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Phytochemical study of different extract from plant *Cistanche phelypaea*

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الملخص

بهدف تثمين المنتجات النباتية البرية النامية في منطقة تمنراست قمنا بدراسة تحديد التركيب الكيميائي للزيوت الأساسية ومركبات المستخلصات بالإضافة الى اعداد دراسة مقارنة عن تأثير تقنية الاستخلاص (استخلاص الزيوت الأساسية "clevenger" وSoxhlet) على مردودية الفحص الفيتوكيميائي وجود العديد من Phenylethanoid glycosides والعديد من المركبات في الزيوت الأساسية polyterpenoid. وتأكدت هذه النتائج من خلال دراسة كمية البوليفينولات وكروماتوغرافيا الطبقة الرقيقة.

انطلاقا من مردود استخلاص السوكسليت واستنادا إلى هذه النتائج يمكننا القول أن تقنية استخراج تلعب دورا هاما في المردودية، التركيبة الكيميائية والآثار البيولوجية حتى و إن استخدمنا كميات مختلفة من المواد النباتية.

Abstract

In order to value the wild plant products growing in Tamanrasset region, we studied the determination of chemical composition of the essential oils and crude extracts and prepared a comparative study on the effect of the extraction technique (extracting the essential oils "clevenger" and soxhlet) on the cost-effectiveness of the phytochemical examination, the presence of many Phenylethanoid glycosides and many in polyterpenoid essential oils.

These results were confirmed by studying the amount of polyphenols and thin layer chromatography. A good percentage of the yield of Soxhlet extraction was observed.

Based on these results, we can say that the extraction technique plays an important role in the yield, chemical composition and biological effects even if we used different amounts of plant materials.

Résumé

Afin de valoriser les produits végétaux sauvages poussant dans la région de Tamanrasset, nous avons étudié la détermination de la composition chimique des huiles essentielles et des composés chimiques et préparé une étude comparative sur l'effet de la technique d'extraction (extraction des huiles essentielles «clevenger» et soxhlet) sur la rentabilité de l'examen phytochimique, la présence de nombreux glycosides phényléthanoïdes et de nombreux Marquises aux huiles essentielles polyterpénoïdes.

Ces résultats ont été confirmés par l'étude de la quantité de polyphénols et la chromatographie sur couche mince. Un bon pourcentage du rendement de l'extraction Soxhlet a été observé.

Basant sur ces résultats, on peut dire que la technique d'extraction joue un rôle important dans le rendement, la composition chimique et les effets biologiques même si l'on utilise différentes quantités de matières végétales.

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List of Abbreviations

PhGs: Phenylethanoid Glycosides

AD: Alzheimer's disease

LDH lactate: dehydrogenase

MAGL/ hMAGL: monoacylglycerol lipase / human monoacylglycerol lipase

IC_{50} : half maximal inhibitory concentration

µM: micrometre

ROS: reactive oxygen species

O^{-2} : superoxide radical

OH^{-}: hydroxyl radical

ROO^{-}: hydroperoxyl radical

RNS: reaction nitrogen species

$ONOO^{-}$: peroxynitrite

NO^{-}: nitric oxide

DNA: deoxyribonucleic acid

ECD: echinacoside

HAPH: high-altitude pulmonary hypertension

%: Percentage

NADPH-CCl_4: nicotinamide adenine dinucleotide phosphate hydrogen with carbon tetrachloride

CCl_4: carbon tetrachloride

ATP: adenosine triphosphate

C_2H_5OH: ethanol

$CHCl_3$: chloroform

Introduction

Natural substances are becoming increasingly interested in the applications of many consumer products. Indeed, their use is encouraged because equivalent products derived from chemical synthesis have, rightly or wrongly, bad press among the general public. Plants are an inexhaustible source of active ingredients whose traditional and medical use has been known for a long time. There is therefore a need for the production of isolated, concentrated and purified bioactive substances to use in a wide range of applications (cosmetics, pharmaceuticals, nutritional additives, etc.)**[1, 2]**.

Exploration of the healing power of natural products is an ancient and established belief. However, the current civilization, which initially depends on the industrial revolution, has developed the organic chemistry and introduced it in pharmacology, resulted in a preference for synthetic drugs in modern medicine over natural products **[3]**. On the other side, medicinal plants still retain its popularity, it was estimated that up to 80% of the world inhabitants depends on plants to cover their primary healthcare needs **[4]**.

