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Phytochemical Screening, Antioxidant Activity and Safety of *Petroselinum crispum* (Mill.) AW Hill Apiaceae Leaves Grown in Benin

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Abstract

Petroselinum crispum (Mill.) AW Hill Apiaceae (Parsley) is a plant species commonly used for its culinary value, but little is known about its phytochemical composition and antioxidant properties. This study aimed to deepen knowledge of the *Petroselinum crispum* leaves (LPc) grown and consumed in Benin. The phytochemical screening of the LPc powder was done according to the method of Houghton and Raman at the Laboratory of Pharmacognosy and Essential Oils of the Institute of Applied Biomedical Sciences in Benin. The antioxidant activity (IC_{50}) was evaluated by the DPPH reduction test. Phytochemical screening revealed the presence of chemical groups such as catechin tannins, gallic tannins, flavones, saponins, mucilages, coumarins, reducing agents and O-heterosides with reduced genins in the LPc powder. The aqueous extract of LPc showed antioxidant activity with an IC_{50} = 63.66 µg/mL and was found to be less active than ascorbic acid (IC_{50} = 0.111 µg/mL). No acute oral toxicity was observed and the LD_{50} of the aqueous extract of LPc is estimated to be greater than 5000 mg/kg in the Wistar rat. The absence of toxicity, its antioxidant activity associated with its richness in active ingredients makes *Petroselinum crispum* a promising species suitable for safe long-term use as food.

Keywords

Parsley, Phytochemistry, Antioxidant Properties, Toxicity, Wistar Rat

1. Introduction

Medicinal plants are used over the world to deal with various health problems. The fact is that aromatic and medicinal plants play a very important role in the food industry, perfumery and especially in the pharmaceutical industry [1]. Actually, plants represent an inexhaustible source of traditional and effective remedies due to active compounds such as alkaloids, flavonoids, glycosides, saponins, quinones [2]. The Apiaceae family, formerly called Umbelliferae, includes edible plants such as carrots, celery, fennel, parsley, caraway, coriander, cumin. This family is known for its richness in essential oils [3]. Parsley, *Petroselinum crispum* (*Mill.*) *Fuss* (*P. crispum*), so-called parsley, is one of the culinary herbs commonly used in Chinese kitchen. In Benin, as in other countries around the world, Parsley is a popular aromatic vegetable, and its forms part of the daily diet in Morocco and around the world [4]. In addition, it has been used in different parts of the world as a carminative, gastrotonic, diuretic, urinary tract antiseptic, anti-urolithiasis, antidote and anti-inflammatory, and for the treatment of amenorrhea, dysmenorrhea, gastrointestinal disorders, hypertension, heart diseases, urinary diseases, ear infections, sniffles, diabetes and also various skin diseases in traditional medicine [5]. A piece of *P. crispum* is a "power" food, and is rich in vitamin B, vitamin C, β-carotene and zinc; it is an important food that helps the bones because it is rich in boron and fluoride. Additionally, it contains iron and calcium in an absorbable structure [6]. The results of a study carried out by Stitou in 2015 [7] revealed a significant antioxidant activity of methanolic and ethanolic extracts of the leaves, seeds and stems of *P. crispum*. For food stability, antioxidants are able to effectively minimize rancidity, delay lipid peroxidation, without affecting the sensory and nutritional properties of the food product [7]. Numerous studies have shown that the presence of natural antioxidants from various aromatic and medicinal plants is closely linked to the reduction of chronic diseases such as DNA damage, mutagenesis and carcinogenesis [8].

In short, the data in the literature relating to the medicinal properties of parsley leaves are very superficial and relate more to ancestral ethnomedicinal knowledge. Few experimental scientific studies have been devoted to these medicinal properties. Several authors including Ajebli and Eddouks [4] have discussed the phytochemical composition of parsley leaves. These studies were carried out in a geographical setting outside Benin, and cannot assume the phytochemical composition of parsley leaves grown and consumed in Benin. So far, this lack of data (phytochemical composition and toxicity of parsley leaves) on the use implies that parsley is less studied in Benin. It is therefore important to determine the phytochemical composition and test the safety of parsley leaves grown in Benin.

The aim of this study was to deepen the knowledge on the parsley leaves grown in Benin, more specifically to determine the phytochemical composition, to assess the antioxidant activity and to test the safety of parsley leaves in the Wistar rat.

