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Phytochemical composition, toxicity and anti-diabetic activity of *Ammodaucus leucotrichus* fruit from Algerian Sahara

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Abstract

Ammodaucus leucotrichus Cosson & Durieu. subsp *leucotrichus* is an endemic plant from the family Apiaceae (Umbelliferae) frequently used by the Arab world for traditional medicine and as a food condiment. This study aims to determine the chemical composition, the toxicity level of an aqueous extract of *Ammodaucus leucotrichus* fruit with different doses, and to evaluate its anti-diabetic activity on normoglycemic rats and rats made diabetic (streptozotocin induced). The results indicated a diminution in blood glucose level recorded with a dose of 500 mg/kg of aqueous extract (4.15 to 2, 08 g/dl) for the rats diabetic which received this amount but for the rats monoglycemic no change of basal glycaemia was detected. Also, a slow and a regular ponderal growth; very significant (p <0, 01), was noted for normal control rats and normal rats treated with aqueous extract (500 mg/kg of body weight). This investigation clearly showed that in addition to its antidiabetic activity, *A. leucotrichus* is not toxic when administered orally to rats and it contains the greatest number of compounds: tannins, flavonoïds, saponins, acids phenolic, coumarins, terpenes and sterols etc.

Keywords:*Ammodaucus leucotrichus*, toxicity, anti-diabetic, streptozotocin, rats

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1. Introduction

Diabetes mellitus a very common disease in the world [1] is defined as a group of metabolic diseases characterized by hyperglycemia as a result of partial or total pancreatic β-cells destruction in insulin synthesis. This disease is treated by insulin and oral ant diabetic agents; however, they are expensive and can cause serious side effects like hepato-toxicity, hypertension, hypoglycemia, diarrhea and dyslipidemia [2].

For this reason, there is research for new anti-diabetic drugs from medicinal plants with anti-diabetic potentials; especially non-toxic plant sources which usually have fewer side effects than synthetic sources [3]. The current research work is concerned with finding the anti-diabetic effect and the toxicity of *Ammodaucus leucotrichus* fruitwhich is widely used in Southern Algeria to treat diabetes in a traditional way.

Ammaudocus leucotrichus Cosson & Durieu subsp. *Leucotrichus* is from Apiaceae family distributed to North African Sahara [4, 5], where it is commonly known under the names of "Moudrayga" or "Nessoufa" (Algerian Dialect), "Kamune es sufi" or "akâman" (Arabic-African) and "Cumin chevelu" (French) [4, 6]. Growing annually, it is a glabrous small plant having small branches and finely divided sheets forming flat narrow ridges with white flowers positioned according to a composed umbel and Mericarps and secondary ribs covered with 8 to 10 mm long silky hair [7, 8]. *Ammodaucus leucotrichus* is an endemic aromatic plant with significant medicinal values. The fruits are prepared in the form of decoction to treat a certain number of diseases such as, diabetes, common cold, blows of cold, fever, vomiting, digestive disorders and allergies particularly with children [9, 10]. In Morocco, for example, the flowers are used for the treatment of the cardiac diseases [11]. The chemical composition of *Ammodaucus leuchotrichus* fruits' essential oil was reported inseveral studies. Essential oils of this plant collected in several areas of the Algerian South (Djelfa, Illizé, Bachar, Ghardaia and Ouargla) are mainly made up from perilla aldehyde (59.13%-84.43%) and limonene (1.71%-29.2%) [12-14].

Thus, the present study aims to determine the acute toxicity and evaluate the anti-diabetic activity of an aqueous extract of the *Ammodaucus leucotrichus* fruit.

2. Materials and methods

2.1. Plant material

The fruits from the plant were harvested in the region of Bechar, (south of Algeria). The plant was authenticated by Professor A. Marouf (Institute of Science and Technology,Department of Natural Sciences and Life, Ctr Univ Naama, Algeria). Dried fruits were shade-dried and pulverized through a crusher to get a fine powder used to prepare different extracts.