It is thought that there are 250 000 to 500 000 known species of plants on earth, these plants are considered as a large diverse resource for biological and chemical products of bioactive effects on the human body **[5]**. Surprisingly, there are 25 to 50% of current drugs prescribed in modern medicine are derived from plants; none of them is used as antimicrobial drug **[6]**.

An increasing recourse to the use of medicinal plants in industrialized societies has been attributed in part to the therapeutic effect of the secondary metabolites extracted from these plants as well as the development of several drugs whose active ingredients were herb**[7, 8]**.

Algeria is one of the countries known for its biodiversity resulting from various geo-climatic phenomena. There are more than 3000 species of plants, 15% of which are endemic and belonging to several botanical families. This floristic potential of medicinal, toxic and condimentary plants is little explored from a chemical and pharmacological point of view. To this end, in our view, it is an significant source of research into natural substances **[9]**.

The Sahara, which occupies 10% of the surface of the African continent, is the largest hot desert in the world. This eco-region includes the northern part of the Sahara where rainfall

occurs during the winter, thus nourishing a variety of plants that bloom before hot and dry summer **[10]**.

As part of the enhancement of Algerian flora by searching new molecules of therapeutic interest, we were interested in studying an endemic plant named *Cistanche phelypaea (C. tinctoria)* growing in a spontaneous state in the Wilaya of Tamanrasset and which, despite its use in the folkloric medicine and as a condiment. Very few studies have been conducted on its phytochemical and biological properties.

This work was aimed to evaluate the preliminary chemical composition of different extracts (chloroform and methanol) obtained by soxhlet extraction together with the essential oil obtained by hydrodistillation (Clevenger) of *Cistanche phelypaea*.

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Chapter I

Bibliographic Synthesis

I.1. Description of the studied plant

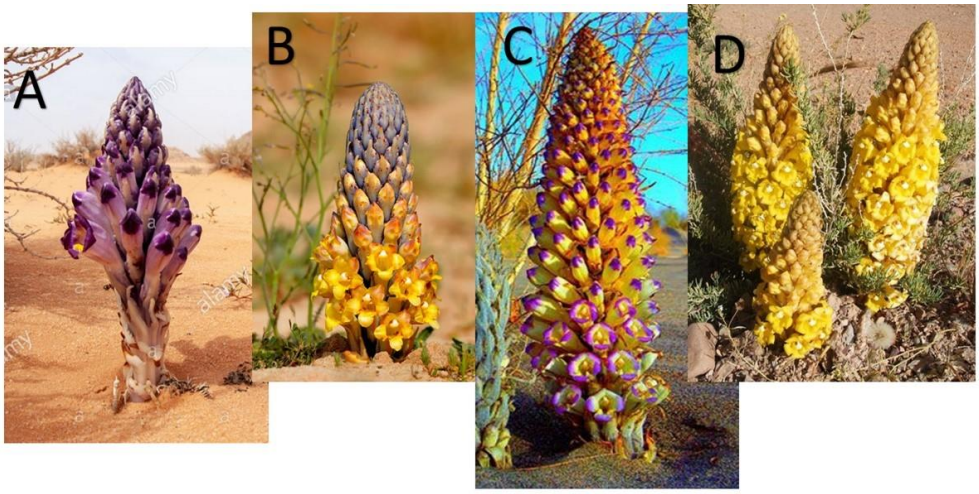
I.1.1. Generality about the Orobanchaceae family

The plant family Orobanchaceae, includes many parasitic weeds that are also impressive invaders and aggressive crop pests with several specialized features (e.g. microscopic seeds, parasitic habits). Although they have provoked several large-scale eradication and control efforts, no global evaluation of their invasive potential is as yet available [1].

Orobanchaceae Vent (the broomrape family) comprise approximately 2060 species in 90 genera that are distributed across all continents and major islands with the exception of Antarctica. Narrow circumscriptions of the family included only no-photosynthetic species (holoparasites) [2, 3], whereas broader circumscriptions have combined Orobanchaceae sensu stricto with parasitic members of von Wettstein's (1891) rhinanthoid Scrophulariaceae [4], which is supported by morphological and anatomical character states [5, 6].

I.1.2. Generality on the *Cistanche* genus

Cistanche is a genus of holophrastic desert plants grow on plants like *Haloxylon*, *Salvadora*, *Reaumuria*, *Kalidium* and *Tamarix*. *Cistanche* species lack chlorophyll and get their nutrients and water from the roots of host plants. This genus is mainly distributed in arid lands and deserts of the northern hemisphere [7].



