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Phytochemical Screening, Hypoglycemic and Antihyperglycemic Effect of Flavonoids from the Leaves of Algerian *Olea europaea* L. in Normal and Alloxan-Induced Diabetic Rats

[1,2]N. Benhabyles, [3]K. Arab, [3]O. Bouchenak and [1]A. Baz
[1]Laboratory of Biology and Animal Physiology, Normal Superior School, Kouba, 16308, Algeria
[2]Faculty of Biological Sciences, University of Science and Technology, Houari Boumediene (USTHB) Bp 32, 16111 Bab Ezzouar EL-Alia, Algier's, Algeria
[3]Laboratory of Valorisation and Conservation of Biological Resources, Department of Biology, Faculty of Sciences, University of M'Hamed Bougara, Boumerdes, 35000, Algeria

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Corresponding Author:
N. Benhabyles,
Laboratory of Biology and Animal Physiology, Normal Superior School, Kouba, 16308, Algeria

ABSTRACT
Phytotherapy has a promising future in the management of diabetes, considered to be less toxic and free from side effects as compared to the use of synthetic drugs. The aim of this study was set out to study the preliminary phytochemical profile and to determine the hypoglycemic and antihyperglycemic activities of the Aqueous Extract (AE) extracted from the leaves of *Olea europaea* L. in normal and alloxan-induced diabetic rats. The aqueous extract was obtained by confrontation with organic solvents method with a yield of 37%. The total phenol and flavonoid contents were determined using designed methods and found to be 378.4±5.2 mg/100 g of gallic acid and 125.13±2.8 mg/100 g of quercetin, respectively. The 100 mg kg^{-1} of aqueous extract was administered intravenously and produced a significant decrease in blood glucose level ($p<0.05$). Plasma insulin levels were also determined and the results showed a significant increase of insulinemia ($p<0.001$). In the other hand, Change in blood glucose in diabetic rats treated in acute treatment (2 h) at doses of 200, 400 and 600 mg kg^{-1} in subchronic treatment (28 days) at a dose of 200 mg kg^{-1} showed a substantial decrease in blood glucose for all groups treated with different doses of flavonoids in both antidiabetic assays ($p<0.001$). From the results it can be concluded that flavonoids of *Olea europaea* can be a potential candidate in treating the hyperglycemic conditions.

Key words: *Olea europaea* L., diabetes mellitus, flavonoids, alloxan, hypoglycemic effect, antidiabetic, Insulin

INTRODUCTION

Diabetes mellitus is a heterogeneous group of metabolic diseases in which the main feature is hyperglycaemia resulting from a lack of secretion, insulin action or both associated anomalies (Sharma *et al*., 2008). It affects nearly 10% of the world population (Burke *et al*., 2003) and is the major source of morbidity in developed countries (Ravi *et al*., 2005). The increasing worldwide incidence of diabetes mellitus constitutes a global public health burden (Wild *et al*., 2004). In some traditional societies (China, some African and Latin American) the medicinal treatment of chronic pathologies, such as diabetes, is largely provided by the use of medicinal plants (Sharma *et al*., 2008; Zhou *et al*., 2009; Singh and Kakkar, 2009). In fact, human life on earth is closely linked to the exploitation of plants. These have the capacity to produce wide range of natural substances. Currently 1200 species of plants are used in traditional treatment of diabetes. The beneficial effects seem to be attributable to its secondary metabolites content. In fact, flavonoids and phenolic alcohols (Bianco *et al*., 2006) a highly diverse class of secondary plant metabolites with about 9000 structures (Martens and Mithofer, 2005). Flavonoids are polyphenolic compounds derived from 2-phenylchromane commonly found in many plants,

vegetables and flowers (Boue *et al*., 2003; Plochmann *et al*., 2007; Androutsopoulos *et al*., 2010). They have been particularly studied for many biological effects, such as their role in protecting against inflammations, cardiovascular disease (Narayana *et al*., 2001) their vasculoprotective action (Vitor *et al*., 2004), antibacterial (Wilson *et al*., 1997) and antidiabetic (Gurib-Fakim, 2006).