2.2. Preparation of aqueous extract

The preparation of the plant aqueous extract was performed as follows: fifty grams of powder were suspended in 500 ml of distilled water, heated under reflux for 30 min and filtered. The filtrate was frozen at -70°C and lyophilized. It was stored at ambient temperature until further use.

2.3. Phytochemical screening

The phytochemical tests consist of detecting the different families of secondary metabolites existing in the fruits of *Ammodaucus leucotrichus* by qualitative reactions. The characterization tests of different chemical groups were carried out according to the methods described by Fry *et al*.[15-18]. The qualitative study of *Ammodaucus leucotrichus* fruits is based on thin-layer chromatography assays and staining or precipitation reactions by chemical reagents.

2.4. Animals

To carry out our study, forty-four healthy Wistar rats weighing between 156 to 312 g were selected. They were raised and housed in an air-conditioned animal room at 24±2°C, and subjected to 12 h light/dark cycle at a room temperature of 27-30°C.

2.5. Acute toxicity study

The aqueous extract acute toxicity of *Ammodaucus leucotrichus* was tested on twenty four Wistar rats (8 females and 16 males) that were summer assigned into six groups of four animals and housed in uniform conditions. These healthy rats fasted for 18 h and were used. A group control was treated with sodium chloride solution (0.9%) and the five groups remaining were treated with aqueous extract (lyophilisat) of *A. leucotrichus* administered orally in graded doses of 1; 1.5; 2; 2.5 and 3 g/kg body weight (B.W), respectively. The rats were monitored for four weeks.

2.6. Induction of diabetes

The anti-diabetic activity was evaluated using the method described by Adolfo Andrade Cetto *et al*. [19]. Diabetes was induced in the 20 normal rats at a dosage of 60 mg/kg body weight at fasting by a single intraperitoneal injection of a freshly prepared sodium citrate buffer (0.1 M, pH 4.5), while 5 of the remaining rats served as a non-diabetic control group. Fasting blood glucose level was measured in the streptozotocin (STZ) treated rats prior and after the feeding using a blood glucose meter, throughout the duration of the experiment. The streptozotocin-treated rats with fasting blood glucose levels ≥2, 5 g/L were considered as diabetic and selected for further experimentation.

2.7. Experimental design and chronic animal treatment

This study was carried out on healthy and diabetic rats which fasted for 16 h. They were divided into four groups with five animals in each.

- A group contains normal control rats which received sodium chloride solution (0.9%)
- A group consists of diabetic control rats untreated, given saline solution and served as a positive control
- A group includes normal rats treated with an aqueous extract (500 mg/kg B.W) and served as normal rats treated
- A group comprises diabetic rats that received aqueous extract (500 mg/kg B.W)

This treatment was maintained for 28 days and the fasting blood glucose level, glycosuria and body weight were weekly measured.

2.8. Analytical techniques

Blood glucose levels were determined by the glucose oxidase method [20] using a reflective glucometer (ACCU-CHEK, Fast Clix, Germany). The body weight of the rats was also measured during the experiment.

2.9. Statistical analysis

Results were expressed as mean±standard error (SEM). Statistical comparison was performed by Student's test. The results were considered statistically significant if the values were 0.05 or less.

3. Results and discussions

3.1. Preliminary phytochemical screening

The results of the chemical screening of *A. leucotrichus* fruits are consigned in table 1. The preliminary tests carried out on our plant revealed the main phytoconstituents of the secondary metabolism: phenolic acids (cinnamic acid, ferulic acid), flavonoids, coumarins, cardiotonic glycosides, and saponins. It also comes out from these preliminary tests which the fruits of *Ammodaucus leucotrichus* contain only very few alkaloids in a state basic, not of anthocyanes, quinones (free or combined), of anthracene derivatives, condensed tannins and hydrolysable tannins and of sesquiterpenes lactones.

3.2. Toxicity evaluation

As regards *Ammodaucus leucotrichus* fruits' acute toxicity, the rats were monitored and observed during a period of 28 days. The aqueous extract oral administration of

A. leucotrichus fruits to the doses of 1; 1.5; 2; 2.5 and 3 mg/kg body weight with the rats did not induce any sign of acute toxicity. The effect of the aqueous extract to the various doses, on the glycemia and the relative weight are illustrated in Figures 1 and 2.

