (08) blood plasma parameters to describe their respective mean responses. Statistical analysis was carried out in R Studio GUI v1.4.1717 using ggstatsplot and ggplot2 packages (Allaire, 2012; Patil, 2021; Wickham, 2016).

RESULTS AND DISCUSSION

Algeria enjoys a diverse climate where plants grow in abundance in coastal, mountainous and Saharans' regions. These plants are potential natural remedies that can be used in preventative or curative treatment. Medicinal plants in Algeria have never been completely abandoned and people have never stopped using traditional medicine, which contributed to maintaining a living therapeutic tradition, especially for diabetes, despite the spectacular development of modern medicine Abo (2008); Azzi *et al.* (2012).

Colocynth (*C. clocynthis*) has an anti-hyperglycemic effect on rats. Indeed, we have found that administering a single, Intraperitoneal injection containing 20 mg/kg-BW of cucurbitacin's seeds glycosides (Alloxan) caused diabetes during the first three hours.

Globally, the data show that the both groups of rats, Diabetic and Rendered diabetic, showed significant blood chemistry change as indicated by their higher means than control; these observations were significantly higher in the case of Glucose, Cholesterol, Creatinine and Urea. The average levels were increased in both: (i) Treated diabetic (Glucose group, 5.32±0.38 g/l, p= 7.46 e^{-5}; Cholesterol, 0.92±0.08 g/l, p= 8.43 e^{-4}; Creatine 0.01±5.4e^{-4} g/l, p= 7.91 e^{-3} and Urea 1.20±0.18 g/l, p= 7.12e^{-5}; respectively compared to control, 1.21±0.18, 0.64±0.09, 4.7 e^{-3}±5 e^{-4} and 0.45±0.025) and (ii) Rendered diabetic groups (Glucose group, 2.24±0.52 g/l, p= 0.038; Cholesterol group, 0.92±0.08 g/l, p= 0.048, Creatine 0.01±4.5e^{-4} g/l, p= 0.043 and Urea 0.62±0.05 g/l, p= 0.035; compared to control).

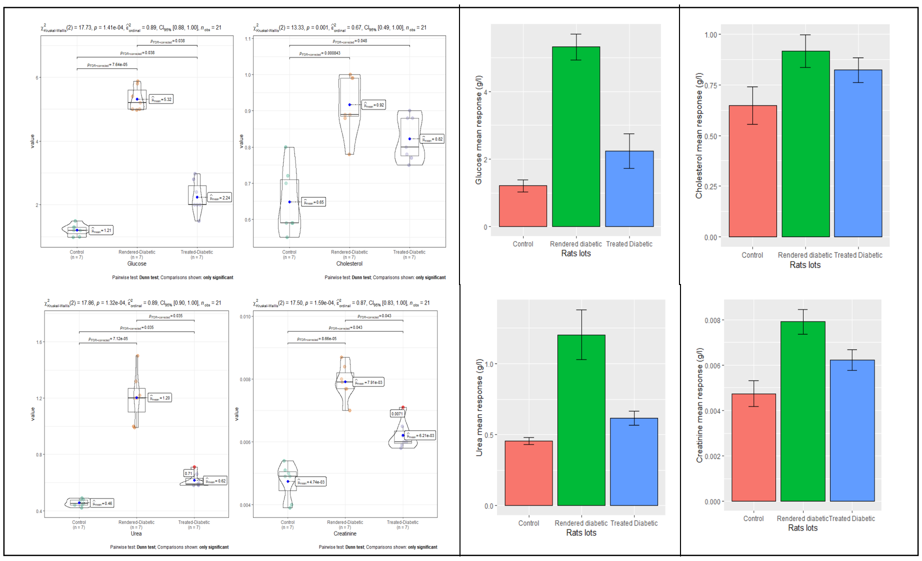
The following Fig 1 and 2 summarize blood biochemical plasma parameters levels in Control, Rendered diabetic and Treated Diabetic rats' lot, along with complete intergroup comparison. Besides, blood plasma parameters mean values±Standard deviation is given within error bar charts; all the variables are given in g/l except for total protein and Triglycerides, reported in units/l.

In rendered diabetic rats lots, plasma biochemical parameters taken before and after sacrifice exhibited severe hyperglycemia due to a lack of secreted insulin. This observation is in agreement with (Venkateswaran and Pari, 2002)and several other recent researchers who confirmed the anti-hyperglycemic effect of the different parts and different excerpts of Colocynth (*C. colocynthis*), which were tested on healthy animals or made diabetic by the action of Alloxan or Streptozotocin (STZ).