Olea europaea L. is among the important and oldest fruit trees in the world, particularly, in the Mediterranean Basin. Olive leaves are one of the by products of farming of the olive grove, they accumulate during pruning of the olive trees (Tabera *et al*., 2004). Several reports have shown that olive leaf extract has the capacity to treat and prevent the intestinal muscle spasms, hypertension and for their hypoglycaemic and antiseptic properties (Bruneton, 1993). Literature data indicate that some flavonoids isolated from medicinal plants significantly reduce the blood glucose levels (Perez *et al*., 2000; Koreca *et al*., 2000; Abdel-Hassan *et al*., 2000). The aim of this report was to evaluate the efficacy of hypoglycemic properties of flavonoids isolated from the leaves of *Olea europaea* L. in normal and alloxan diabetic rats.

MATERIALS AND METHODS

Plant material: Experiments were carried out on the leaves of *Olea europaea* commonly cultivated in different areas of Algeria. Leaves were obtained by handpick and collected during March 2014 near Issers, Boumerdes, East of Algiers, Algeria. Leaves were allowed to air dry at room temperature and then ground using a microwave (waring). Dried leaves were powdered and stored in a dry and dark place.

Extraction procedure: The extract was obtained as follows, 30 g of leaves powder was soaked in methanol (85%, 1: 10 w/v) for 72 h at 4°C. The macerate was filtered on a Buchner under reduced pressure and then subjected to evaporation at low pressure at 50°C (Stuart RE300DB, UK). The aqueous phase obtained was preserved for 48 h at 40°C to accelerate the diffusion of molecules in the solvent and then filtered by using Whatman No. 1 filter paper. The concentrated extract was then washed three times with petroleum ether (v/v).

The resulting aqueous phase was extracted three times with by n-butanol (v/v). The Aqueous Extract (AE) were concentrated by evaporation at low pressure at 35°C and then weighed to calculate the efficiency of extraction and finally stored at 4°C until use. The percentage of crude dry extract was determined as follows:

$$\text{Y extract}(\%) = \frac{\text{Mextract}}{\text{m feed}} \times 100$$

Determination of total phenolics content: The total phenolics content of the plant extract was determined by Folin-Ciocalteu Reagent (FCR) method (Gao *et al*., 2000). The extract was mixed with 0.2 mL Folin-Ciocalteau reagent, 2 mL of distilled water and 1 mL of 15% Na_2CO_3. The absorbance was measured at 765 nm (using a UV Optizen 2120 spectrophotometer, Korea) after 2 h incubation at room temperature. The levels of total phenolics content were determined in triplicate. Total phenolics content was expressed as µg of gallic acid equivalent/g of dry extract (µg GAE g^{-1}).

Determination of total flavonoids content: Total flavonoids content of the plant extracts was determined using aluminum chloride colorimetric method and using standard solutions (20, 40, 80 and 100 µg mL^{-1} of quercetin in 80% methanol) (Basma *et al*., 2011). One milliliter of the extract was added to a 10 mL volumetric flask. Distilled water was added to make a volume of 5 mL. At time zero, 0.3 mL of 5% (w/v) sodium nitrite was added to the flask. After 5 min, 0.6 mL of 10% (w/v) $AlCl_3$ was added and then at 6 min, 2 mL of 1 M NaOH were also added to the mixture, followed by the addition of 2.1 mL distilled water. Absorbance at 510 nm was read immediately. Quercetin was used as the standard for the quantification of total flavonoid. Results were expressed as milligrams of quercetin equivalent per gram of dry weight extract (mg QE g^{-1}). Total content of flavonoids was calculated as follows:

$$\text{Total flavonoid content} = \text{QE} \times \frac{\text{V}}{\text{m}}$$

where, QE is the quercetin equivalence (mg mL^{-1}) or concentration of quercetin solution established from the calibration curve; V is the volume of extract (mL) and m is the weight (g) of the dry extract. Data were reported as arithmetic Mean±SD for three replications.

Animals: Adult male wistar rats, weighing 120-200 g, purchased from the Pasteur Institute of Algeria were used in the experiments. Water and food were provided *ad libitum*. Prior to the experiments, rats were fed with standard food for one week in order to adapt to the laboratory conditions. Sixteen hours before the experiments, they were fasted overnight, but allowed free access to water. The experiment was conducted at room temperature (20°C) with a natural cycle of light and darkness.

Oral glucose tolerance test: The rats were fasted for 14 h prior OGTT. The AE of the leaves of *O. europaea* was administrated at a dose of 100 mg kg^{-1}. At 10 min after the administration of the treatments, 4 g kg^{-1} of glucose was administered to each rat. The blood glucose level was measured using a glucometer (On Call Plus, ACON Laboratories, Inc. USA) before the treatment and every 30 min for 2 h of treatment.