3.3. Antidiabetic effect

3.3.1. Effect of the aqueous extract on glycaemia

The results of the study *in vivo* of the aqueous extract effect on the hyperglycemia caused by streptozotocin on the rats are illustrated in Figure 3. The blood glucose levels of the control rats receiving only sodium chloride solution (0.9%) and the control rats receiving the 500 mg/kg aqueous extract did not vary significantly ($p>0.05$) throughout the duration of this study that are ranged between 0.90 to 1.05 g/L. Figure 2 shows that a decrease in basal blood sugar was recorded in the first week after treatment with the dose of 500 mg/kg of aqueous extract which goes from 4.15 to 2.08 g/L in rats diabetics. In contrast, the untreated rats showed no change in their blood level, which was too high and exceeded 6 g/L.

3.3.2. Body weight evolution of rats

According to the results obtained [figure 3], we noted a slow and a regular growth of body weight; very significant ($p<0.01$) in the normoglycemic rats and in the non-diabetic ones treated by 500 mg/kg of aqueous extract from *Ammodaucus leucotrichus*, while the diabetic control rats had decreases in their body weight.

Nowadays, medicinal plants represent an unavoidable source for the discovery of new therapeutic molecules very efficacious against many degenerative diseases. This has been the subject of interest given recently by scientific researchers. The present work focused essentially on the phytochemical composition, acute toxicity and ant diabetic activity from the fruits of *Ammodaucus leucotricus* Coss. & Dur.

The phytochemical screening of extracts from the fruits of *Ammodaucus leucotrichus* by precipitation reactions and thin-layer chromatography analysis revealed the presence of flavonoids, coumarins, cardiotonic glycosides, saponins and phenolic acids (cinnamic acid, ferulic acid). On the other hand, this plant does not contain alkaloids in a state basic, anthocyans, quinones (free or combined), of anthracene derivatives, condensed tannins, hydrolyzable tannins and of sesquiterpenes lactones indicating importance of plant extracts. This matches with the results found by Gherraf *et al*., who also found the presence of volatile oils, flavonoids, saponins, tannins, carotenoids, and coumarins [21].

The study of the aqueous extract acute toxicity of *Ammodaucus leucotrichus* in rats has shown that this extract, administered orally, does not cause rat mortality for doses ranging from the limit dose of 3 g/kg of body weight.

Diabetes mellitus was induced in rats by single intraperitoneal injection of streptozotocin (60 mg/kg body weight). This substance produced an increase in plasma glucose level with a diabetic state, resulting from partial or total destruction of the pancreatic β-cells in insulin synthesis [22]. After streptozotocin induction, the hyperglycemic rats were treated with a single dose of *Ammodaucus leucotrichus* fruit aqueous extract (500 mg/kg) for 28 days.

The study of antidiabetic activity from the aqueous extract of *Ammodaucus leucotrichus* fruits has shown that it induces a significant reduction ($P>0.05$) in blood glucose levels compared to streptozotocin-induced diabetic control rats, and a regular growth in body weight; very meaningful. This decrease in blood sugar in rats could be linked to the presence of natural molecules (secondary metabolites). Several studies have shown that the therapeutic activity of certain plants is linked to the presence of chemicals, for example polyphenols can influence blood sugar at different levels and can also help prevent and control complications of diabetes. These phenolic compounds have been the subject of several experiments carried out *in vivo* and *in vitro* and they have shown the protective activity of the capillaries [23, 24]. These properties intervene much in the prevention against diabetes degenerative complications. In fact, the phytochemical study of the various extracts of the fruits of *Ammodauchus leucotrichus*, shows the presence of a multitude of varieties of these polyphenols including flavonoids, coumarins and phenolic acids (cinnamic acid, ferulic acid) which confirms the traditional use of this plant in the treatment of diabetes.