We quote, as an example, the work of Dashti (2012), who found a significant decrease of about 12% in blood glucose levels, one week after oral administration of 100 mg/kg-BW of Colocynth seeds to rabbits made diabetic through Alloxan. Even more, Agarwal V (2012) showed that an oral administration of 200 mg/kg-BW of aqueous ethanolic or chloroform extracts of the roots of *C. colocynthis* induced a significant decrease in blood glucose levels in a range oscillating between 34.72% to 58.70%, in Wistar rats made diabetic through Alloxan. Similarly, they reported a decrease in cholesterol and triglyceride levels and a loss in rats body weight.

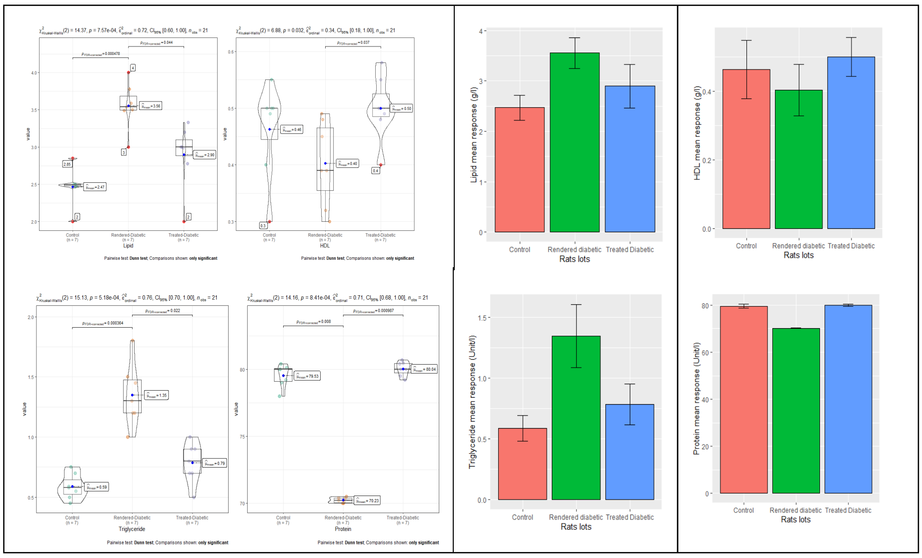
Thus, for rats made diabetic by Alloxan, fruits and seeds of *C. Colocynthis* can have several effects through active constituents that effectively regulate blood glucose levels in diabetic rats and rabbits (glucose homeostasis). These active molecules can exert their effect by potentiating secretion of residual insulin or by increasing cell capacity to maintain glucose: amino acid levels (insulinotropic effect), alkaloids, saponins and glycosides, fixed oil (protective and/or regenerative effect toward the mass of the β-pancreatic cells) (Abdel-Hassan 2000; Azzi *et al*. 2012; Benmehdi 2011; Sebbagh 2009.

Several studies have shown the nephrotoxicity of Alloxan on animal's kidneys (nephropathy) (Almdal 1988;



(A) (B)

Fig 1: (A) Kruskal-Wallis violin-box rank pairwise comparison for control, Rendered diabetic and treated diabetic groups. PFDR adjusted < 0.05; show significant differences between all groups or versus control. • red dots indicate outlier measurements. (B) In between rats' lots error bar charts for Glucose, Cholesterol, Urea and Creatinine.



(A) (B)

Fig 2: (A) Kruskal-Wallis violin-box rank pair wise comparison for control, Rendered diabetic and treated diabetic groups. PFDR adjusted<0.05; show significant differences between all groups or versus control. •red dots indicate outlier measurements. (B) In between rats' lots error bar charts for Lipid, HDL, Triglycerides and Protein.

Ogbeifun *et al*. 2020; Ørskov 1966). In rendered diabetic rats' lot, the concentration of urea and creatinine is higher in comparison to the treated diabetic rats' lot. The rise of the concentration of these two significant markers of renal function is due to renal dysfunction in diabetic rats Eidi 2007). Thus, it appears that the experienced therapy causes a decrease in uremia and creatinine, suggesting adequate protection of kidneys.

In the case of proteinemia, total protein level was significantly lowered in the diabetic group (70.23±0.21 g/l, p= 0.008); compared to the control, 79.52±0.84) (Fig 2).

The in-between rendered diabetic and treated diabetic groups comparison showed significant differences for plasma Triglyceride, Lipid, HDL and protein levels (p<0.022, 0.044, 0.037 and 9.87 e-4). Indeed, mean values were respectively 1.70 and 1.22-fold increased for the couple Triglyceride and lipid and lowered by a factor of -1.25, -1.13 for HDL and Protein compared to the diabetic group. Finally, the latter treated diabetic biochemical indicator mean levels did not fluctuate much when compared to the control group as they all ranged near-control levels (p>0.05 vs. control) (Fig 2).

The total protein profile conforms with protein metabolism who does not show significant changes if there is a short exposition to Alloxan. In this situation, no severe harm is caused to the insulin secretion process (Biolo 1992). In contrast, if the exposition is prolonged, some of the proteins' serum leaks into the urine, resulting in their decline (Hong 2005).