2. Materials and Methods

2.1. Plant Material

- Obtaining fine powder from parsley leaves

The parsley leaves (Figure 1) were removed from their stems with a pair of scissors, then washed with water, dried in the open for 24 hours and then in the shade for 4 days [9] to dry them completely. They were then grounded with an electric mixer to obtain a fine powder.

- Preparation of the aqueous extract of parsley leaf powder

For the preparation of the aqueous extract, 50 g of powdered parsley leaves were poured into 200 ml of distilled water. The mixture obtained after stirring was heated at 100˚C for 15 minutes, then filtered. The filtrate obtained corresponds to a concentration of 0.25 g of LPc powder per millilitre. It was evaporated in an oven at 60˚C. for 24 hours. The extract obtained was stored in a hermetically sealed bottle and stored in the freezer at −20˚C. It was used for the evaluation of the antioxidant power of parsley leaves.

2.2. Phytochemical Screening

Phytochemical screening is a means of highlighting the groups of chemical families present in a plant organ. It was carried out using Houghton and Raman method [10] revised and adapted to the conditions of the Laboratory of Pharmacognosy and Essential Oils (LAPHE). Alkaloids, polyphenolic compounds, tannins, catechic tannins, gallic tannins, flavonoids, anthocyanins, triterpenoids, steroids, cardenolides, cyanogenic derivatives, mucilages, reducing agents, free anthracene derivatives and glycosides were researched (See **Supplemental Data**).

2.3. Determination of the Antioxidant Capacity of Parsley Leaves

Antioxidant power indicates the ability of a substance to neutralize free radicals. It is therefore an antiradical test using a substance that generates free radicals, here DPPH (2, 2'-diphenyl-1-picrylhydrazyl). For the evaluation of the antioxidant effect of aqueous extracts of parsley leaves, the isolation method guided by

(a) (b)

Figure 1. Leaves (a) and tufts of leaves (b) of *P. crispum*. © Germaine Gnitoungbé, 2022.

Biossay was used. For the test, the samples were prepared using dissolution in ethanol method. For each extract, a 1 mg/ml stock solution was prepared with distilled water. The solution was diluted in a geometric series of ratio 2 to have different concentrations. In dry and sterile test tubes, 1 mL of the solution of the extract to be tested was added, 3 mL of DPPH solution (0.04 mg/mL) was also added. After vortexing, the tubes were placed away from light at laboratory temperature for 30 min. For each dilution, a blank was prepared consisting of 1 mL of the test solution added with 3 mL of ethanol. The positive control was ascorbic acid (200 µg/mL). Absorbance was measured at 517 nm using a spectrophotometer (Biomate UV/VIS).

2.4. Safety of Parsley Leaves in Wistar Rats

The acute oral toxicity study was conducted in accordance with OECD 423 standards [11].

2.4.1. Plant Material

The plant material consists of the fine powder of parsley leaves.

2.4.2. Animal Material

Batch sizes are defined according to OECD 423 standards [11].

Batch 1 = 3 control female rats;

Batch 2 = 3 treated female rats.

2.4.3. Acute Toxicity Study Procedure

Batch 2, at the end of the acclimatization period, received by oral gavage with an intragastric tube, a single dose of 5000 mg/kg of the aqueous extract of parsley leaves.

Batch 1 received 1 ml of distilled water. The experiment lasted 14 days. After gavage, the rats have free access to water and pellets. The consumptions were recorded every day and the rats were weighed on the 7th and 14th day. The rats were constantly observed during the first hours following force-feeding, then every 24 hours for the duration of the experiment, in order to record deaths and clinical signs (vomiting, asthenia, dyspnoea, excessive sweating, convulsions, stupor, disorientation, coma, etc. described by Chaouali *et al.* [12]. For each extract, a stock solution was prepared in distilled water at 1 mg/ml. This solution was then diluted in a geometric series of ratio 2 to have different concentrations.

In dry and sterile test tubes, 1 mL of the solution of the extract to be tested was introduced, 3 mL of solution with DPPH (0.04 mg/mL). After vortexing, the tubes were placed away from light, at laboratory temperature for 30 min. The absorbance is measured at 517 nm using a spectrophotometer (UV/VIS biomate).

For each dilution, a blank was prepared, consisting of 1 mL of the solution tested to which 3 mL of ethanol was added. The positive control is represented by ascorbic acid (200 µg/mL) and is treated under the same conditions as the test sample.

