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Original article

In *vitro* alpha-amylase and alpha-glucosidase inhibitory activity and in *vivo* antidiabetic activity of *Quercus coccifera* (Oak tree) leaves extracts

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ABSTRACT

Quercus species are group of plants known as oak which represent important genus of Fagaceae family. These species are widely distributed in Mediterranean countries. Many of those species used in traditional medicine to treat and prevent various human disorders such as diabetes. Exhausted extraction for *Quercus coccifera* leaves were carried out using n-hexane, chloroform, methanol, boiled water and microwaved water. Extracts were subjected to phytochemical screening, acute toxicity study, and in vitro and in *vivo* animal model to evaluate antidiabetic activity of the produced extracts. The highest in *vitro* activity against α-amylase and α-glucosidase activity was obtained from methanolic extract with an IC_{50} of 0.17 and 0.38 μg/ml respectively and better than the positive control acarbose. While the rest of the extract was either with moderate or low activity. Similarly, in the in *vivo* study, methanolic extract with a concentration of 200 mg/kg/day was able to reduce the blood glucose level for the diabetic mice to 146.8 mg/dL with normal bodyweight and biochemical signs when compared to the normal mice group. While the rest of the extracts were either with moderate or low ability to maintain blood glucose level for diabetic mice with few signs of hepatic and renal toxicity and weight loss. All data were statistically significantly different with p-value of less than 0.001 at confidence interval of 95% with high variance homogeneity. In conclusion, methanolic plant leaves extract of *Q. coccifera* can possibly be used alone to control the elevation of blood glucose level with a renal and hepatic protective property.

1. Introduction

Diabetes Meletus (DM) is a chronic disease which is caused by the elevation of glucose level in blood, leading to macro and micro-complications. This disease is spreading widely between individuals all around the world with two major types 1 and 2 (Furman et al., 2020, Dey et al., 2020). The first type is caused by an autoimmune disease which is caused by the destruction of pancreas cells ending up with no insulin secretion (Dey et al., 2020). On the other hand, the second type is caused by the lack of insulin ability to transfer glucose found in blood to interstitial tissues. In addition to diet and lifestyle changing for controlling DM, many synthetic antidiabetic drugs are used to control glucose level by enhancing insulin sensitivity and secretion with high risk of side effects on patients (Zeng et al., 2016, Abu-Odeh et al., 2022). For those two reasons (the spreading of DM and the safety of the treatments), searching for safe and natural antihyperglycemic agents is pushing the pharmaceutical companies to work with natural products (Ota and Ulrih, 2017). Natural sources used to be a major player in the treatment of diseases, and an important source for a drug discovery starting point. Thus, natural source is trusted by both patients and healthcare provider as its side effects are considered to be lower than other synthetic treatments (Karimi et al., 2015). *Quercus coccifera* (Kermes Oak) is a species in Fagaceae family widely grown in Jordan. (Genç et al., 2012). The decoction of different part obtained from Kermes oak have been used for different pathological diseases like hemorrhage, chronic diarrhea, wounds, and burns (Anlas et al., 2019, Söhretoglu et al., 2014). The antidiabetic activity of *Quercus coccifera* have been reported and described by Taib et al., 2020 (Taib et al., 2020). This research was designed to evaluate the possible antidiabetic activity of *Quercus coccifera* extracts obtained from its dried leaves in animal model. The animal model based on rats with destructed β-cells used exhausted

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extraction using n-hexane, chloroform, methanol and water extracts.

2. Materials and methods

2.1. Materials

Alpha-glucosidase, alpha-amylase, alloxan monohydrate, glucose, acarbose were obtained from Sigma-Aldrich (St. Louis, MO, USA). Glibenclamide was purchased from a local pharmacy (Saif pharmacy) Amman, Jordan, and all other chemicals and reagents used in this study were analytical grade.

2.2. Plant material collection and preparation

Quercus coccifera leaves have been collected from the east part of Amman, Jordan and stored in a canvas bag between 3rd and the 4th of May 2022. The botanical identification of the plant leaves was done by Professor Jameel Allaham, Faculty of science, Al Yarmouk University, Irbid, Jordan. The collected leaves were lifted to dry in a well oxygenated and covered place from sun for 3 days.