Concerning lipid parameters, there is a very highly significant increase in triglycerides and total lipids of the rendered diseased lot compared to the treated diabetic. In addition, an apparent decrease in total lipids amount was observed on treated batches and this is because of the ability of Colocynth to decrease triglyceride and total cholesterol level (decrease in hyper-cholesterolemia).

Lowering triglyceride levels in diabetic batch inhibits the activity of the lipases (active lipolytic enzyme in the adipocytes), which leads to its Internalization (Babu *et al*. 2007; Gaw and Cowan 2004; Rigelsky and Sweet 2002).

Besides, significant HDL increase in the treated diabetic group with comparison to diabetic ones is probably due to the ability of Colocynth to restore insulin secretion by stimulation of the activity of Lecithin Cholesterol Acyl-transferase (LACT) (Enzyme inside the pre-toxicity of Alloxan that causes a highly significant decrease in emerging HDL particles). This enzyme forms the cholesterol esters of pre-HDL from fatty acids and cholesterol free-forming at the end tail of the HDL molecules (Babu *et al*. 2007; Marshall 2005).

Finally, several studies have pinpointed the active role of flavonoids, triterpenes and saponins in Colocynth and *Crataegus Oxyacantha*, which exert an anti-hyper-cholesterolemia role on diabetic rats (Rigelsky and Sweet 2002).

CONCLUSION

As a conclusion of the present pharmacological analysis, intraperitoneal injection of 20 mg/kg of cucurbitacins glycosides of *C. colocynthis* exhibited significant antidiabetic, anti-hyperureimia, anti-proteinemia, anti-hyperlipidemia and anti-hypercholesterolemia activity on Alloxan induced diabetic Wistar laboratory rats. These extracts also showed improvement in lipide, protein, creatinine and urea profiles. It appears that *C. colocynthis* carry out its hypoglycemic action via insulinotropic impact in addition to the inhibitory impact on glucose absorption, gluconeogenesis and/or glycogenolysis pathways. Moreover, these results consolidate our observations on why phytotherapy remains a widely used practice in third-world countries (*i.e.*, the Algerian population) to treat many diseases, including diabetes mellitus, even if they experience significant socio-economic development and better access to modern medical care. Finally, more thorough investigations are required to pinpoint the specific active molecules (phytoconstituents) directly involved in type II diabetes treatment.

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Declarations

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

REFERENCES

Abdel-Hassan, I.A., Abdel-Barry, J.A., Mohammeda, S.T. (2000). The hypoglycaemic and antihyperglycaemic effect of (*Citrullus colocynthis)* fruit aqueous extract in normal and alloxan diabetic rabbits. Journal of ethnopharmacology. 71: 325-330. DOI: 10.1016/s0378-8741(99)00215-9.

Abo, K.A. (2008). Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. Journal of Ethnopharmacology. 115: 67-71. DOI: 10.1016/j.jep.2007.09.005.

Agarwal, V., Upadhyay, A., Singh, G., Gupta, R. (2012). Hypoglycemic effects of *Citrullus colocynthis* roots. Acta Pol Pharm. 69: 75-79.

Allaire, J. (2012). RStudio: Integrated Development Environment for R. Boston, MA, 770: 165-171.

Allali, H., Benmehdi, H., Dib, M., Tabti, B., Ghalem, S., Benabadji, N. (2008). Phytotherapy of diabetes in west Algeria. Asian Journal of Chemistry. 20: 2701.

Almdal, T.P. (1988). Strict insulin therapy normalises organ nitrogen contents and the capacity of urea nitrogen synthesis in experimental diabetes in rats. Diabetologia. 31: 114-118. DOI: 10.1007/BF00395558.

Azzi, R., Djaziri, R., Lahfa, F., Sekkal, F., Benmehdi, H., Belkacem, N. (2012). Ethnopharmacological survey of medicinal plants used in the traditional treatment of diabetes mellitus in the North Western and South Western Algeria. Journal of Medicinal Plants Research. 6: 2041-2050. DOI: 10.13040/IJPSR.0975-8232.5(5).2006-13.

Babu, P.S., Prabuseenivasan, S., Ignacimuthu, S. (2007). Cinnamaldehydea potential antidiabetic agent. Phytom -edicine. 14: 15-22.

Benmehdi, H., Rachid, A., Rabah, D., Farid, L., Nabila, B., Boufeldja, T. (2011). Effect of saponosides crude extract isolated from *Citrullus colocynthis* (L.) seeds on blood glucose level in normal and streptozotocin induced diabetic rats. Journal of Medicinal Plants Research. 31: 6864-6868. DOI: 10.5897/JMPR11.1369.