The AA% antioxidant activity is given by the following formula:

1 − AA% = 100 − {[(Abs_{test} − Abs_{blanc}) * 100]/$Abs_{control}$}. Or again: 2-Inhibition% = ($Abs_{control}$ − Abs_{test})/$Abs_{control}$ * 100.

Either: AA: Antioxidant activity; Abs: Absorbance at the wavelength of 517 nm.

The following parameters were determined:

- IC_{50}: concentration of the substrate which causes the loss of 50% of the activity of DPPH (color).
- EC_{50}: effective concentration of the substrate which causes the loss of 50% of the activity of DPPH and EC_{50} = (IC_{50}/mg DPPH/mL)].
- Antiradical power (APR) which is inversely proportional to the EC_{50}; (APR = 1/EC_{50}).

2.4.4. Biochemical, Hematological and Histological Tests

1) Biochemical and hematological tests

At the end of the treatment, the blood of each rat was taken by puncture of the retro orbital sinus under light anesthesia with ether [13]. The blood was taken in an EDTA tube for the complete blood count (NFS), and in a dry tube for the determination of the biochemical parameters (ASAT, ALAT, urea, creatinine).

2) Histological examinations

The technique for making tissue sections used in this study is formalin fixation combined with hematoxylin-eosin staining adapted for optical microscopy observations reported by many researchers [14]. It requires seven (07) steps, namely: sampling, fixation, progressive dehydration, inclusion in paraffin, sections, staining and mounting. Thus, following the blood sample, the rats were sacrificed by asphyxiation. The kidneys and the liver were removed, weighed immediately and introduced into physiological water for rinsing. The organs were then fixed for ten days by immersion in 10% buffered formalin intended to immobilize the cellular and tissue structures, in a state as close as possible to their living state. This fixing phase was followed by dehydration by passing the fragments through successive ethanol baths of increasing degree then through a series of toluene baths for cleaning. Dehydration was succeeded by inclusion in paraffin, a hardening and preservative substance that allows the realization of fine and regular cuts. The paraffin block produced in cassettes was obtained. Thin sections (2 to 5 µm thick) of the paraffin block were made with a microtome. These sections collected on pre-numbered glass slides were placed in a bath of absolute alcohol and water (v/v) and then in gelatinous water. The slides are incubated for 24 hours. The staining, the step that follows that of the sections, accentuates the contrasts to better recognize the different elements (nucleus, cytoplasm and fibers) of the preparation. It was performed after a series of treatments (dewaxing, hydration, rinsing and cleaning) followed by different specific colorings.

3. Results

Phytochemical study of *P. crispum* leaves

Table 1 indicates the different chemical groups present in the leaves of *P. crispum*.

The phytochemical analysis of *P. crispum* leaves used in Benin reveals that this species is made up of different chemical groups: catechic tannins, gallic tannins, flavonoids, saponosides, mucilages, coumarins, reducing agents and O-glycosides (Table 1).

Evaluation of the antioxidant activity of *P.crispum* leaves

Figure 3 shows the antioxidant activities of the aqueous extract of *P. crispum* leaves (A) and ascorbic acid (B) in the presence of DPPH. The antioxidant activity of the extract and of the standard was determined from the equation of the correlation line obtained on each chart. The values of the IC50, EC50 and APR parameters of the extract and of the standard were determined from the equations obtained on each correlation curve plotted using Excel. The Chart A in Figure 2 shows that as the concentration of the aqueous extract increases, the absorbance decreases. This confirms the presence of so-called antioxidant molecules in the leaves of *P. crispum*. The same observation was made on Chart B which shows the variation in the absorbance of ascorbic acid as a function of its concentration. In addition, it noted that a low concentration of ascorbic acid lowered the absorbance more than the concentration of the aqueous extract.

Table 1. Phytochemical screening of *P. crispum* leaves.

Phytochemical groups	Results
Catechic tannins	+
Gallic tannins	+
Flavonoids	+ Flavones
Alkaloids	–
Anthocyanins	–
Leuko-anthocyanins	–
D. quinones	–
Saponosides	+
Mucilage	+
Coumarins	+
Reducing agents	+
Cyanogenic derivatives	–
Triterpenoids	–
Steroids	–
Cardenolides	–
Free anthracenes	–
O-heterosides	+ with reduced genins
C-glycosides	–

Legend: + = Presence; – = Absence.

