# Anticoagulant Activities of *Centaurea macrocephala* L.

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## Abstract

Our study is based on the biological activities of the plant *Centaurea macrocephala* L., from the Mechtat Seraghna in the wilaya of Mila (Algeria).This work consists to study the anticoagulant activity of the extracts of the seeds and leaves of *Centaurea macrocephala* L., obtained by maceration in the solvent of ethanol. The extraction yield showed values between31% for the leaves and 25% for the seeds. The anticoagulant activity was evaluated by the testof prothrombin levels (TP) and the blood of a normal man, the results obtained indicate avery important anticoagulant activity of the two extracts on the exogenous pathway of coagulation (TQ of 47,26 s in the leaves and 29,23s for the seeds).

**Keywords:** *Centaurea macrocephala*, polyphenols, anticoagulant activity.

## 1. Introduction

*Centaurea macrocephala* L. is from to the Caucasus, is a perennial plant, with robust, leafy stems, which reaches a height of 1 m and bears yellow flowers, of the *Centaurea* genus, the Asteraceae family, includes about 700 species in the world. In Algeria, this genus is represented by 45 species **(Quezel and Santa, 1963; Mabberley, 1987).**

The genus *Centaurea* has been the subject of many phytochemical studies, these works allowed the isolation of various secondary metabolites, citing; phenolic compounds, flavonoids, lignans, tannins, saponins, alkaloids, terpenes and sesquiterpene lactones **(Sarker et *al.*, 1997)**, the latter generally considered as constituents of the genus *Centaurea* and in particular act as primary antioxidants and stabilized radicals **(Formisano et *al.*,2012)**. Many species of the genus *Centaurea* have been used in traditional medicine. In this context this study was inscribed aiming to evaluate *in vitro* the anticoagulant activity of the polyphenolic extracts of the leaves and fruits of *Centaurea*.

## 2. Materials and Methods

### 2.1.Plant material

The plant material used in our study is constituted of the leaves and seeds of *Centaurea macrocephala* L. They were collected from Mechtat Seraghnain the wilaya of Mila (Algeria).

### 2.2.Methods

We took forty leaves and seeds in a random manner for each organ, and then each material for extract (leaves and seeds) is placed in the oven at 40°C for 5 days to dry. The dehydrated samples were triturated to obtain homogeneous samples.

### 2.3. Extraction of polyphenols

Having the objective to make an extraction of phenolic compounds, 5g powder obtain from homogeneous samples were added to a mixture of methanol and water (100ml, 70:30(vv)). The mixture was allowed to stand for 5 days at room temperature in the dark. Then, it was filtered using a 0.45µm filter **(Abaza et *al*., 2007)**. After filtration, the filtrate was evaporated using a rotavapor at a temperature of 60°C for a total elimination of methanol.

### 2.4. Anticoagulant activity

The anticoagulant activity of the polyphenolic extracts of the leaves and seeds of *Centaurea macrocephala* L. was evaluated *in vitro* by the exogenous pathway of the coagulation on anormal plasma using a global test chronometric; the Quick time (QT).The blood is obtained from a young adult as voluntary healthy non treaty, whose QT is normal, by venipuncture in sodium citrate 3.2% tube (9:1 v/v, blood: anticoagulant). The blood is then centrifuged for 5 minutes at 2500 rpm to obtain platelet poor plasma.

#### 2.4.1. Quicktime

The prothrombin time (PT), was developed by Armand Quick in1935 for investigating patients with liver disease **(Quick, 1935)**. PT measures the time to form the initial clot after tissue thromboplastin is added to the recalcified, citrated blood specimen, and is an expression of the extrinsic pathway. The PT is responsive to congenital or acquired deficiencies off actors VII, X, V, and II and fibrinogen **(Wiesner, 2003)**.An elongated time of coagulation compared to that of witness explained that the sample exercises an anticoagulant effect in this pathway of coagulation. The effect of olive polyphenols on the exogenous pathway of coagulation has been evaluated according to the protocol described by Athukorala and his collaborators, with some changes **(Athukorala et*al*.,2007).**

On the one hand, we put 100µl of plasma already obtained in a witness tube which is then incubated for 2 minutes at 37°C. On the other hand, 50 µl of the polyphenolic extracts diluted with distilled water to 50% (1:1 (v/v)) was added to 100µl of plasma in each of the analyzetubes, then incubated at 37°C during an optimal time of 15 minutes. After the incubation, thromboplastine (200µl) pre-incubated at 37°C for 15 minutes was added and clotting time was recorded. The results are expressed by the clotting time in second (s). The same operation was repeated three times in the same conditions for each organ.

### 2.5.Statistical analysis

Statistical analyses were performed by the SPSS 21 software. The results are expressed using means ± standard deviations (SDs). Parameters were compared between groups using analysis of variance (ANOVA) and the values of $p \leq 0.05$was considered statistically significant.

## 3. Results and Discussion

The anticoagulant capacity of the polyphenolic extracts of the seeds and leaves of *Centaurea macrocephala* L. against the exogenous pathway of coagulation by the TQ assay was evaluated at the fixed optimal incubation time of 15minutes.We used the prothromb in count (PT) test, which explores the extrinsic pathway of blood clotting where tissue factor (thromboplastin) is the trigger for this pathway **(Tripodi, 2009).**The results obtained for the anticoagulant activity are grouped together in the table1.