2.3. Preparation of dried powder extracts

700 g of dried plant leaves powder were subjected to multiple steps extraction using n-hexane, chloroform, methanol, boiled water and microwaved water starting from the lowest to the highest polarity. All solvents were purchased from Sigma-Aldrich, Montana, US. Boiled and microwaved water extracts were obtained separately from two different steps extraction.

2.4. Phytochemical screening

Different phytochemical screening tests were carried out to the produced extracts using Mayer's, Dragendorff's tests for alkaloid detection (Kognou et al., 2016). While other tests were used for the detections of tannins, glycosides, and terpenoids (Uddin and Rauf, 2012, Elezabeth and Subramanian, 2013).

2.5. In-vitro alpha-amylase and alpha-glucosidase activity

For α-amylase, 100 µl of plant extracts dissolved in its suitable solvent were mixed with 100 µl of α-amylase enzyme followed with incubation for 30 min at 37 °C (Tamil et al., 2010). 1% starch solution was added to the mixtures and incubated for 1 h at 37 °C. The reaction was stopped using 200 µl of DNS (Dinitrosalicylic acid) color reagent after being placed in a boiling water for 5 min followed by cooling at a room temperature (Tamil et al., 2010). Acarbose was used as a positive control, while percentage of inhibition for all extracts were obtained by comparing the absorbance results obtained at 540 nm for tests samples and control. The concentrations of 0.001, 0.005, 0.01, 0.05, 0.1, 0.5 and 1 mg/ml were used as a final concentration of plant extracts for the determination of IC_{50}. On the other hand, α-glucosidase inhibitory assay was carried out using Pistia-Brueggeman and Hollingsworth protocol (Pistia-Brueggeman and Hollingsworth, 2001). In a similar manner to α-amylase, 50 µl of different plant leaves extracts with a final concentration of 0.001, 0.005, 0.01, 0.05, 0.1, 0.5 and 1 mg/ml were added to 96-well plates containing 10 µl of α-glucosidase (1U/ml) and 125 µl of phosphate buffer of pH 6.8, and incubated for 20 min at 37 °C. After the 20 min an addition of 20 µl of 1 M pNPG (4-Nitrophenyl-D- glucopyranoside) as a substrate was done followed by incubation for 30 min. 50 µl of 0.1 N Na_2CO_3 was added to terminate the reaction, while the optical density of each well was measured using microplate reader at a wavelength of 405 nm. Again, acarbose was used as a positive control and extracts activity were measured using the equation below:

$$Extractinhibitoryactivity = \left[\frac{Xa - Xb}{Xa}\right] \times 100\%$$

Xa: absorbance of control, and Xb: absorbance of sample.

2.6. Acute toxicity study

Acute toxicity study for *Q. coccifera* leaves extracts was performed according to OECD-423 guidelines produced by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). During the experiment, a doses of 100, 200, 300, and 500 mg/kg b.wt (b.wt: body weight) from each extract was administered to the mice by mixing it with the food (Ghosh M, 1984). All gross behavioral, neurological, autonomic, and toxic effects at short and long intervals of 1 and 28 days were monitored for each mice participated in acute toxicity study.

2.7. Animal study preparation

Eighty Swiss albino mice were obtained from the animal house at the University of Jordan, Amman, Jordan, and used in the study. Mice were aged between 7 and 8 weeks with a weight range of 25–27 g and placed in a room with a temperature of 24 ± 1 °C with a light–dark cycle of 12 h. Mice in this research were subjected to 180 ng/kg alloxan monohydrate injection after 12 h of fasting to induce the apoptosis process for beta cells (Revisiting Experimental Models of Diabetic Nephropathy) (Carvalho et al., 2003). To avoid liberation of insulin levels in the blood which can cause a severe hypoglycemic effect, 0.2 ml of a glucose syrup with a concentration of 4 g/L was administered. Five days later, stabilization of blood glucose was obtained with a fasting glucose level of more than 400 mg/dL to be selected.