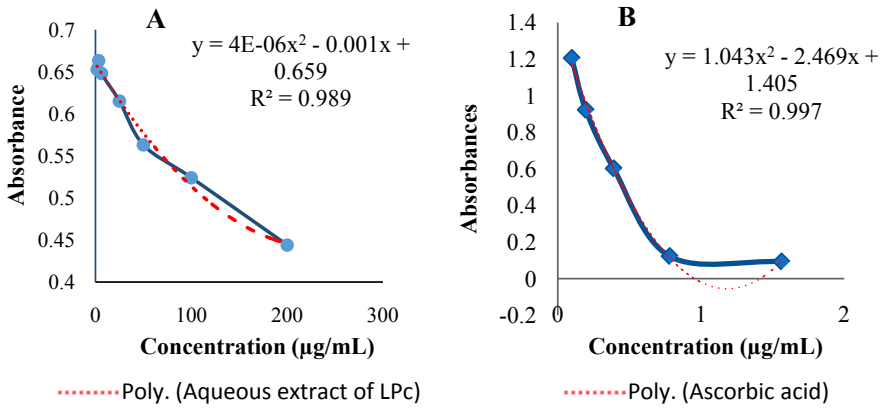

Figure 2. Antioxidant activities of the aqueous extract of the leaves of *P. crispum* (A) and of ascorbic acid (B) in the presence of DPPH. Poly. (Aqueous extract of LPc): Trend curve; Poly. (Ascorbic Acid): Trend curve; LPc = Leaves of *P. crispum*.

The concentration of the aqueous extract of the leaves of *P. crispum* (63.66 µg/mL) which caused the 50% reduction in DPPH (IC50) was significantly higher than that of ascorbic acid (0.111 µg/mL). In addition, the effective concentration (EC50) and the antiradical potency (APR) the leaves of *P. crispum* aqueous extract were lower than those of ascorbic acid (Table 2).

Exploration of acute oral toxicity

Hematology cytology

The hematological parameters of the treated rats were compared with those of the control rats (Table 3).

The acute oral toxicity test showed no significant difference between the concentrations of the various hematological parameters sought.

Biochemical examinations

The biochemical parameters (urea, creatinine, AST, ALT) were measured at the end of the acute oral toxicity test and the results of the treated batch were compared with those of the control batch (Table 4).

For the acute toxicity test, there was no significant difference between the concentration of the biochemical parameters assayed in the control rats and the rats treated at the single dose of 5000 mg/Kg.

Liver histology

In the acute oral toxicity test (AOT), the hepatic parenchyma of the rats of the treated group is typical as in the rats of the control group. The hepatocytes (arrows) are well organized in bays around the centrilobular veins (CV) and the sinusoids (S) are visible (Figure 3). These observations testify to good hepatic functioning of the treated rats. This really confirms that the aqueous extract of parsley leaves has almost no toxicity at the doses applied to liver cells.

Kidney histology

In the acute oral toxicity test (AOT), the renal parenchyma of the rats of the treated group is typical as in the rats of the control group. The glomeruli (G), the proximal (TP) and distal (TD) tubes have the characteristic appearance (Figure 4). These observations testify to good renal functioning of the treated rats. This

Table 2. Parameters of antioxidant activity of *P. crispum* and ascorbic acid.

	IC50	EC50	APR
Aqueous extract of LPc	63.66 µg/mL	2122×10^3 µg/mL	0.0157
Ascorbic acid	0.111 µg/mL	11.1×10^3 µg/mL	9.0090

LPc = Leaves of *P. crispum*.

Table 3. Determination of hematological parameters (acute toxicity).

Settings hematological	Batch x	Batch y	t	p
GB (10^3/µL)	8.35 ± 1.2	7.6 ± 6.22	0.17	0.894
RBC (10^6/µL)	7.22 ± 0.68	7.295 ± 0.54	−0.12	0.923
HGB (g/dl)	13.5 ± 0.57	13.1 ± 0.71	0.62	0.645
HCT (%)	41.95 ± 3.32	41.9 ± 1.7	0.02	0.988
MCV (fL)	58.15 ± 0.92	57.5 ± 1.98	0.42	0.746
MCHC (g/dl)	32.25 ± 1.2	31.3 ± 0.42	1.05	0.483
TCMH (pg)	18.75 ± 0.92	17.95 ± 0.35	1.15	0.456
PLT (10^3/µL)	492 ± 121.62	627.5 ± 36.06	−1.51	0.372

Batch x = control batch; Batch y = treated batch (5000 mg/kg) t = t-student value, p = p-value.