**Table**1. The results obtained for the anticoagulant activity (s)

| *macrocephala* L. | **100%** | **50%** | **25%** | Witness |
|---|---|---|---|---|
| **Leaves** | 65,55± 2,27 | 23,30± 2,30 | 19,18± 0,53 | 18,29 |
| **Seeds** | 47,52± 2,04 | 43,04± 0,63 | 21,03± 2,47 | 18,29 |
| **Signification(AVI)** | 001 | 000 | 274 | |

The analysis of these results reveals the existence of a large variability between the QT values of the seeds and leaves of *Centaurea macrocephala* L. This is confirmed by the analysis of variance (ANOVA); it emerges a highly significant difference in the QT in the presence of the polyphenolic extracts the seeds and leaves in al concentration ($p<0.001$). The results of polyphenolic extracts of the leaves, we note that there is a greater lengthening of TQ of the order of (47.26s) for the 100% concentration in compared to that of the control(18.29 s) and a less significant elongation of TQ of the order of (5.01s and 0.89s) for the 50%and 25% concentrations respective Moreover, for the polyphenolic extracts of the seeds, it is observed that the highest TQ elongations are recorded for 100% and 50% concentrations with the order of 29.23s and 24.75s and a less significant elongation of TQ of the order of 2.47s for the 25%concentration.

The blood coagulation cascade is a physiological phenomenon that comprises intrinsic, extrinsic, and common pathways. Briefly, the activation of the intrinsic pathway occurs because of trauma and contact between kininogen, prekallikrein, and factor XII with underlining collagen on endothelium **(Hood and Eby, 2008)** In addition, studies by **Lemaoui (2011)** concerning the evaluation of the anticoagulant *in vitro* activity of the essential oils of *Nigella sativa* L. seeds have shown that the seoils richin polyphenols may cause aprolongation at the level of the clotting time **(Abdallah et *al*.,2022)** mentioned that has in the results, *Centaurea hyalolepis* Boiss extracts prolonged a PTT values, which in contrast, demonstrated a pronounced decreasing effect on the a PTT at the studied concentration. The noticed anticoagulation effect of these plant species may be related to inhibition of the contact factors of intrinsic pathway **(HoodandEby, 2008).**

## 4. Conclusion

The present work is within the framework of *in vitro* evaluation of the anticoagulant activity of polyphenols extracted from leaves and seeds of *Centaurea macrocephala* L. The anticoagulant activity of the polyphenolic extracts was evaluated *in vitro* using the Test QT who says that the polyphenols exert a great anticoagulant activity on the exogenous pathway of the coagulation with a difference very highly significant between the four varieties studied. In effect, the results showed that the two extracts of *C. macrocephala* L. presents a good activity on the exogenous path of coagulation, with an elongation of the important time in the leaves (TQof47.26s) than the seeds (TQof29.23s).

## Conflict of Interest

The authors declare that there are no conflicts of interest.

## References

**ABDALLAH L, SURAKJI I, TQAWASME T, AYYASH D, SHHADEH R, OMAR G, and BARAKAT A. 2007.** Induction of Growth Inhibition and Differentiation of Human Leukemia HL-60Cells by Tunisian Gerboui Olive Leaf Extract. Bioscience Biotechnology

and Biochemistry,71:1306-1312.

**ABDALLAH L, SURAKJI I,TQAWASME T, AYYASHD, SHHADEHR, OMARG, and BARAKAT A.**,2022 *In Vitro* Activity of Some Medicinal Plants on Blood Coagulation Turk J Pharm Sci.2022 Jun;19(3):330–335.

**ATHUKORALAY, LEE K.W, KIM S.K, JEON, Y.J, 2007**. Anticoagulant activity of marine green and brown algae collected from Jeju Islandin Korea. Bioresource Technology,98:1711–1716.

**FORMISANO C, RIGANO D, SENATORE F, BANCHEVA S, MAGGIO A, ROSSELLI S. AND BRUNO M., (2012).** Flavonoidsin subtribe Centaureinae (Cass.) Dumort. (Tribe Cardueae, Asteraceae) : Distribution and 13C-NMR spectral data. Chemistry & Biodiversity.9,2096-2158.

**HOOD JL, EBY CS. 2008** Evaluation of a prolonged prothrombin time. Clin Chem.54:765–769.

**MABBERLEY D J. (1987)**. The Plant Book. Cambridge: Cambridge University Press**.**

**QUÉZEL P. &SANTA S.(1963)**. Nouvelle flore de l'Algérie et des régions désertiques méridionales. CNRS, Paris, Tome 2:978-979.

**MABBERLEY D J. (1987).** The Plant Book .Cambridge Cambridge University Press.

**SARKER SD, SAVCHENKO T, WHITING P, SIK V, DINAN LN., (1997)** Moschamine, *cis* moschamine, moschamindole and moschamindolol; Four novel indole alkaloids from *Centaurea moschata*. Natural Product Letter; 9: 189-199.

**TRIPODIA, (2009)**. Tests of Coagulation in Liver Disease. Clinics in Liver Disease, 13: 55–61.

**WIESNER R, EDWARDS E, FREEMAN R and *al*. (2003**). United Network for Organ Sharing Liver Disease Severity Score Committee. Model for end-stage liver disease (MELD) and allocation of donor liver. Gastroenterology; 124: 91-6.