2.8. Blood glucose analysis and evaluation

The effect of single day and 28 days effects of different plant extracts were evaluated after the administration of plant extracts by injecting different doses presented in Table 1.

The single-day effect was monitored at 1, 3, 5, 12, and 24 h of treatment administration by using Accu-Chek Performa strips and instruments for insulin level analysis. While BD U-100 Pet Insulin Syringe for sampling was used for blood sample provided for analysis.

Table 1
Groups distribution with types of treatments used for mice samples or control.

Groupe number	Type of mice	Treatment	Dose
Group 1	Normal (control)	Distilled water (DW)	0.2 ml/day
Group 2	Diabetic (control)	Distilled water (DW)	0.2 ml/day
Group 3	Diabetic	Glibenclamide	2 mg/Kg/day
Group 4	Diabetic	n-Hexane extract	200 mg/Kg/day
Group 5	Diabetic	Chloroform extract	200 mg/Kg/day
Group 6	Diabetic	Methanol extract	200 mg/Kg/day
Group 7	Diabetic	Boiled water extract	200 mg/Kg/day
Group 8	Diabetic	Microwaved water extract	200 mg/Kg/day

2.9. Statistical analysis

All graphs with means and SD were calculated and prepared using Graph Pad Prism 5. While one way ANOVA was used to assess the difference between the different groups using the SPSS X28 program.

3. Results

3.1. Plant leaves drying and extracts preparation

7.5 g of plant leaves were collected and grinded using a Powder Grinding Pulverizer Stainless Steel Machine of 2500 W. The grinding process resulted in 6.9 g of powder, 3.45 g were subjected to multistep endded with boiled water and 3.45 g were subjected to multistep extraction ended with microwaved water. The extraction process resulted in weights presented in Table 2.

3.2. Phytochemical screening

According to the phytochemical screening of some classes known to exert antidiabetic activity, methanolic extract indicated the presence of the highest concentration of them between all extracts (Alam et al., 2022). Furthermore, boiled water extract, show to be positive with all calsses but in a lower concentration. The rest of the extracts show the existing of a fewre phytochemical classes. All phytochemical screening resultes are presented in Table 3.

3.3. In vitro Alpha-amylase and glucosidase

According to α-amylase and α-glucosidase, methanol extract was found to exert the best inhibitory activity with a concentreation of 0.17 and 0.38 mg/ml when compared to acarbose (positive results) that exerted inhibitory effect of 0.59 and 1.01 respectevly. Boild water extract was found to have a lower IC_{50} (inhibitory concentration 50%) compared to the microwaved water by around 1 mg/ml, while microwaved water IC'_{50}s were higher than 2 mg/ml. On the other hand, the lowest polar extract (n-hexane), was found to exert the lowest inhibitory effect with IC_{50} of greater than 100 mg/ml. In *vitro* results are presented in Table 4 and Fig. 1.

3.4. Acute toxicity results

According to the toxicity study for doses 100, 200, 300, and 500 mg/kg b.wt, no changes in behavior and no mortality were observed. On the 29th day, no macroscopic pathology observations indicated no visible lesion in 3 mice of each extract, and the plant extracts can be safely used up to 500 mg/kg b.wt.

3.5. In vivo blood glucose analysis and evaluation

3.5.1. Plant leaves extracts effect on biochemical parameters and body weight

According to bodyweight and biochemical results for the control and the treated mice samples presented in Table 5, all groups with uncontrolled glucose levels (groups 2 (negative control), 4, 5, and 8) showed decrease in bodyweights concomitant with hyperglycemia. While groups 3 (diabetic mice treated with glibenclamide), 6, and 7 show a better control on glucose level and have improved (normal) bodyweights. On the other hand, ASAT (aspartate transaminase) and ALAT (alanine transaminase) biochemical results for groups 2, 4, 5, 8 were elevated as a result of hepatic damage, while groups 3, 6, and 7 show restored ASAT and ALAT as a result of restored hepatic function. Urea and creatinine levels for groups 2, 4, 5, and 8 which failed to maintain blood glucose levels have been elevated. While groups with maintained blood glucose levels have normal levels or close values to normal group (positive control).