Biolo, G., Tessari, O., Inchiostro, O., Bruttomesso, A., Sabadin, I., Fongher, O., Tiengo, O. (1992). Fasting and postmeal phenylalanine metabolism in mild type 2 diabetes. American Journal of Physiology-Endocrinology and Metabolism, 263: E877-E883. DOI: 10.1152/ajpendo.1992.263. 5.E877.

Dashti, N., Zamani, M., Mahdavi, R., Ostad Rahimi, A. (2012). The effect of *Citrullus colocynthis* on blood glucose profile level in diabetic rabbits. Journal of Jahrom University of Medical Sciences. 9: 24-28. DOI: 10.29252/jmj.9.4.27.

Eddouks, M., Ouahidi, O., Farid, A., Moufid, A., KhalidiLemhadri, A. (2007). L'utilisation des plantes médicinales dans le traitement du diabète au Maroc. Phytothérapie. 5: 194-203. DOI: 10.1007/s10298-007-0252-4.

Eidi, A., Eidi, M., Sokhteh, M. (2007). Effect of fenugreek (*Trigonella foenum-graecum* L) seeds on serum parameters in normal and streptozotocin-induced diabetic rats. Nutrition Research. 27: 728-733. DOI: 10.1016/j.nutres.2007.09.006.

Gaw, A.M.M. and Cowan, R. (2004). Métabolisme du Glucose et Diabète Sucré; Diagnostic et Surveillance du Diabčte. O'reilly St JD, Stewart JM, Sheperd J (ed). ElsevierMasson.

Grimaldi, A. (2009). Traité de diabétologie. Flammarion médecine-sciences.

Hatam, N.A., Whiting, D.A.,Yousif, N.J. (1989). Cucurbitacin glycosides from *Citrullus colocynthis*. Phytochemistry. 28: 1268-1271. DOI: 10.1016/0031-9422(89)80230-4.

Hong, C., Shandong, Y., Aihong, F. (2005). Relationship between of VEGF and diabetic nephropathy. Acta Universitis Medicinalis Nahui, 6.

Huseini, H.F., Darvishzadeh, F., Heshmat, R., Jafariazar, Z., Raza, M., Larijani, B. (2009). The clinical investigation of *Citrullus colocynthis* (L.) schrad fruit in treatment of Type II diabetic patients: A randomized, double blind, placebo controlled clinical trial. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 23: 1186-1189.

Marshall, W.J., Bangert, S.K., Raynaud, E. (2005). Biochimie médicale: Physiopathologie et diagnostic. Elsevier.

Ogbeifun, H., Peters, D.Monanu, M. (2020). Ameliorative Effect of *Citrullus lanatus* (Water Melon) Seeds on Alloxan Induced Hepato and Nephro Toxicity. Asian Journal of Advanced Research and Reports: 1-10.

Ørskov, H., Olsen, T.S., Nielsen, K., Rafaelsen, O.J., Lundbaek, K. (1966). Kidney lesions in rats with severe long-term alloxan diabetes. Diabetologia. 1: 172-179.

Patil, I. (2021). Visualizations with statistical details: The'ggstatsplot' approach. Journal of Open Source Software. 6. DOI: 10.21105/joss.03167.

Perez, G.R., Zavala, S.M., Perez, G.S., Perez, G.C. (1998). Antidiabetic effect of compounds isolated from plants. Phytomedicine. 5: 55-75. DOI: 10.1016/S0944-7113(98)80060-3.

Rigelsky, J.M. and Sweet, B.V. (2002). Hawthorn: Pharmacology and therapeutic uses. American Journal of Health-System Pharmacy. 59: 417-422. DOI: 10.1093/ajhp/59.5.417.

Sebbagh, N., Ouali, F., Berthault, M.F., Rouch, C., Sari, D.C., Magnan, C. (2009). Comparative effects of *Citrullus colocynthis*, sunflower and olive oil-enriched diet in streptozotocin-induced diabetes in rats. Diabetes Metab. 35: 178-184. DOI: 10.1016/j.diabet.2008.10.005.

Venkateswaran, S. and Pari, L. (2002). Effect of *Coccinia indica* on blood glucose, insulin and key hepatic enzymes in experimental diabetes. Pharmaceutical Biology. 40: 165-170. DOI: 10.1076/phbi.40.3.165.5836.

Wang, H. and Ng,T. (1999). Natural products with hypoglycemic, hypotensive, hypocholesterolemic, antiatherosclerotic and antithrombotic activities. Life Sciences. 65: 2663-2677. DOI: 10.1016/S0024-3205(99)00253-2.

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. https://ggplot2.tidyverse.org

Ziyyat, A., Legssyer, H., Mekhfi, A., Dassouli, M., Serhrouchni, W. Benjelloun. (1997). Phytotherapy of hypertension and diabetes in oriental Morocco. Journal of Ethnopharmacol. 58: 45-54. DOI: 10.1016/S0378-8741(97)00077-9.