Effect of flavonoids on insulin secretion: To evaluate the effect of flavonoids on insulin secretion, a normoglycemic group of rat was treated with 100 mg kg^{-1} of AE. The tow other groups controls and standards have received,

respectively saline solution and glucophage (2.5 mg kg^{-1}). The insulinemia was measured before and after 60, 90 min of treatment using an enzyme immunoassay method ELISA type (AxSYM Insulin reagent pack, Abbot Laboratory, USA).

Effects on alloxan-induced diabetic rats (non-insulin dependent diabetes model, NIDDM)

Alloxan induced diabetic rats: To assess the antidiabetic activity of flavonoids, induction of experimental diabetes is made by injection, intraperitoneally, of 125 mg kg^{-1} of alloxan (A7413-25G SIGMA-ALDRICH, US) (Diatewa *et al*., 2004), which will cause selective necrosis on pancreatic beta cells giving chronic insulin deficiency (Dhanabal *et al*., 2007). The monohydrate alloxan is reconstituted prior to administration in a physiological solution (NaCl 0.9%) to provide the concentration described previously to be subsequently injected in rats. One week after injection, an evaluation of blood glucose is performed and the rats, whose blood glucose is higher than 2 g L^{-1} were considered diabetic and randomly divided into groups of 5.

Acute effect of AE on diabetic rats: To evaluate the antidiabetic effect of AE, 6 groups of 5 rats were used: group 1, control diabetic, receiving orally 1 mL of saline solution (0.9% NaCl), groups 2, 3 and 4, respectively receive the AE in a single dose of 200, 400 and 600 mg kg^{-1}, group 5 standard control receives glucophage at a dose of 2.5 mg kg^{-1} per os. Blood glucose of animals was measured using a glucometer (On Call Plus, ACON laboratories, Inc. USA) before gavage and 30, 60, 90 and 120 min after treatment.

Subacute effect of AE on diabetic rats: To assess the antidiabetic effect of the aqueous extract on diabetic rats for 28 days, three groups of rats were used: A diabetic group and a standard group received orally a daily dose of respectively 200 mg kg^{-1} of flavonoids and 2.5 mg kg^{-1} of glucophage, diabetic control group received saline solution (NaCl 0.9%). The blood glucose of animals was measured at D+7, D+14, D+21 and D+28.

Statistical analysis: Data were expressed as Means±SE of Mean (SEM). The statistical significance was evaluated by one-way using the statistical software Statistica® version 6 (Genistat Conseils Inc, Montréal) followed by the Dunnett's Multiple Comparisons Test or T test. At a 95% confidence interval, p-value = 0.05 was considered statistically significant.

RESULTS

Extraction yield and total flavonoids and phenols content: In this study and from the data presented in Table 1, AE containing soluble flavonoids was obtained with a yield of 37%. The total flavonoids of the aqueous extract of *Olea europaea* was determined using method based on the formation of a flavonoid-aluminium complex. As evidenced in data reported in Table 1, The total phenolics content of AE was 378.4±5.2 mg/100 g of gallic acid equivalent of plant extract with reference to gallic acid standard curve ($y = 0.004x+0.521$ and $R^2 = 0.996$). These observations suggest that *Olea europaea* plant is rich in phenolics that have potential as value added products. The total flavonoid content of AE was 125.13±2.8 mg/100 g of quercetin equivalent of plant extract with reference to quercetin standard curve ($Y = 0.05x -0.375$ and $R^2 = 0.991$).

Table 1: Extraction yield and total polyphenols and flavonoids contents of aqueous extracts of *Olea europaea*

AE contents	Values
Extraction yield (%)	37
Phenols (mg GAE/100 g)[a]	378.40±5.2
Flavonoids (mg EQ/100 gE)[a]	125.13±2.8

[a]Each value was expressed as Mean±SD (n =3), AE: Aqueous extracts

Hypoglycemic activity: As shown in Fig. 1, the oral administration of saline solution did not alter blood glucose on normoglycemic rats. Blood glucose remains stable effect after 2 h of observation (0.80±0.01 vs. 0.82±0.02 g L^{-1}) ($p>0.05$). In the group of hyperglycemic rats, the oral administration of glucose at a dose of 4 g kg^{-1} causes a significant increase of blood glucose ranging from 0.84±0.01 to 1.2±0.02 g L^{-1}, with a peak of 1.54±0.01 g L^{-1} noted after 90 min. In the group pre-treated with glucophage at a dose of 2.5 mg kg^{-1} rats, there has been a highly significant decrease in blood glucose compared to hyperglycemic control after 2 h of administration (0.81±0.01 vs. 0.34±0.01 g L^{-1}) ($p<0.001$). The AE declined sharply blood glucose in rats. The same observation was made about the effect of glucophage on the content of the glucose-treated rats. This reduction is probably the result of glucose uptake on the OH functions of flavonoids.