3.5.2. One-Day effect

According to the in *vivo* biological assay, the highest one-day effect was obtained after the use of methanol extract with a lowest glucose level of 206.8 mg/dL after 5 h of extract administration. In addition, boiled water extract shows a promising one-day effect with a lowest blood glucose level of 333.5 mg/dL after 12 h. On the other hand, microwaved water extract shows lower biological activity than boiled water extract with a blood glucose level of 389.2 mg/dL. Both n-hexane and chloroform extracts' one-day effect show very weak activity with a blood glucose level of more than 400 mg/dL during all intervals. One way ANOVA with 95% confidence show p-value of less than 0.001, while variance homogeneity test shows high samples homogeneity. All results of one-day effect are presented in Fig. 2.

3.5.3. Twenty-eight days effect

According to the 28-days effect of plant leaves extracts presented in Fig. 3, n-hexane extract shows no change in the blood glucose level with a concentration of more than 400 mg/dL. While the rest of the plant extracts show lower blood glucose level and methanol extract shows the best biological activity with a blood glucose level of 146.8 mg/dL after 28 days. Methanol extract shows similar biological activity of positive control (Glibenclamide). While the rest of the plant extracts show lower biological activity. According to Fig. 3, boiled water extract shows better biological activity with blood glucose level of 238.7 mg/dL when compared to 331.5 mg/dL produced after the consumption of microwaved water extract.

4. Discussion

The current investigation was done in order to evaluate the potential antidiabetic activity of *Q. coccifera* plant leaves extract through in *vitro* inhibition of α-amylase and α-glucosidase enzymes and in *vivo* blood lowering activity on animal model. Different phytochemical tests were performed on the produced extracts and both methanolic and boiled water extracts were found to have different classes of compounds (alkaloids, tannins, glycosides, and terpenoids). All the tested classes have been previously studied and found to exert a potential antidiabetic activity either in in *vivo* or in *vitro* model (Ye et al., 2021, Fraga-Corral et al., 2021, Pałasz et al., 2019, Singh et al., 2022). Different species from the same family have been studied before, and the phytochemical testing of plants extracts detected the presence of alkaloids, tan-

Table 2
Extracts yield resulted from multistep extraction.

Solvent	Extract weight (mg)	% yield	Solvent	Extract weight (mg)	% yield
n-Hexane	371.9	10.78	Boiled water	1627.3	47.17
Chloroform	214.6	6.22	Microwaved water	1814.8	52.6
Methanol	1033.7	29.96			

Table 3
Phytochemical screening of plant leaves crude extracts.

Extract type	Type of compounds Alkaloid	Tannins	Glycosides	Terpenoid
n-hexane	–	–	–	–
Chloroform	+	+	–	+ +
Methanol	+ + +	+ + +	++	+ +
Boiled water	+ +	+ +	++	+
Microwaved water	+	–	+	–

+: indicates the presence of compound after 10–15 min, + +: indicates the presence of compounds after 5–10 min, + + +: the presence of the compounds before 5 min, and -: the absence of the compounds after 1 h.

Table 4
Alpha-amylase and glucosidase IC_{50} results.

Plant Extract	α-amylase IC_{50} (mg/ml)	α-glucosidase IC_{50} (mg/ml)
n-haxane	> 100	> 100
Chlorofom	2.41	4.81
Methanol	0.17	0.38
Boiled water	1.02	0.98
Microwaved water	2.08	2.13
Acarbose	0.59	1.01