These results are similar to those of El-Ouady et al. who showed that the fruits of *A. leucotrichus* aqueous extract, administered orally at a dose of 10 mg/kg of body weight caused a significant drop in the blood sugar of diabetic rats for a period of 15 days. In addition, this extract may have shown a remarkable influence on glucose tolerance [25].

Table 1. Phytochemical screening by color reactions of *A. leucotrichus* fruits

Compound groups	Results
-Cardiotonic glucoses	+
-Saponins	+++
-Flavonoids	+++
-Coumarins	+++
-Tannins	+
-Quinones	-

-Anthracenes compounds	++
-Sterols and triterpenes	+++
-Sesquiterpene lactones	-
-Reducing compound	+
-Alkaloids	-/+
-Phenol acids	Cinnamic acid and Ferulic acid

Key: (-) absent; (+): low quantity; (++): average quantity; (+++): high quantity

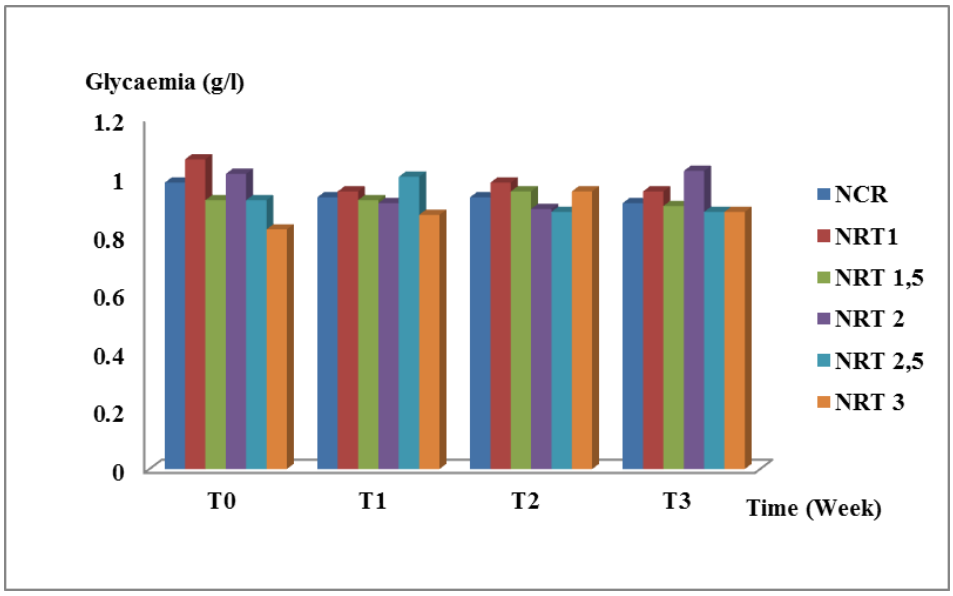

Figure 1: Fasting blood sugar levels of control and treated groups of rats during the study period. Values are expressed as mean±standard deviation (n=4). NCR: normal control rats; NRT1: normal rats treated with aqueous extract (1g/kg b.w); NRT1,5: normal rats treated with aqueous extract (1.5 g/kg b.w); NRT2: normal rats treated with aqueous extract (2g/kg b.w); NRT2,5: normal rats treated with aqueous extract (2,5g/kg b.w); NRT3: normal rats treated with aqueous extract (3g/kg b.w).