Effect of AE on insulin secretion: The results showed that there is a positive variation of insulinemia in the blood of rats treated with flavonoids (Fig. 2). The insulinemia values were significantly higher both for the animals treated with the flavonoids that those treated with glucophage compared with those obtained on the serum of untreated rats ($p \leq 0.001$).

Antihyperglycemic activity: The results of the acute effect of the AE of *Olea europaea* on diabetic rats are illustrated in Fig. 3. There's been a significant decrease in blood glucose during the 120 min for all the groups receiving treatment with different doses of flavonoids. Indeed, from 30 min, the difference of blood glucose levels of treated diabetics groups is already significant ($p<0.05$) compared to the initial blood glucose of diabetics control. This drop in blood sugar continuous in time depending on the doses of flavonoids administered to reach the limit of 1.99±0.018 g L^{-1} to 600 mg kg^{-1}, 2.79±0.075 g L^{-1} to 400 mg kg^{-1} and 3.05±0.086 g L^{-1} to 200 mg kg^{-1}. All these values are highly significant in relation to the initial glucose of the same group or the control group. It is noted that the smaller blood glucose after 120 min of treatment has no significant difference with that obtained with the glucophage in the same period (1.82 ±0.013 g L^{-1}) (Fig. 4). The variation of blood glucose in subchronic treatment with AE showed that there is a very significant and/or a highly significant

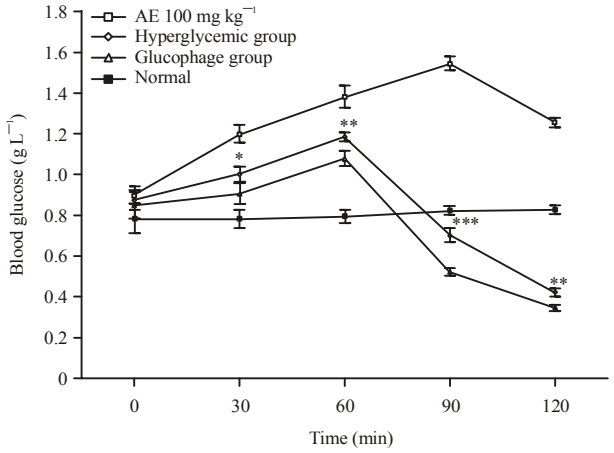

Fig. 1: Percentage reduction of blood glucose produced by AE of the leaves of *O. europaea* after oral administration in glucose load rats. Each point represents the Means±SEM (n = 5). *p<0.05 the difference is significant, **p<0.01 the difference is highly significant, ***p<0.001 the difference is very highly significant

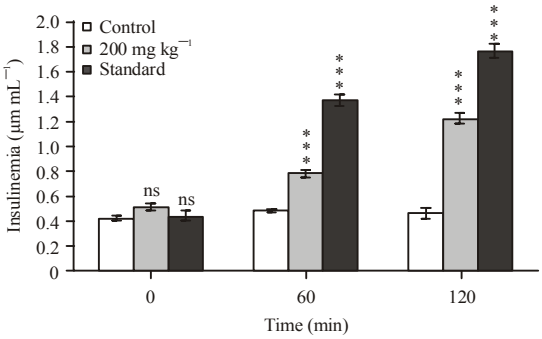

Fig. 2: Variation of insulinemia in normoglycemic rats treated with flavonoids. Each point represents the Means±SEM (n = 6). ns: Non significant, ***p<0.001 the difference is very highly significant

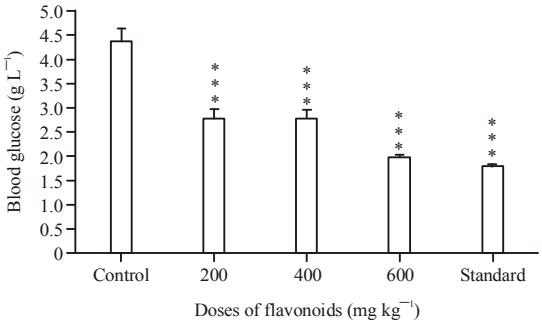

Fig. 4: Effect of AE on blood glucose in diabetic rats throughout 120 min of treatment. Data represent Means±SEM (n = 5). ***p<0.001 the difference is very highly significant, compared to the control group