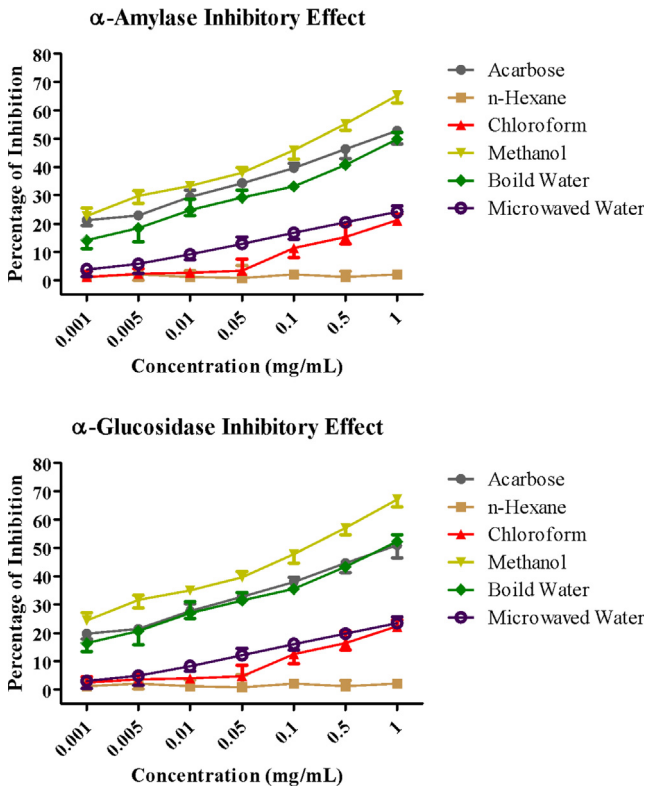

Fig. 1. In *vitro* α-amylase and α-glucosidase results of plant leaves extracts.

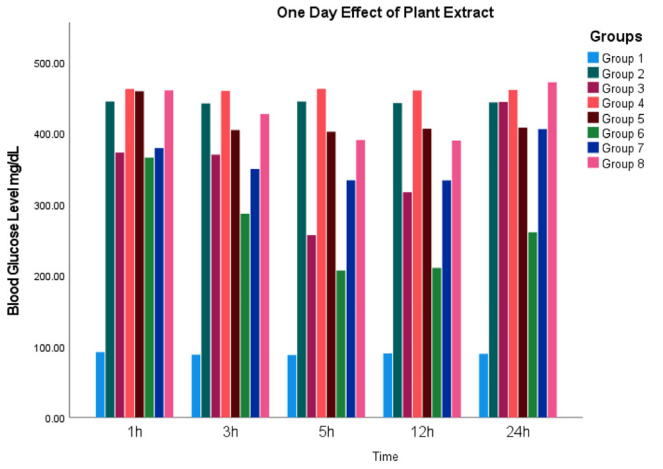

Fig. 2. In vivo biological assay of different plant leaves extracts with one-day effect.

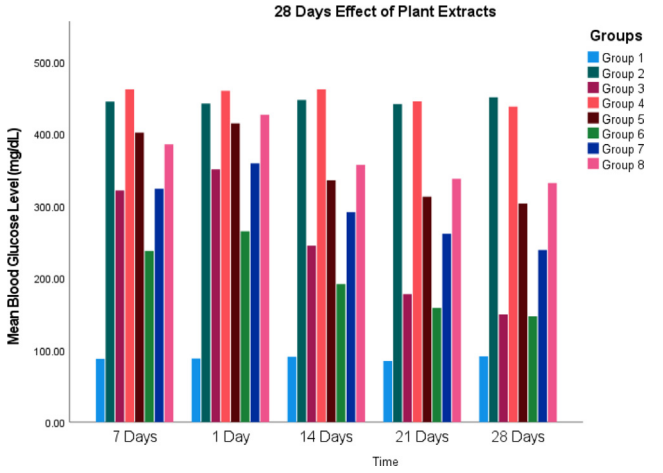

Fig. 3. In *vivo* biological assay of different plant leaves extracts with 28-days effect.

Table 5
The 28-days effect of different plant leaves extracts on bodyweight and biochemical parameters.