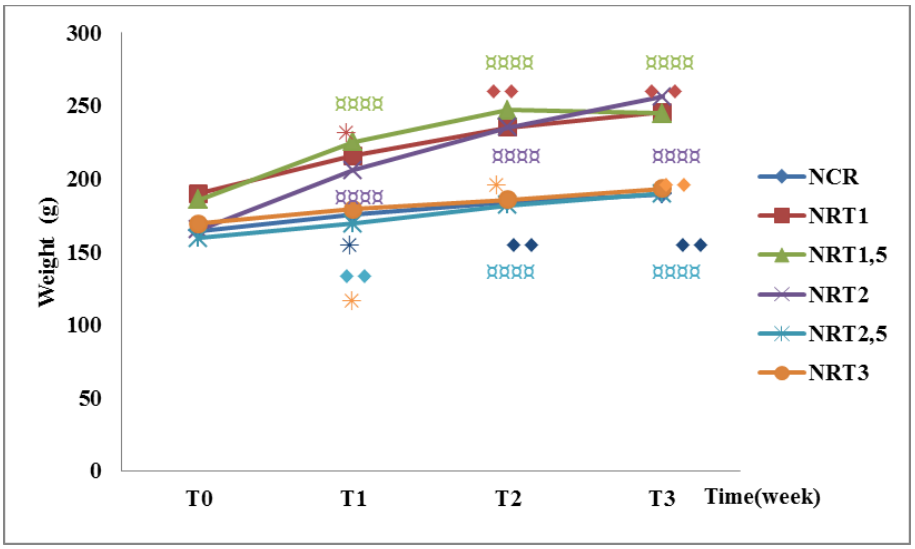

Figure 2: Evaluation of the body weight and change in weight of rats. Values are expressed as mean±standard deviation (n=4). àP≤0.05; ®®P≤0.01; P≤0.0005: Significance by rapport T0

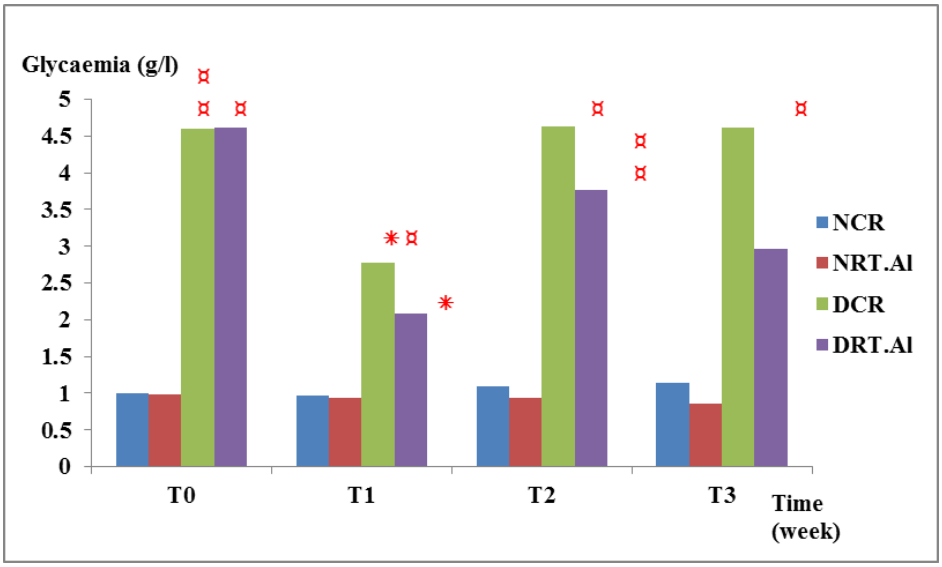

Figure 3: Effect of aqueous extract (500 mg/kg b.w) on blood glucose level of normal and STZ diabetic rats during 28 days. Values are expressed as mean±standard deviation (n=5). NCR: normal control rats; DCR: diabetic control rats; NRT *A.l*: normal rats treated with aqueous extract (500 mg/kg b.w); DRT *A.l*: diabetic rats treated with aqueous extract (500 mg/kg b.w). ✳, ¤: slightly significant (P≤0.05); ¤ ¤: significant (P≤0.01). ✳: Significance by rapport T_0. ¤: Significance by rapport (NCR)