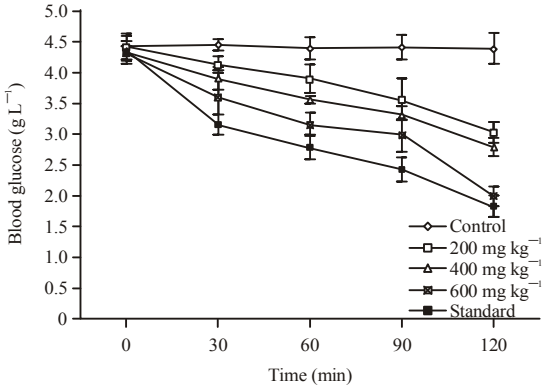

Fig. 3: Effect of AE on blood glucose at different doses in diabetic rats. The values are Mean±SEM (n = 5)

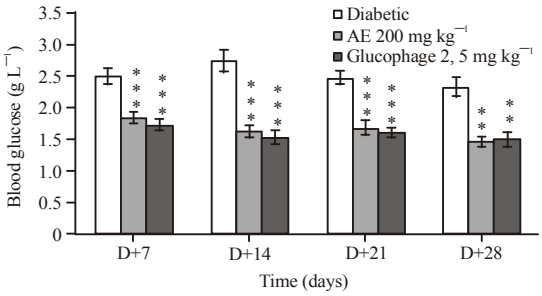

Fig. 5: Change in blood glucose in diabetic rats during 28 days of treatment by AE. Values are given as Mean±SEM (n = 5). **p<0.01 the difference is highly significant, ***p<0.001 the difference is very highly significant

reduction of blood glucose (p<0.001) in diabetic rats in the four weeks of treatment (Fig. 5). These glucose levels are not significantly different from those obtained with the standard group.

DISCUSSION

Diabetes is one of the most challenging diseases facing health care professionals today. Current oral antidiabetic

agents represented by the insulin secretors, insulin sensitizers and glucosidase inhibitors have a modest action with limited effectiveness (Yu *et al*., 1999). For example, several studies have indicated that metformin had a little or no effect on peripheral glucose uptake stimulated by insulin in diabetic's obese subjects (Inzucchi *et al*., 1998; Yu *et al*., 1999). In addition, the antidiabetic drugs, generally, have some side effects, decreased effectiveness over time, inefficiency against diabetic complications in the long term and a low cost-effectiveness (Grover *et al*., 2002). Consequently, the discovery and development of new antidiabetic drugs is even more indispensable. Hence, plants have been suggested as a rich as yet unexplored source of potentially useful antidiabetic drugs (Koehn and Carter, 2005; Frode and Medeiros, 2008). However, only a few have been subjected to detailed scientific exploration due to a lack of mechanism based available in vitro assays (Saxena and Vikram, 2004). Folk medicine performed by humans throughout the world is mainly based on the use of plants as sources of natural active substances. Among these substances, phenolic compounds and including flavonoids are typically known for health promoting properties such as antioxidant, anti-allergic, anti-inflammatory, antimicrobial and anticancer properties (Aiyelaagbe and Osamudiamen, 2009).

In our present study, the choice of the plant is based on the results of an ethnobotanical survey at the local level, which highlighted the wide use of *Olea europaea* by populations to treat diabetes. The method of flavonoids extraction adopted was used to extract the maximum amount of compounds and prevent their denaturation or modification likely due to the high temperatures used in other extraction methods. Therefore, it is difficult to compare our results with those of the bibliography. Indeed, the yield is only dependent on the geographical origin of the plant and the method and the conditions under which the extraction was performed (Lee *et al*., 2003). But for this preliminary experiment the yield obtained was considered for further analysis. The established health benefits of phenolics due to their biological activities, necessitates their quantification. The total soluble phenols and flavonoids of the leaves of *Olea europaea* were determined by Folin–Ciocalteau assay and using a method based on the formation of a flavonoid-aluminium complex, respectively. The observed results suggest that the leaves of *olea europaea* were rich in phenolics and flavonoids that have potential as value added products. Like any plant products, the total phenolic and flavonoids contents and the radical inhibitory effect can be influenced by the plant source and environment where the plant is collected and cultivated. On the other hand, using different methods of extraction reduces the possibility of any comparison between studies.