A : *Cistanche Violet* **B :** *Cistanche Tubulosa* **C :** *Cistanche Deserticola* **D :** *Cistanche Phelypaea*

Figure I.1. The diversity of the *Cistanche* genus

The genus *Cistanche* includes 16 species forming an attractive group of phanerogamic root parasites. The occurrence of this genus is restricted to certain arid and semi-arid regions of Africa, Asia and the Mediterranean area including parts of Southern Europe **[8]**. the genus *Cistanche* is represented in Algeria by three species which are *C. phelypaea*, *C. violacea* and *C. tubulosa* **[9]**.

I.1.3. *Cistanche phelypaea*

Cistanche phelypeae (L.) Coutinho [syn.: *C. tinctoria*] is a plant known with various names such ترثوث دانون (in Arabic), ahléwan (in Targui), broomrape (in English) and Cistanque (in French) **[10]**. *C. phelypeae* is a perennial parasite with a height of the plant is about 0.2 to 1m. The width of the plant ranges from about 3 to 5 cm. The leaves turn into brown crusts. With the absence of roots, the plant attaches itself to its host via small tubers. In most cases, the main host plants are *Tamarix gallica*, *Calligonum comosum* and *Pulicaria* species. Its numerous flowers form a dense spike, with yellow petals, shaped like a tube with a 3-4 cm, opening at the top. Since the plant appears after rain, flowering occurs soon afterwards; this can happen at any time of the year in high mountains of the central Sahara. Flowering in the more northern zones of the Algerian Sahara is usually in early spring **[10]**.

Figure I.2. Airial parts of *Cistanche phelypeae* (syn.: *C. tinctoria*)

This plant occurs in an arid climate with a low rainfall (under 100 mm per year) and favors the sandy-loamy soils. It is mainly found in large valley and can tolerate moderate amounts of salt [11].

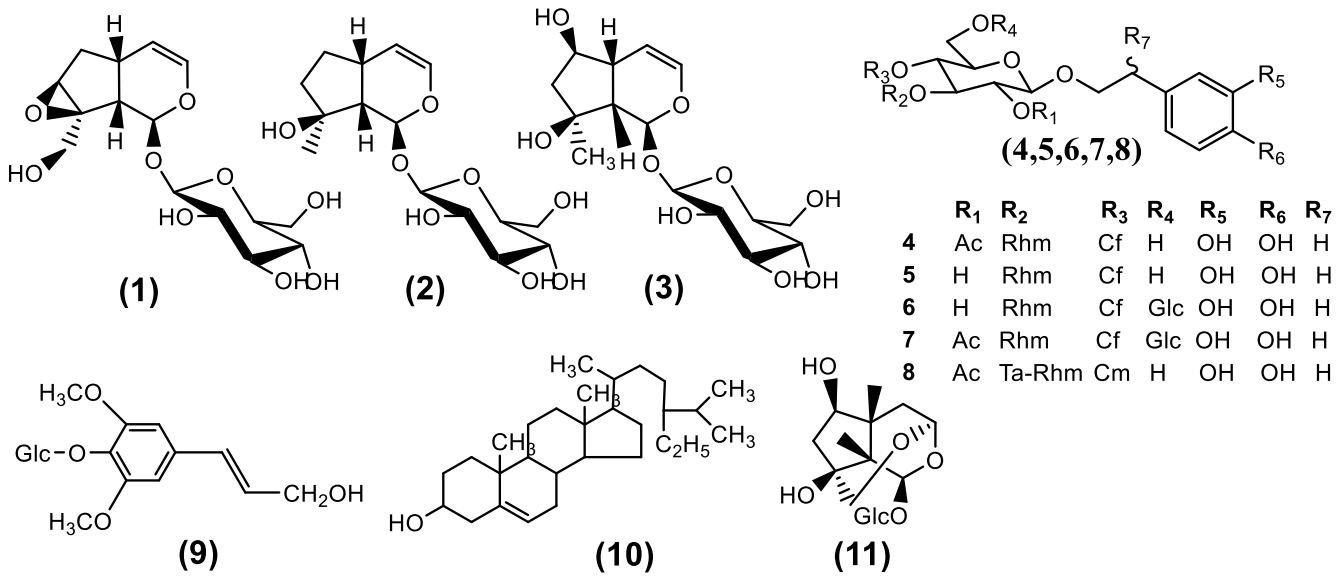
I.2. Traditional medicine and local knowledge