Group	Body weight ± SD	Biochemical parameters			
		ASAT ± SD	ALAT ± SD	Urea ± SD	Creatinine ± SD
1	24.7 ± 1.7	249.7 ± 22.4	43.1 ± 3.8	0.21 ± 0.01	3.1 ± 0.23
2	17.8 ± 1.3	487.6 ± 19.8	99.7 ± 7.9	0.73 ± 0.07	5.9 ± 0.42
3	25.3 ± 0.81	287.2 ± 22.7	76.2 ± 9.4	0.31 ± 0.03	3.9 ± 0.38
4	16.9 ± 2.7	491.3 ± 17.1	103.6 ± 13.3	0.71 ± 0.02	6.1 ± 0.39
5	20.3 ± 0.79	398.2 ± 20.7	88.5 ± 10.9	0.42 ± 0.04	4.7 ± 0.61
6	26.7 ± 2.6	268.3 ± 18.4	53.1 ± 6.4	0.23 ± 0.01	3.3 ± 0.17
7	22.1 ± 1.8	324.9 ± 16.4	80.1 ± 10.8	0.39 ± 0.03	4.3 ± 0.23
8	19.8 ± 0.93	401.5 ± 23.7	91.8 ± 6.7	0.46 ± 0.01	4.9 ± 0.18

nins, glycosides, and terpenoids (Yusof and Abdullah, 2020). According to the in *vitro* α-amylase and α-glucosidase results presented in Fig. 1 and Table 4, methanol extract was found to exert the best inhibitory effect with an IC_{50} of 0.17 and 0.38 mg/ml respectively. In another species (*Quercus robur*) from the same family, ethanolic extract was found to exert the best antidiabetic activity as reported by Ștefănescu et al (Ștefănescu et al., 2022). This could be a result of the presence of the highest concentration of all the chemical classes that reported to exert the most promising antidiabetic activity as mentioned in Table 3 and reported before (Ye et al., 2021, Fraga-Corral et al., 2021, Pałasz et al., 2019, Singh et al., 2022). Using of plant leaves extracts was found to be safe for use up to 500 mg/kg, this finding was similar to previous finding about extracts from different *Quercus* species that were used previously as complementary and alternative medicine and food mentioned in literature (Morales, 2021). According to the animal study, group 6 which used methanolic extract were able to maintain an average body weight similar to the normal mice positive control group (group1) and it was better than the positive control group which used glibenclamide as a treatment (group 3). On the other hand, the rest of the groups had either a small reduction in the bodyweight like boiled water extract, or large reduction in the bodyweight in the other groups like the negative control group (group 2: diabetic mice treated with distilled water). All extracts were unable to reduce the blood glucose level for mice to a normal level with a peak activity of 5hs. While after 28 days, methanol and boiled water extracts were able to exert the best antidiabetic biological activity as shown in Figs. 2 and 3. The decline in the bodyweight in the groups failed to maintain blood glucose level, resulted from the excessive catabolism of fats and structural proteins to be used as a source of energy due to the lack of carbohydrates, and due to the absence of insulin which plays a vital role in regulating proteins synthesis (Hatting et al., 2018, Ludwig and Ebbeling, 2018). In addition to bodyweight sign, biochemical indicators like ASAT, ALAT, creatinine, and urea elevation in groups failed to maintain blood glucose level (groups 4, 5, 7, and 8), were another indicator of the inability of the mentioned extracts to maintain a healthy life of mice. While methanol extract was able to maintain blood glucose level in addition to the biochemical indicators which reflect a normal life style for the tested mice group. The elevation of ASAT and ALAT was an indicator of cellular leakage and hepatocellular injury, while high creatinine and urea levels indicated a renal injury (Contreras-Zentella et al., 2016, Vaidya et al., 2008). The normal level of the biochemical markers is a result of the possible protective property of methanolic extract that resulted from the antioxidant, anti-inflammatory and healing properties (Makhlouf et al., 2018, Burlacu et al., 2020, Anlas et al., 2019).

5. Conclusion

The results in this study confirmed the traditional use of *Q. coccifera* for patients suffering from diabetes. It was observed that methanolic extract which contain the major chemical classes with a possible antidiabetic activity resulted in the highest antidiabetic activity in *vitro* and *vivo*. In addition, boiled water extract resulted in a promising biological activity with a moderate ability to reduce blood glucose level. Methanolic extract was able to produce lower IC_{50} in α-amylase and α-glucosidase inhibitory activity when compared to the positive control sample (Acarbose). Similarly, in *vivo* results of methanolic extract indicate a better blood glucose lowering activity than glibenclamide positive control group, and similar to the normal mice group over 28 days period with normal biochemical and bodyweight sign. Thus, methanolic extract can possibly be used alone without any drug, with a possibility to inhibit the two tested enzymes which are responsible for the breakdown and absorption of carbohydrates.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Saif Aldeen Jaber reports was provided by Middle East University. Saif Aldeen Jaber reports a relationship with Middle East University that includes: funding grants.