The purpose of the hypoglycemic activity study was to evaluate the glucose tolerance by peripheral tissues of animals treated with flavonoids. The hypoglycemic effect of AE of the leaves of *O. europaea* can be explained by an increase in glucose tolerance in peripheral tissues, i.e., a very efficient use of glucose by these tissues and the increase in insulin secretion. Our results are in agreement with the work of some authors on the flavonoids extracted from *Biophytum sensitivum* (Puri, 2001) *Musanga cecropioide* (Adeneye *et al*., 2007), *Nymphaea stellataqui* (Dhanabal *et al*., 2007) and *Leandra lacunose* (Cunha *et al*., 2008). In addition, other authors assumed that the fraction of ethyl acetate of *Euonymus alatus*, rich in flavonoids, can be equipped with stimulant properties on the release of insulin and is therefore responsible for the hypoglycemic effect in normoglycemic mice and anti hyperglycemic mice with non-insulin dependent diabetes (Fang *et al*., 2008). Therefore and for our study, the significant hypoglycemic effect of AE could probably be linked to a mechanism involving the secretion of insulin. The significant increase of insulinemia is explained by a stimulation of the β cells by flavonoids and increasing the secretion of insulin during the 90 min of treatment. This hyperinsulinemia is suggested at the origin of hypoglycemia, that is why, it is preponderant to administer a smaller flavonoids dose to prevent the drop in blood sugar below the normoglycemia. In this context, a number of other plants have proven to have antihyperglycemic activity with a stimulating effect on the regulation and release of insulin (Sharma *et al*., 2006; Esmaeili and Yazdanparas, 2004).

Alloxan is known for its selective pancreatic islet β cell cytotoxicity and has been extensively used to induce a hyperglycemic state in animals (Lenzen, 2008). Our results showed that *O. europaea* can compensate the metabolic alterations occurring in alloxan induced hyperglycemic rats. A sustainable normalization of blood glucose decreases the risk of micro-vascular disease and reduces the complications of diabetes. Conventional therapies for diabetes have many shortcomings, for example the secondary effects such as oxidative stress and insulin intolerance (Punitha *et al*., 2005). It has been shown that plant extracts have the same efficiencies as antidiabetic drugs without secondary effects (Kim *et al*., 2006).

In this study, the AE after its administration at a dose of 200 mg kg^{-1} on diabetic rats during 28 days of treatment caused a significant hypoglycemic effect ($p<0.01$). The same effect has been demonstrated by other authors with the treatment of diabetic rats with alcoholic extracts of other plants (*Musanga cecropioides*, *Berberis aristata*) (Singh and Kakkar, 2009). Some authors have had a positive correlation of the decrease in blood glucose in insulin secretion (Cunha *et al*., 2008). Other authors (Esmaeili and Yazdanparas, 2004; Gupta *et al*., 2009; Sharma *et al*., 2008; Dhanabal *et al*., 2007) have shown that flavonoids extracts of *Eugenia jambolana* plants, *Cassia auriculata* and *Teucrium polium* stimulate and regenerate β cells of the pancreas.

CONCLUSION

This study provides scientific evidence for the ethnobotanical use and action of flavonoids of *Olea europaea*, which helps its research and development for type 2 diabetes. Further studies need to be conducted to elucidate the bioactive compounds and molecular mechanisms.

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REFERENCES

Abdel-Hassan, I.A., J.A. Abdel-Barry and S.T. Mohammeda, 2000. The hypoglycaemic and antihyperglycaemic effect of *Citrullus colocynthis* fruit aqueous extract in normal and alloxan diabetic rabbits. J. Ethnopharmacol., 71: 325-330.

Adeneye, A.A., O.P. Ajagbonna and O.W. Ayodele, 2007. Hypoglycemic and antidiabetic activities on the stem bark aqueous and ethanol extracts of *Musanga cecropioides* in normal and alloxan-induced diabetic rats. Fitoterapia, 78: 502-505.

Aiyelaagbe, O.O. and P.M. Osamudiamen, 2009. Phytochemical screening for active compounds in *Mangifera indica* leaves from Ibadan, Oyo State. Plant Sci. Res., 2: 11-13.