This genus is known for different uses such in food and medicinal applications. For example, some of the *Cistanche* species are used as tonic in the traditional Chinese medicine for the deficiency of the kidney [11]. Some of these plant species is also used for treating diarrhea, diabetes, intestinal troubles, infection (abscesses) and as a diuretic. The lower part of the plant is an essential ingredient in southern Morocco and Algeria Nomads food where they use it as a sort of porridge or flatbread; It is also known for its aphrodisiac properties. *C. phelypaea* is also used for tanning and dyeing skins. In the tissint region (Morocco), the powder of the plant is applied to wounds as a hemostat agent. A preparation made from the dried lower part of the broomrape, combined with honey and leaves of the olive tree is used as a cream for hemorrhoids, also the dried powdered plant mixed with camel's milk is used to poultice contusions [11].

I.3. Chemical composition

I.3.1. Isolated compounds from *Cistanche phelypaea*

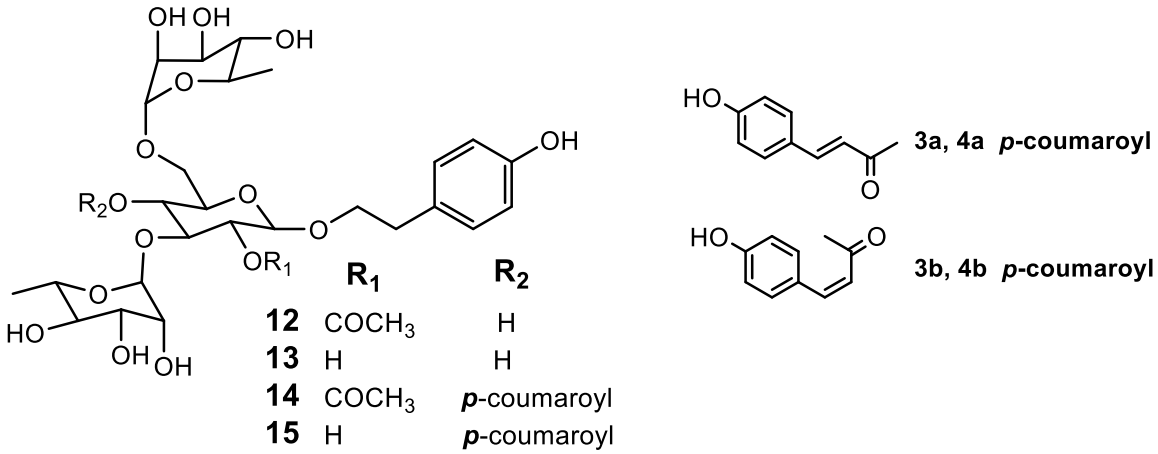
The phytochemical analysis of the methanolic extract obtained from the aerial parts of *C. phelypaea* led to the identification of 6-deoxycatalpol **(1)**, gluroside **(2)**, ajugol **(3)**, 2'-acetylacteoside **(4)**, acteoside **(5)**, echinacoside **(6)**, tubuleside A **(7)**, tubuloside E **(8)**, syringin **(9)**, *β*-sitosterol **(10)** and phelypaeside **(11)** [12, 13].



	R_1	R_2	R_3	R_4	R_5	R_6	R_7
4	Ac	Rhm	Cf	H	OH	OH	H
5	H	Rhm	Cf	H	OH	OH	H
6	H	Rhm	Cf	Glc	OH	OH	H
7	Ac	Rhm	Cf	Glc	OH	OH	H
8	Ac	Ta-Rhm	Cm	H	OH	OH	H

Figure I.3. Chemical structures obtained of the methanolic extract

Another study on the *n*-butanol extract of *C. phelypaea* aerial parts, reported the isolation of four new phenylethanoid glycosides named 1-*β*-*p*-hydroxyphenyl-ethyl-2-*O*-acetyl-3,6-di-*α*-L-rhamnopyranosyl-*β*-D-glucopyranoside **(12)**, 1-*β*-*p*-hydroxyphenyl-ethyl-3,6-*O*-di-*α*-L-rhamnopyranosyl-*β*-D-glucopyranoside **(13)**, 1-*β*-*p*-hydroxyphenyl-ethyl-2-*O*-acetyl-3,6-di-*α*-L-rhamnopyranosyl-4-pcoumaroyl-*β*-D-glucopyranoside **(14)**, and 1-*β*-phydroxyphenyl-ethyl-3,6-di-*α*-L-rhamnopyranosyl-4-*p*-coumaroyl-*β*-D-glucopyranoside **(15)** [14].