Table 4. Determination of biochemical parameters (acute oral toxicity).

Settings	Batch x	Batch y	Ref.	t	p
Urea (g/L)	0.58 ± 0.06	0.78 ± 0.06	0.12 - 0.24	−3.40	0.182
Creatinine (mg/L)	7.21 ± 0.30	8.07 ± 0.11	10 - 12	−3.88	0.161
AST (IU)	137 ± 12.73	196.5 ± 26.16	74 - 143	−2.89	0.212
ALT (IU)	150.5 ± 36.06	224.5 ± 89.80	18 - 45	−1.08	0.475

Batch x =control batch; Batch y = treated batch (5000 mg/kg) t = t-student value, p = p-value.

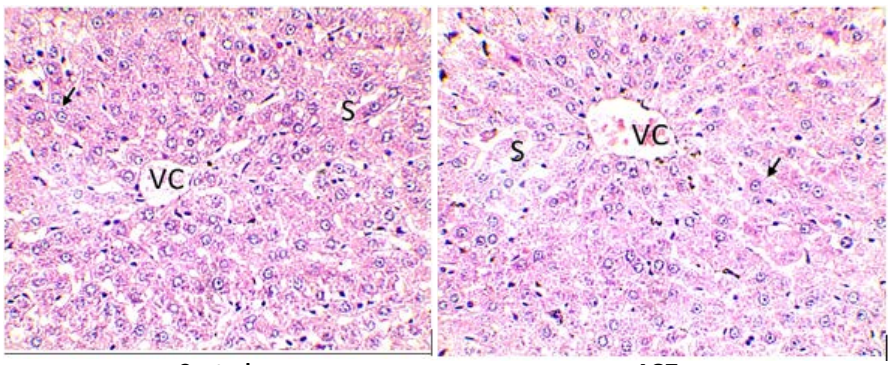

Figure 3. Liver of rats in acute oral toxicity test (AOT).

really confirms that the aqueous extract of parsley leaves exhibits almost no toxicity at the doses applied to the renal cells.

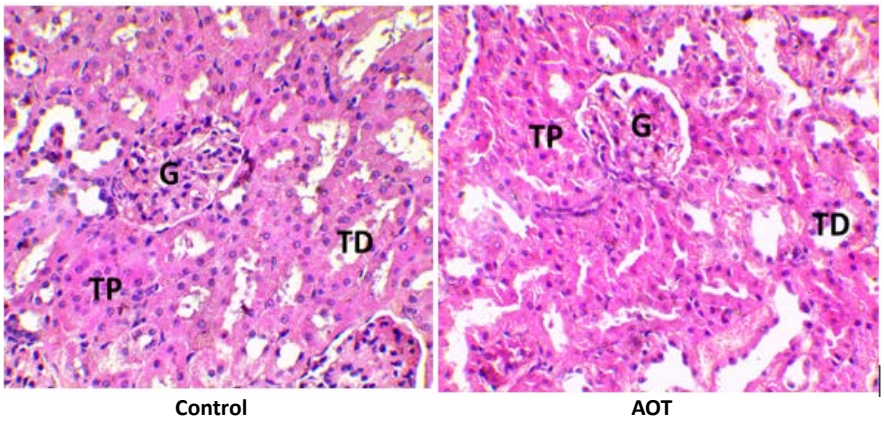

Figure 4. Kidneys of rats in the acute oral toxicity test (AOT).

4. Discussion

At the end of the phytochemical analysis, the aqueous extract of the parsley leaves revealed the presence of catechic tannins, gallic tannins, saponosides, mucilages, coumarins, the reducing agents O-heterosides and flavonoids. In the literature, the chemical composition of parsley leaves varies according to the authors. Kouar *et al.* [15], during a comparative study of the phytochemical composition of the leaves of *P. crispum*, *Tymus satureioides* and *Spirulina platensis* carried out in Morocco identified the presence of tannins, flavonoids, sterols and triterpenes. According to other authors, parsley leaves contain flavonoids. These are apiin, luteolin and apigenin-glycosides [16]. Volatile compounds (myristicine, apiole), and coumarins (bergaptene, imperatorine) have been identified by Fejes *et al.* [16], phthalides, furanocoumarins, and sesquiterpenes by Spraul *et al.* [17], flavonoids, polyphenolic acids, vitamin C, and carotenoids [18]. As with most authors, the phytochemical study of *P. crispum* leaves revealed many polyphenolic compounds (tannins, flavonoids and coumarins).