Androutsopoulos, V.P., A. Papakyriakou, D. Vourloumis, A.M. Tsatsakis and D.A. Spandidos, 2010. Dietary flavonoids in cancer therapy and prevention: Substrates and inhibitors of cytochrome P450 CYP1 enzymes. Pharmacol. Ther., 126: 9-20.

Basma, A.A., Z. Zakaria, L.Y. Latha and S. Sasidharan, 2011. Antioxidant activity and phytochemical screening of the methanol extracts of *Euphorbia hirta* L. Asian Pac. J. Trop. Med., 4: 386-390.

Bianco, A., M.A. Chiacchio, G. Grassi, D. Iannazzo, A. Piperno and R. Romeo, 2006. Phenolic components of *Olea europea*: Isolation of new tyrosol and hydroxytyrosol derivativess. Food Chem., 95: 562-565.

Boue, S.M., C.H. Carter-Wientjes, B.Y. Shih and T.E. Cleveland, 2003. Identification of flavone aglycones and glycosides in soybean pods by liquid chromatography-tandem mass spectrometry. J. Chromatogr. A., 991: 61-68.

Bruneton, J., 1993. Pharmacognosie: Phytochimie-Plantes Medicinales. 2nd Edn., Tec. et Doc. Lavoisier, Paris.

Burke, J.P., K. Williams, K.M.V. Narayan, C. Leibson, S.M. Haffner and M.P. Stern, 2003. A population perspective on diabetes prevention: Whom should we target for preventing weight gain. Diabetes Care, 26: 1999-2004.

Cunha, W.R., G.M. Arantes, D.S. Ferreira, R. Lucarini and M.L.A. Silva *et al*., 2008. Hypoglicemic effect of *Leandra lacunose* in normal and alloxan-induced diabetic rats. Fitoterapia, 79: 356-360.

Dhanabal, S.P., M.K.M.M. Raja, M. Ramanathan and B. Suresh, 2007. Hypoglycemic activity of *Nymphaea stellata* leaves ethanolic extract in alloxan induced diabetic rats. Fitoterapia, 78: 288-291.

Diatewa, M., C.B. Samba, T.C.H. Assah and A.A. Abena, 2004. Hypoglycemic and antihyperglycemic effects of diethyl ether fraction isolated from the aqueous extract of the leaves of *Cogniauxia podoleana* Baillon in normal and alloxan-induced diabetic rats. J. Ethnopharmacol., 92: 229-232.

Esmaeili, M.A. and R. Yazdanparast, 2004. Hypoglycemic effect of Teucrium polium: Studies with rat pancreatic islets. J. Ethnopharmacol., 95: 27-30.

Fang, X.K., Y. Gao, H.Y. Yang, S.M. Lang, Q.J. Wang, B.Y. Yu and D.N. Zhu, 2008. Alleviating effects of active fraction of *Euonymus alatus* abundant in flavonoids on diabetic mice. Am. J. Chin. Med., 36: 125-140.

Frode, T.S. and Y.S. Medeiros, 2008. Animal models to test drugs with potential antidiabetic activity. J. Ethnopharmacol., 115: 173-183.

Gao, X., M. Ohlander, N. Jeppsson, L. Bjork and V. Trajkovski, 2000. Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation. J. Agric. Food Chem., 48: 1485-1490.

Grover, J.K., S. Yadav and V. Vats, 2002. Medicinal plants of India with anti-diabetic potential. J. Ethnopharmacol., 81: 81-100.

Gupta, S., S.B. Sharma, S.K. Bansal and K.M. Prabhu, 2009. Antihyperglycemic and hypolipidemic activity of aqueous extract of *Cassia auriculata* L. leaves in experimental diabetes. J. Ethnopharmacol., 123: 499-503.

Gurib-Fakim, A., 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Mol. Aspects Med., 27: 1-93.

Inzucchi, S.E., D.G. Maggs, G.R. Spollett, S.L. Page, F.S. Rife, V. Walton and G.I. Shulman, 1998. Efficacy and metabolic effects of metformin and troglitazone in type II diabetes mellitus. N. Eng. J. Med., 338: 867-872.

Kim, S.H., S.H. Hyun and S.Y. Choung, 2006. Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. J. Ethnopharmacol., 104: 119-123.

Koehn, F.E. and G.T. Carter, 2005. The evolving role of natural products in drug discovery. Nat. Rev. Drug Discov., 4: 206-220.

Koreca, R., K.H. Sensch and T. Zoukas, 2000. Effects of the neoflavonoid coutareagenin, one of the antidiabetic active substances of *Hintonia latiflora*, on streptozotocin-induced diabetes mellitus in rats. Arzneeimittel-Forschung, 50: 122-128.