	R_1	R_2
12	$COCH_3$	H
13	H	H
14	$COCH_3$	*p*-coumaroyl
15	H	*p*-coumaroyl

Figure I.4. Chemical structures obtained of the *n*-butanol extract

Moreover, the *Cistanche* species are rich sources of PhGs, iridoids, lignans, and polysaccharides, and PhGs are the characteristic principles of this genus. To date, 120 compounds have been isolated from *C. deserticola*, 75 compounds from Chinese *C. tubulosa*, 21 compounds from Pakistani *C. tubulosa*, 31 compounds from *C. salsa*, 11 compounds from *C. phelypaea* and 20 compounds from *C. sinensis* **[14, 15]**.

I.3.1. Isolated compounds from other *Cistanche species*

I.3.1.1. Phenylethanoid glycosides (PhGs)

This class of compounds has been regarded as the primary active components of *Cistanche* species where 70 PhGs have been reported, including monoglycosides like salidroside **(16)** from *C. deserticola*, diglycosides such as tubuloside C **(17)** and triglycosides like cistanoside A **(18)** from *C. deserticola* **[15, 16]**.

	R_1	R_2	R_3	R_4	R_5	R_6	R_7
16	H	H	H	H	H	OH	H
17	Ac	TA-Rha	Cf	Glc	OH	OH	H
18	H	Rha	Glc	Cf	OMe	OH	H

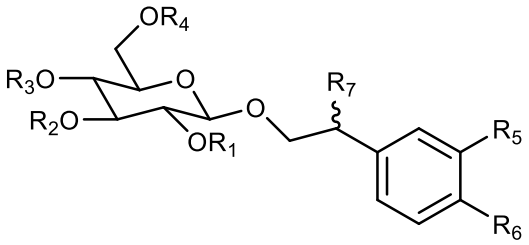


Figure I.5. Phenylethanoid glycosides of *Cistanche* species

I.3.1.2. Iridoids

To our best knowledge, 26 iridoids were isolated from different *Cistanche* species such as Cistanin **(19)** from *C. deserticola,* Kankanoside A **(20)** from *C. tubulosa* and Kankanoside D **(21)** from *C. tubulosa* **[15, 16]**.

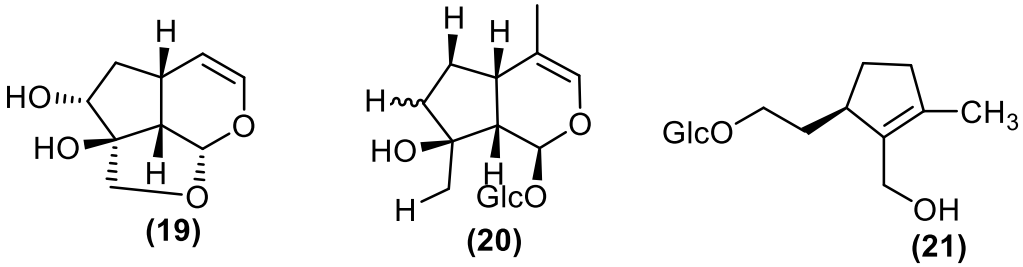


Figure I.6. Chemical structures iridoids of *Cistanche* species

I.4. Bioactivities of the *Cistanche* species

Many biological activities were reported for different species of the Orobanchaceae family, including antioxidant, anti-inflammatory [17], antispasmodic and smooth muscle relaxant [18]. On the other hand, extracts from *Cistanche* plants have an extensive range of biological effects, comprising the treatment of kidney deficiency and senile constipation, for learning improvement, relief of symptoms related with Alzheimer's disease (AD), management of menopausal symptoms, enhancement of immunity, anti-aging and anti-fatigue [19, 20].

These properties are ascribed to the presence of different types of bioactive molecules, such as phenylethanoids glycosides (PhGs), iridoids, flavonoids and polysaccharides [21].