Parsley leaves showed antioxidant activity in this study, although this was lower than that of ascorbic acid. Indeed, according to Lee *et al.* [19], any extract with a half inhibition concentration (IC50) ≤ 10 mg/mL has antioxidant activity. The IC50 of the parsley leaf extract in this study is 0.064 mg/mL. These antioxidant properties are confirmed by the presence of polyphenolic compounds identified at the end of the phytochemical screening (tannins, coumarins, flavonoids), compounds known for their antioxidant power [20].

Stitou also studied the antioxidant power of methanolic and ethanolic extracts of the leaves of *P. crispum* [7]. He found IC50 values of 0.359 mg/mL and 0.019 mg/mL respectively for methanolic and ethanolic extracts, by the DPPH radical inhibition method. These results are comparable to those obtained in this study. The slightly low IC50 obtained by this author on the ethanolic extract may be related to the polarity of the ethanolic solvent which extracts and concentrates the polyphenols more than the aqueous extraction solvent used in this study. Hinneburg *et al.* [21] found, by the same method, an IC50 of 12.0 ± 0.10 mg/mL higher than that obtained in this study in an aqueous extract of parsley leaves.

This more attenuated antioxidant power obtained by these authors may be due to the difference in geographical locations and environmental conditions specific to the plant studied.

After the acute toxicity test, the *P. crispum* leaves did not show any toxicity in Wistar rats through the comparison of blood count and biochemical parameters (urea, creatinine, ASAT, ALAT) with the values of control rats in this study. Conversely, Abdel-Mobdy *et al.* [22] reported a significant decrease in AST, ALT levels in hypercholesterolemic albino rats treated with Petroselinum crispum extract. Similarly, Mahmoud *et al.* [23] reported a significant decrease in creatinine and urea in rats with gentamicin-induced nephrotoxicity treated with *P. crispum* extracts.

The decrease in serum levels of these parameters in these authors is not a sign of lesion or organ failure. It therefore confirms the absence of toxicity of the extracts of *P. crispum* leaves on hematological and blood biochemical parameters. Histological tests confirmed this absence of toxicity in this study.

The OECD [11] specifies that when no toxic effect is observed in the Wistar rat subjected to a dose of 5000 mg/kg of a plant extract, within the framework of an acute toxicity test, it can be concluded that this plant extract is not toxic.

5. Conclusion

P. crispum leaves are widely used in food in Benin. Phytochemical analysis of *P. crispum* leaves used in Benin reveals the presence of different chemical groups such as: catechic tannins, gallic tannins, flavonoids, saponosides, mucilages, coumarins, reducing agents and O-heterosides. In this study, they present antioxidant properties. The absence of toxicity, the rich phytochemical composition and antioxidant activity make *P. crispum* leaves suitable for long-term use for food.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplemental Data

Search for alkaloids

This was done using two tests:

- General acid test

5 g of the powder were mixed with 25 mL of 5% hydrochloric acid. The mixture was macerated for 24 hours. 1 mL of the filtrate obtained was collected, to which 5 drops of Mayer's reagent were added. If alkaloids are present, a yellow or cloudy precipitate will appear in the tube.

- Test in basic medium

5 g of powder were mixed with 5 mL of 50% ammonia. 25 mL of chloroform ether were added to the mixture and the whole was left to macerate for 24 hours in a stoppered bottle. The filtrate was dried over anhydrous sodium sulphate and then exhausted twice in succession with 5 mL of 5% hydrochloric acid. To the exhausted filtrate, 5 drops of Mayer's reagent were added. If an alkaloid is present, a precipitate will be observed in the tube.

Search for polyphenolic compounds

To 5 g of powder in an Erlenmeyer flask were added 100 mL of boiling water. The infused mixture was left for 15 minutes with continuous stirring, then filtered. This filtrate divided into 2 portions was used for the following research:

* Search for tannins

To the first portion of the filtrate was added a few drops of 1% ferric chloride. The observation of a dark blue, green or black color indicates the presence of tannins.

* Search for catechin tannins

To 30 ml of the second portion was added 15 ml of Stiasny's reagent and the mixture was heated in a water bath at 90˚C for 15 minutes. The appearance of a pink precipitate indicates the presence of catechin tannins.

Search for gallic tannins

The filtrate obtained previously was saturated with sodium acetate to which a few drops of 1% ferric chloride were added. A blue or black tint indicated the presence of gallic tannins.