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References

Abu-Odeh, A., Shehadeh, M., Suaifan, G. A. R. Y., Karameh, N., Abdel Rahman, D. & Kandil, Y. 2022. In Vitro and In Vivo Antidiabetic Activity, Phenolic Content and Microscopical Characterization of Terfezia claveryi. Molecules [Online], 27.
Alam, S., Sarker, M.M.R., Sultana, T.N., Chowdhury, M.N.R., Rashid, M.A., Chaity, N.I., Zhao, C., Xiao, J., Hafez, E.E., Khan, S.A., Mohamed, I.N., 2022. Antidiabetic Phytochemicals From Medicinal Plants: Prospective Candidates for New Drug Discovery and Development. Front Endocrinol (Lausanne) 13, 800714.
Anlas, C., Bakirel, T., Ustun-Alkan, F., Celik, B., Yuzbasioglu baran, M., Ustuner, O., Kuruuzum-uz, A., 2019. In vitro evaluation of the therapeutic potential of Anatolian kermes oak (Quercus coccifera L.) as an alternative wound healing agent. Ind. Crop. Prod. 137, 24–32.
Burlacu, E., Nisca, A., Tanase, C., 2020. A Comprehensive Review of Phytochemistry and Biological Activities of Quercus Species. 11, 904.
Carvalho, E.N.D., Carvalho, N.A.S.D., Ferreira, L.M., 2003. Experimental model of induction of diabetes mellitus in rats. Acta Cirúrgica Brasileira 18.
Contreras-Zentella, M. L., Hernández-Muñoz, R. J. O. M. & Longevity, C. 2016. Is liver enzyme release really associated with cell necrosis induced by oxidant stress? Oxidative medicine and cellular longevity, 2016.
Dey, P., Singh, J., Suluvoy, J.K., Dilip, K.J., Nayak, J., 2020. Utilization of Swertia chirayita Plant Extracts for Management of Diabetes and Associated Disorders: Present Status, Future Prospects and Limitations. Natural Products and Bioprospecting 10, 431–443.
Elezabeth, D.V., Subramanian, A, 2013. Identification of phytochemical constituents and antimicrobial activity of Indigofera suffruticosa leaves. Int. J. Curr. Biotechnol 1, 6–10.
Fraga-Corral, M., Otero, P., Echave, J., Garcia-Oliveira, P., Carpena, M., Jarboui, A., Nuñez-Estevez, B., Simal-Gandara, J., Prieto, M.A., 2021. By-Products of Agri-Food Industry as Tannin-Rich Sources: A Review of Tannins' Biological Activities and Their Potential for Valorization. 10, 137.
Furman, B.L., Candasamy, M., Bhattamisra, S.K., Veettil, S.K., 2020. Reduction of blood glucose by plant extracts and their use in the treatment of diabetes mellitus; discrepancies in effectiveness between animal and human studies. Journal of Ethnopharmacology 247, 112264.
Genç, Y., Yüzbasioglu, M., Harput, Ü.S., Kuruüzüm-Uz, A., 2012. Antioxidant activity and total phenolic content of Quercus coccifera L. 37, 17.
Ghosh M, N. 1984. Toxicity studies. Fundamentals of experimental pharmacology.
Hatting, M., Tavares, C.D.J., Sharabi, K., Rines, A.K., Puigserver, P., 2018. Insulin regulation of gluconeogenesis. Ann. N. Y. Acad. Sci. 1411, 21–35.
Karimi, A., Majlesi, M., Rafieian-Kopaei, M., 2015. Herbal versus synthetic drugs; beliefs and facts. J Nephropharmacol 4, 27–30.
Kognou, A. L. M., Rajesh Singh, P., Rosalie Annie, N., Pradeep Pateriya, P. N. P., Alembert Tiabou, T., Gabriel Agbor, A., Rebecca Madeleine, E., Raymond Simplice, M., Albert Sone, M. & Cecile Enguehard, G. J. B. J. O. P. R. 2016. Antioxidant, analgesic and antipyretic activities of ethanol extract of the stem bark Dichaetanthera africana (Hooker F.) Jacq. Felix.(Melastomataceae). British Joornal of Pharmaceutical Research, 14, 1-12.
Ludwig, D.S., Ebbeling, C.B., 2018. The Carbohydrate-Insulin Model of Obesity: Beyond "Calories In, Calories Out". JAMA Intern. Med. 178, 1098–1103.
Makhlouf, F.Z., Squeo, G., Barkat, M., Trani, A., Caponio, F., 2018. Antioxidant activity, tocopherols and polyphenols of acornoil obtained from Quercus species grown in Algeria. Food Res. Int. 114, 208–213.
Morales, D., 2021. Oak trees (Quercus spp.) as a source of extracts with biological activities: A narrative review. Trends Food Sci. Technol. 109, 116–125.
Ota, A. & Ulrih, N. P. 2017. An Overview of Herbal Products and Secondary Metabolites Used for Management of Type Two Diabetes. 8.
Pałasz, A., Cież, D., Trzewik, B., Miszczak, K., Tynor, G., Bazan, B., 2019. In the Search of Glycoside-Based Molecules as Antidiabetic Agents. Top. Curr. Chem. 377, 19.
Pistia-Brueggeman, G., Hollingsworth, R.I., 2001. A preparation and screening strategy for glycosidase inhibitors. Tetrahedron 57, 8773–8778.