I.4.1. Anti-cancer

Cancer is a general term applied to malignant diseases characterized by rapid and uncontrolled abnormal cells formation which may mass together to form a growth or proliferate throughout the body and it may progress until it causes death. Medicinal plants are the most exclusive source of life saving drugs for the majority of the world's population. Medicinal herbs have been widely used for treatment of diseases in traditional way for several generations [22].

An interaction between traditional medicine and modern biotechnological tools is to be established towards new drug development. The interference between cell biology, *in vitro* assays and structural chemistry will be the best way forward to obtain valuable leads [23].

The *n*-butanol extract obtained from *C. phelypaea* was tested for its lactate dehydrogenase (LDH) and monoacylglycerol lipase (MAGL) inhibitory activity [24]. In hMAGL enzymatic assays, only two derivatives (compound **15** and apigenin 7-*O*-*β*-glucuronopyranoside **(22))** proved to be inactive, whereas pinoresinol 4-*O*-*β*-D-glucopyranoside **(23)** and brandioside **(28)** exhibited IC_{50} values of 130.2 and 156.1 µM, respectively. Compounds **13** and **14** showed a better inhibition activity, with similar IC_{50} values of 117.4 and 113.9 µM, respectively. The best inhibition potency on hMAGL was demonstrated by compound **12**, with an IC_{50} value in the low micromolar range (88.0 µM), and it proved to be selective for hMAGL over hLDH [14]. These

dysregulated metabolic pathways are typical features of cancer, and at the same time, they may offer therapeutic windows for anticancer agents that target them. In this context, LDH and MAGL are two enzymes overexpressed in tumor tissues, which play key roles in the typical glycolytic and lipidic cancer metabolism, respectively [23, 25].

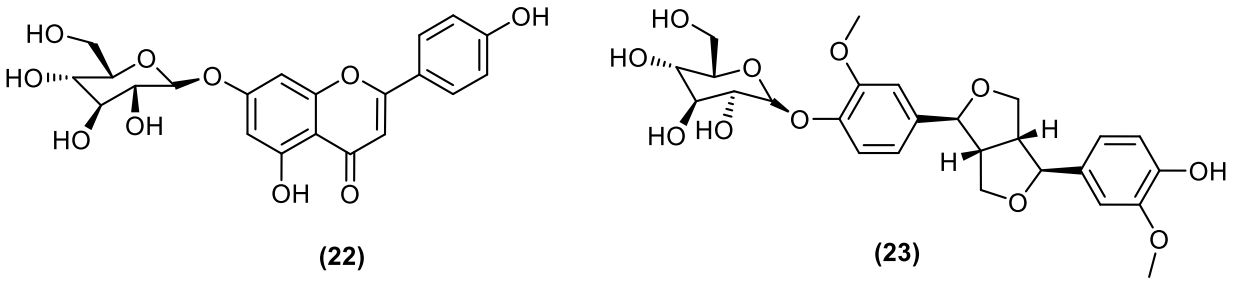


Figure I.7. Bioactive compounds from some *Cistanche* species

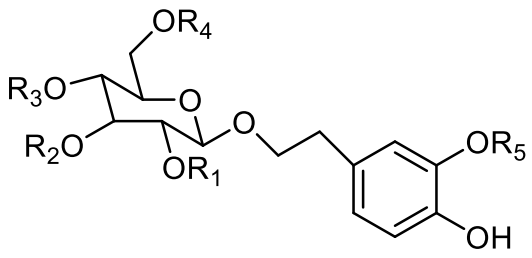
I.4.2. Antioxidants

Antioxidants are a group of substances that are useful for fighting cancer and other processes that potentially lead to diseases such as atherosclerosis, Alzheimer's, Parkinson's, diabetes and heart disease [26].

Unlike cytotoxic agents that damage tumor cells, antioxidants act by preventing the onset of cancer during carcinogenesis, and they are generally beneficial to cells. Oxidants such as reactive oxygen species (ROS) that include the superoxide radical (O^{-2}), hydroxyl radical (OH^{-}), hydroperoxyl radical (ROO^{-}) and nitrogen species(RNS) such as peroxynitrite ($ONOO^{-}$) and nitric oxide (NO^{-}) damage macromolecules, including proteins, lipids, enzymes and deoxyribonucleic acid (DNA) [27].