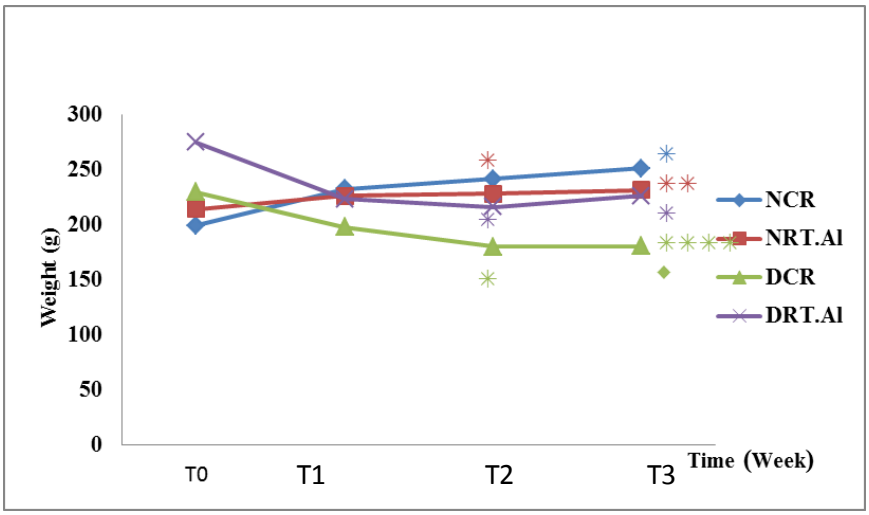

Figure 4: Evaluation of the body weight and change in weight of rats.✳, ◆: slightly significant *(P≤0.05)*;✳✳: significant *(P≤0.01)*; ✳✳✳✳: high significant *(P≤0.0005)*.✳: Significance by rapport T_0. ◆: Significance by rapport (NCR)

Conclusions

The present investigation shows that aqueous extract of *Ammodaucus leucotrichus* fruit is not toxic by oral way with doses superior or equal to 3 g/kg of body weight. The results of the current study provided basic information about the toxicity and the ant diabetic activity of *A. leucotrichus* fruit that might be helpful in planning future pre-clinical experiments on this potent plant. More advanced studies are necessary to better valorize the effect of *A. leucotrichus* against diabetes mellitus, and find the responsible molecules.

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Conflict of interest

There are no conflicts of interest.

References

[1] S.M. Divi, R. Bellamkonda, S.K. Dasireddy. (2012). Evaluation of antidiabetic and antihyperlipedemic potential of aqueous extract of *Moringa oleifera* in fructose fed insulin resistant and STZ induced

diabetic Wistar rats: A comparative study. Asian Journal of Pharmaceutical and Clinical Research. 5: 67-72.

[2] A.J. Krentz, C.J. Bailey. (2005). Oral anti-diabetic agents: current role in type 2 diabetes mellitus. Drugs. 65: 385-411.

[3] L. Pari, S. Suman. (2010). Efficacy of Naringin on hepatic enzymes of carbohydrate metabolism in streptozotocin-nicotinamide induced type 2 diabetic rats. International Journal of Pharmaceutical & Biological Archive. 1: 280-286.

[4] D.J. Mabberley. (1997). The Plant-Book: A portable dictionary of the vascular plants, 2nd ed.

[5] S.C. Fry. (1988). The Growing Plant Cell Wall: Chemical and Metabolic Analysis, Longman scientific and technical. Harlow, U.K.

[6] M. Bernard, D. Fouzia, Le. N. Didler, F.T. Souad, R. Jean-Pertte. (1997). Ammolactone, un guaianolide d'une plante médicinale, *Ammodaucus leucotrichus*. Phytochemistry. 44. 907-910.

[7] P. Ozenda. (1977). Flore de Sahara septentrional et central, Centre national de la recherché scientifique, Paris.

[8] P. Quézel and S. Santa (1963). Nouvelle flore de l'Algérie et des régions désertiques méridionales. Tome II, Editions C.N.R.S, Paris.

[9] A. Velasco-Negueruela, M.J. Pérez-Alonso, P.L. Pérez de Paz, J. Palá-Paúl, J. Sanz. (2006). Analyse par chromatographie en phase gazeuse-spectrométrie de masse des huiles essentielles des fruits *d'Ammodaucus leucotrichus* Cosson & Durieu subsp. *Leucotrichus* une espèce endémique d'Afrique du Nord et *Ammodaucusleucotrichus* subsp. *Nanocarpus* E. Beltrán une espèce endémique des îles Canaries, Espagne. Journal of Chromatography A. 1108: 273-275.