* Search for flavonoids

To 5 ml of the second portion, 5 ml of hydrochloric alcohol (Shinoda's reagent) and a pinch of magnesium powder were added: this is the cyanidin reaction, known as Shinoda's reaction. The appearance of an orange (flavones), red (flavonols) or purple (flavonones) color indicates the presence of flavonoids.

Search for anthocyanins

A few drops of 5% hydrochloric acid were mixed with 1 mL of the second portion. The mixture was made alkaline by adding a few drops of chloroform ammonia ($CHCl_3$). The chloroform solutions were dried over anhydrous sodium sulphate then divided into three portions and evaporated to dryness (sand bath)

* Search for triterpenoids

A few drops of acetic acid dissolved the first portion. To the resulting mixture

was added 3 mL of a mixture of acetic anhydride-sulfuric acid. A purple, blue or green color indicates the presence of triterpenoids.

* Search for steroids

To the second portion, 2 drops of a 2% alcoholic solution of dinitrobenzoic acid and 2 drops of 1N sodium hydroxide were added. The appearance of a purple or wine red color indicates the presence of steroids. This is the Kedde reaction (also for cardenolides).

* Search for cardenolides

Raymond reaction for cardenolides: it consists of adding to the third portion successively 2 drops of an alcoholic solution containing 1% of metadinitronenzene and 2 drops of 20% NaOH. A blue color indicates a positive reaction.

* Search for cyanogenic derivatives

To 15 mL of distilled water, 2 g of the powder were added then immediately corked and left to macerate for 1 hour. The neck of the Erlenmeyer flask was covered with paper soaked in picric acid and heated for a few minutes. The appearance of a brown color indicates the release of HCN.

* Search for mucilage

1 ml of 10% decoction is introduced into a test tube to which 5 ml of absolute alcohol has been added. The appearance of a fluffy precipitate indicates the presence of mucilage after about ten minutes.

* Search for reducing agents

The 10% decoction was obtained by moderate boiling for 3 minutes of a mixture of 50 mL of distilled water and 5 g of powder. After cooling, the filtrate was adjusted to 50 mL with distilled water. 5 mL of filtrate was introduced into a test tube. After heating in a water bath at 90˚ C. for a few minutes, 1 mL of Fehling's reagent (Fehling's liquor A + Fehling's liquor B in equal volume) was added. The filtrate was warmed up a few minutes later. The observation of a bright red precipitate indicates the presence of reducing agents.

Search for anthracene derivatives

* Search for free Anthracenics

To 1 g of powder was added 10 mL of chloroform and heated carefully for 3 minutes in a water bath. After hot filtration, the mixture was made up to 10 mL with chloroform. 1 mL of the chloroform extract was added with 1 mL of ammonia diluted to 1/2 then stirred. The appearance of a more or less intense red color indicates the presence of free anthracenes.

* Search for heterosides

To part of the chloroform-depleted residue was added 10 mL of distilled water and 1 mL of concentrated hydrochloric acid. The test tube kept in a boiling water bath for 15 min was then cooled under a stream of water. The hydrolyzate was obtained after filtration and adjustment to 10 mL. 5 mL of the hydrolyzate was taken and stirred with 5 mL of chloroform. The drawn off organic phase was introduced into a test tube and added with 1 mL of ammonia diluted to 1/2 then stirred (the aqueous phase is kept). The presence of anthracene is revealed by the

more or less intense red coloring.

If the reaction is negative or weakly positive, the O-heterosides with reduced genins are sought. To do this, 5 mL of hydrolyzate was taken and 3 to 4 drops of $FeCl_3$ (ferric chloride) at 10% was added. The mixture, heated in a water bath for 5 min, is then cooled under a stream of water and then stirred with 5 mL of chloroform. To the chloroform phase drawn off and introduced into a test tube, 1 mL of ammonia was added to 1/2 and then stirred. A more or less intense red coloration indicates the presence of O-heterosides with reduced genins C-heterosides.

To the aqueous phase preserved above, 1 mL of 10% $FeCl_3$ was added. The mixture was brought to the boil in a boiling water bath for 30 minutes and then cooled. After stirring with 5 mL of chloroform, the chloroform phase was drawn off and collected in a test tube. To this was added 1 mL of ammonia diluted 1/2 and stirred. A more or less intense red color indicates the presence of C-heteroside genins.