Phenylethanoid glycosides (PhGs) have been regarded as the major active components of *Cistanche* species especially *C. phelypaea*. The pharmacological activity studies of PhGs have demonstrated that they have various functions, such as antioxidation, neuroprotection, enhancing immune and sexual function, hepatoprotection [16].

The isolated PhGs from C. *phelypaea* named 2'-acetylacteoside**(4)**, acteoside **(5)**, echinacoside **(6)**, tubuloside B **(24)**, tubuloside A **(25)**, isoacteoside **(26)** and castanoside A **(27)**, showed strong DPPH radical scavenging activity (IC_{50}=3.30, 3.36, 3.29, 2.99, 3.34, 3.49 and 4.87 µM, respectively) than caffeic acid (IC_{50}=4.79 µM) [28].



	R_1	R_2	R_3	R_4	R_5
(24)	Ac	Rha	H	Cf	H
(25)	Ac	Rha	Cf	Glc	H
(26)	H	Rha	H	Cf	H
(27)	H	Rha	Cf	Glc	Me

Figure I.8. Antioxidant agents from *Cistanche* species

I.4.3. Anti-fatigue Activity

Physical fatigue is the transient inability of muscles to maintain optimal physical performance, and is made more severe by intense physical exercise **[29-31]**. Mental fatigue is a transient decrease in maximal cognitive performance resulting from prolonged periods of cognitive activity. Mental fatigue can manifest as somnolence, lethargy, or directed attention fatigue **[32]**.

Few numbers of studies have focused on the antifatigue activity. A previous study reported that the polysaccharide-rich extract contributed little to the antifatigue activity of *C. deserticola*. It was hypothesized that phenylethanoid-rich extract, containing another major group of chemical constituents, may play an important role in its antifatigue activity. The purpose of this study was to evaluate the effect of ECD on the physical strength **[33]**.

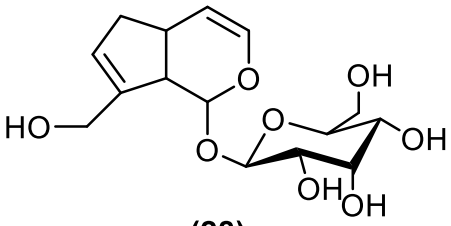
I.4.4. antidiabetic activity

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels **[34]**.

Different *Cistanche* species are used in traditional medicine as antidiabetic agents, and show antidiabetic features on animal models. For example, the administration of the aqueous extract of *C. tubulosa* to mice significantly suppressed the high fasting blood glucose and postprandial blood glucose levels, improved insulin resistance and dyslipidemia, and suppressed body weight loss **[35]**.

The phytochemical analysis of *C. phelypaea* water extracts (roots, stems and flowers) allowed the identification of two classes of compounds, namely iridoids and

phenylethanoid glycosides. The water root extract was dominated by phenylethanoid glycosides PhGs while the flowers extract was rich in iridoids. The stem extracts evidenced resonances of both iridoids and PhGs. The major molecules identified were gluroside **(2)** acteoside **(5)**, echinacoside **(6)**, tubuloside A **(25)** and bartioside **(28)**. Computational studies indicated that compounds **5**, **6** and **25** may behave as a competitive inhibitor of α-glucosidase and tyrosinase. The results suggest that roots and flowers of *C. phelypeae* could be considered as a source of innovative herbal products with pharmaceutical and biomedical applications, with antidiabetic and anti-melanogenic assets. Assays are in progress aiming to determinate toxicity, bioavailability and *in vivo* efficacy [21].



(28)

Figure I.9. Chemical structure of bartioside

I.4.5. Vasorelaxant activity

Vascular endothelial dysfunction is an important risk factor that causes cardiovascular diseases, such as hypertension. Vascular endothelial cells play an important role in respond to various hormone stimuli by releasing endothelium-dependent vasodilators. Decrement of the endothelial NO has been observed in clinical patients and in hypertensive animal models. Therefore, the vasodilator drugs targeting vascular endothelial system should contribute to the relief of vascular pathology-associated diseases. Jin et al. explored the effects of PhGs from *Cistanche* herbs on high-altitude pulmonary hypertension (HAPH) rat models [36]. The data showed that PhGs treatment markedly decreased the pulmonary vascular thickening and inflammatory infiltration. Also reduced the mean pulmonary artery pressure, right ventricular systolic pressure, and right ventricular hypertrophy index. Therefore, PhGs could be clinically used to improve the hemodynamics and right ventricular hypertrophy in high-altitude pulmonary hypertension [36].