Lee, K.W., Y.J. Kim, H.J. Lee and C.Y. Lee, 2003. Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. J. Agric. Food Chem., 51: 7292-7295.

Lenzen, S., 2008. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia, 51: 216-226.

Martens, S. and A. Mithofer, 2005. Flavones and flavone synthases. Phytochemistry, 66: 2399-2407.

Narayana, K.R., M.S. Reddy, M.R. Chaluvadi and D.R. Krishna, 2001. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian J. Pharmacol., 33: 2-16.

Perez, R.M.G., H. Cervantes, M.A.S. Zavala, J.A. Sanchez, S.G. Perez and C.G. Perez, 2000. Isolation and hypoglycemic activity of 5, 7,3 -trihydroxy-3,6,4-trimethoxyflavone from *Brickellia veronicaefolia*. Phytomedicine, 7: 25-29.

Plochmann, K., G. Korte, E. Koutsilieri, E. Richling and P. Riederer *et al*., 2007. Structure-activity relationships of flavonoid-induced cytotoxicity on human leukemia cells. Arch. Biochem. Biophys., 460: 1-9.

Punitha, I.S.R., K. Rajendran, A. Shirwaikar and A. Shirwaikar, 2005. Alcoholic stem extract *Coscinium fenestratum* regulates carbohydrate metabolism and improves antioxidant status in streptozotocin-nicotinamide induced diabetic rats. Evid. Based Complement. Alternat. Med., 2: 375-381.

Puri, D., 2001. The insulinotropic activity of a Nepalese medicinal plant *Biophytum sensitivum*: Preliminary experimental study. J. Ethnopharmacol., 78: 89-93.

Ravi, K., S. Rajasekaran and S. Subramanian, 2005. Antihyperlipidemic effect of *Eugenia jambolana* seed kernel on streptozotocin-induced diabetes in rats. Food Chem. Toxicol., 43: 1433-1439.

Saxena, A. and N.K. Vikram, 2004. Role of selected Indian plants in management of type 2 diabetes: A review. J. Alter. Complement Med., 10: 369-378.

Sharma, S.B., A. Nasir, K.M. Prabhu and P.S. Murthy, 2006. Antihyperglycemic effect of the fruit-pulp of *Eugenia jambolana* in experimental diabetes mellitus. J. Ethnopharmacol., 104: 367-373.

Sharma, B., G. Viswanath, R. Salunke and P. Roy 2008. Effects of flavonoid-rich extract from seeds of *Eugenia jambolana* (L.) on carbohydrate and lipid metabolism in diabetic mice. Food Chem., 110: 697-705.

Singh, J. and P. Kakkar, 2009. Antihyperglycemic and antioxidant effect of *Berberis aristata* root extract and its role in regulating carbohydrate metabolism in diabetic rats. J. Ethnopharmacol., 123: 22-26.

Tabera, J., A. Guinda, A. Ruiz-Rodriguez, F.J. Senorans, E. Ibanez, T. Albi and G. Reglero, 2004. Countercurrent supercritical fluid extraction and fractionation of high-added-value compounds from a hexane extract of olive leaves. J. Agric. Food Chem., 52: 4774-4779.

Vitor, R.F., H. Mota-Filipe, G. Teixeira, C. Borges, A.I. Rodrigues, A. Teixeira and A. Paulo, 2004. Flavonoids of an extract of *Pterospartum tridentatum* showing endothelial protection against oxidative injury. J. Ethnopharmacol., 93: 363-370.

Wild, S., G. Roglic, A. Green, R. Sicree and H. King, 2004. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care, 27: 1047-1053.

Wilson, C.L., J.M. Solar, A. El Ghaouth and M.E. Wisniewski, 1997. Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. Plant Dis., 81: 204-210.

Yu, J.G., Y.T. Kruszynska, M.I. Mulford and J.M. Olefsky, 1999. A comparison of troglitazone and metformin on insulin requirements in euglycemic intensively insulin-treated type 2 diabetic patients. Diabetes, 48: 2414-2421.

Zhou, J., S. Zhou, J. Tang, K. Zhang and L. Guang *et al*., 2009. Protective effect of berberine on beta cells in streptozotocin- and high-carbohydrate/high-fat diet-induced diabetic rats. Eur. J. Pharmacol., 3: 262-268.