Singh, S., Bansal, A., Singh, V., Chopra, T., Poddar, J., 2022. Flavonoids, alkaloids and terpenoids: a new hope for the treatment of diabetes mellitus. J. Diabetes Metab. Disord. 21, 941–950.
Söhretoglu, D., Kuruüzüm-Uz, A., Simon, A., Patócs, T., Dékány, M., 2014. New secondary metabolites from Quercus coccifera L. Rec. Nat. Prod. 8, 323.
Ștefănescu, R., Ciurea, C.N., Mare, A.D., Man, A., Nisca, A., Nicolescu, A., Mocan, A., Babotă, M., Coman, N.-A., Tanase, C., 2022. Quercus robur Older Bark—A. Source of Polyphenolic Extracts with Biological Activities. 12, 11738.
Taib, M., Rezzak, Y., Bouyazza, L., Lyoussi, B., 2020. Medicinal Uses, Phytochemistry, and Pharmacological Activities of Quercus Species. Evid. Based Complement. Alternat. Med. 2020, 1920683.
Tamil, I.G., Dineshkumar, B., Nandhakumar, M., Senthilkumar, M., Mitra, A., 2010. In vitro study on α-amylase inhibitory activity of an Indian medicinal plant, Phyllanthus amarus. Indian J Pharmacol 42, 280–282.
Uddin, G., Rauf, A., 2012. Phytochemical screening, antimicrobial and antioxidant activities of aerial parts of Quercus robur L. Middle-East journal of medicinal plants research 1, 1–4.
Vaidya, V.S., Ferguson, M.A., Bonventre, J.V., 2008. Biomarkers of Acute Kidney. Injury 48, 463–493.
Ye, G., Huang, C., Li, J., Chen, T., Tang, J., Liu, W. & Long, Y. 2021. Isolation, Structural Characterization and Antidiabetic Activity of New Diketopiperazine Alkaloids from Mangrove Endophytic Fungus Aspergillus sp. 16-5c. 19, 402.
Yusof, W., Abdullah, H., 2020. Phytochemicals and Cytotoxicity of Quercus infectoria Ethyl Acetate Extracts on Human Cancer Cells. Trop Life Sci Res 31, 69–84.
Zeng, Z., Shuai, T., Yi, L.-J., Wang, Y., Song, G.-M., 2016. Effect of case management on patients with type 2 diabetes mellitus: a meta-analysis. Chin. Nurs. Res. 3, 71–76.