[10] E. Idm'hand, F. Msanda, K. Cherifi. (2020). Medicinal uses, phytochemistry and pharmacology of *Ammodaucusleucotrichus*. Clinical Phytoscience. 6(1): 6.

[11] P. Ozenda. **(**2004). Flore and vegetation of the Sahara. 3th ed. Editions of the national center of scientific research.

[12] M.H. Abu Zarga, I.A.J. Hala, Y.B.A. Zahra, S. Lakhdar. (2013). Chemical Composition, Antimicrobial and Antitumor activities of Essential Oil of *Ammodaucus leucotrichus* Growing in Algeria. Journal of Biologically Active Products from Nature. 3: 224-231.

[13] D.Dahmane, D. Tahar, K. Soumeya, N. Ahmed. (2017). Chemical composition, antioxidant and antibacterial activities of the essential oils of medicinal plant *Ammodaucus leucotrichus* from Algeria. Journal of Essential Oil Research. 29. 48-55.

[14] H. Neghliz-Benabdelkader, T. Benabdelkader, F. Halladj, F. Jullien. (2021). Characterization of the Chemical Diversity in Essential Oils from Vegetative and Reproductive Organs of *Ammodaucus leucotrichus* subsp. leucotrichus Coss. & Dur. Growing in Algeria. Journal of Essential Oil Bearing Plants. 24(1): 75-85.

[15] H. Wagner, S. Bladt. (1996). Plant Drug Analysis: A Thin Layer Chromatography Atlas. Springer-Verlag: Berlin. Heidelberg. New York.

[16] F. Haddouch, T.M. Chaouche, N. Halla. (2016). Phytochemical screening, antioxidant activities and hemolytic power of four Saharan plants from Algeria. Phytothérapie. 1-9.

[17] R.S. Kumar, C. Venkateshwar, G. Samuel, R.S. Gangadhar. (2013). Phytochemical Screening of some compounds from plant leaf extracts of *Holoptelea integrifolia* (Planch.) and *Celestrus emarginata* (Grah.) used by Gondu tribes at Adilabad District, Andhra Pradesh, India. International Journal of Engineering Science. 2(8): 65-70.

[18] E. Iqbal, K.A. Salim, L.B.L. Lim. (2015). Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. Journal of King Saud University - Science. 27(3): 224-232.

[19] A. Andrade-Cetto, H. Wiedenfeld, C. Revilla Ma, S. Islas. (2000). Hypoglycemic effect of *Equisetum myriochaetum* aerial parts on streptozotocin diabetic rats. Journal of Ethnopharmacology. 72. 129-133.

[20] P. Trinder. (1969). Determination of blood glucose using 4-amino phenazone as oxygen acceptor. Journal of Clinical Pathology. 22. 246.

[21] G. Noureddine, Z. Amar, K. Azeddine, L. Mesbah, S. Ramdane, R. Salah. (2017).Chemical constituents and antimicrobial activity of essential oils of *Ammodaucus leucotricus*. Arabian Journal of Chemistry. 10: S2476-S2478.

[22] M.D. Mythili, R. Vyas, G. Akila, S. Gunasekharan. (2004). Effect of Streptozotocin on the ultra-structure of rat pancreatic islets. Microscopy Research and Technique. 63: 274-281.

[23] A. Braca, M. Politi, R. Sanogo, H. Sanou, I. Morelli, C. Pizza and N. De Tommasi. (2003). The chemical composition and antioxidant activity of phenolic compounds from wild and cultivated *Sclerocarya birrea* (Anacardiaceae) leaves. Journal of Agricultural and Food Chemistry. 51: 6689-6690.

[24] O. Potterat and K. Hostettmann. (1995). Plant sources of natural drugs and compounds, [In] Encyclopedia of Environmental Biology, vol. 3, Editor, Academic Press Inc, London.

[25] F. El-Ouady, M. Eddouks. (2020). Glucose Lowering Activity of Aqueous *Ammodaucus leucotrichus* Extract in Diabetic Rats. Cardiovascular & Hematological Disorders-Drug Targets. 20(2): 152-159.