I.4.6. Antitumor activities

It has been reported that both the ethyl acetate and aqueous extracts from three kinds of *Cistanche* plants (*C. tubulosa*, *C. deserticola, and C. salsa*) showed obvious peripheral blood lymphocyte activating effects, leading to the killing of human leukocythemia K562 cells. In particular, the extract of *C. tubulosa* showed the most significant effect [15].

In addition, treatment with polysaccharides from *C. deserticola* for 10 days significantly inhibited the growth of mouse Lewis lung cancer and tumor S180 *in vivo*, and the tumor inhibitory rates were 42 and 46%, respectively [37].

According to reported data, acteoside **(5)** is a potent inhibitor of protein kinase C, with an IC_{50} value of 25 μM [38]. The published results indicated that at doses of 10 and 20 μmol/L, it could improve MGc80-3 cells morphology towards normalization rather than by killing tumor cells with high cytotoxicity or other side-effects [39]. It could induce promyelocytic leukemia HL-60 DNA degradation with an IC_{50} value of 26.7 μM [40].

Acteoside **(5)** (25-100 μM) promoted apoptosis by regulating HIPK2-p53 signaling in human colorectal cancer cell line. In addition, further in vivo study also found that it inhibited the growth of mice tumor and the inhibition rate up to 60.99% on the concentration of 80mg/kg [41].

I.4.7. Hepatoprotection activity

Four PhGs from *C. deserticola* have been reported to show inhibitory effects on lipid peroxidation of NADPH-CCl_4-induced hepatomicrosomes and to suppress the release of aspartate aminotransferase *in vitro*. Studies showed that 2′-acetylacteoside **(4)**, acteoside **(5)**, echinacoside **(6)** and isoacteoside **(26)** from *C. deserticola* exhibited significant protective effects on liver. These compounds inhibited the lipid peroxidation in liver cell microsomes, blocked the aspartate aminotransferase release, and reduced CCl_4 and d-aminogalactose-induced hepatotoxicity [43, 44].

In recent years, it is reported that PhGs, such as 2'-acetylacteoside **(4)**, acteoside **(5)**, echinacoside **(6)**, cistanoside A **(18)**, tubuloside B **(24)** and isoacteoside **(26)** exerted positively hepatoprotective effects through multiple mechanisms, including scavenging free radicals, blocking cytochrome P450 biotransformation, and strengthening antioxidant defense system, etc. [43]. PhGs (echinacoside **(6)** 42.71 ± 0.42 %, acteoside(5) 14.27 ± 0.18

%, IC_{50} = 119.125 µg/mL), acteoside **(5)** (IC_{50} = 6.999 µg/mL) and echinacoside**(6)** (IC_{50} = 520.345 µg/mL) inhibited TGF-β1/smad signaling pathway in hepatic stellate cell, which showed hepatoprotective activity *in vitro* [45]. Compound **5** (30, 100 mg/kg, s.c.) showed hepatoprotective activity against CCl_4-induced live damage in rat [43], this effect maybe associated with the reduced P450 2E1 level and antioxidation [46].

Cistanoside A **(18)** alleviated alcohol-induced hepatotoxicity in mice through increasing the activities of mitochondrial antioxidant enzymes (GST, SOD and CAT) and energy metabolism enzymes (total ATPase, Na^+–K^+–ATPase, Ca^{2+}–Mg^{2+}–ATPase), as well as antioxidant defense system, besides, it inhibited apoptosis and necrosis of the primary cultured hepatocytes through upregulating Bcl-2 and downregulating c-fos expression [47, 48]. Besides PhGs, *C. deserticola* polysaccharide (0.11, 0.33, 1.00, 3.00 mg/mL, Mw = 1300 kDa), which contained higher proportion of galacturonic acid, can inhibit the growth and proliferation of HepG2 cell line, furthermore, it (200, 600, 1800 mg/kg) showed hepatoprotective activity against liver injury induced by alcohol in ICR mice [49].

I.4.9. Anti-myocardial ischemia activity

The reported data on the isolated PhGs from *C. salsa* indicated that they could increase the activity of superoxide dismutase and glutathione peroxidase in the mouse cardiac muscle after ischemia reperfusion [52, 53]. Moreover, *β*-sitosterol **(10)** isolated from some *Cistanche* species presented the protection activity on myocardial cells by upregulating mitochondrial glutathione redox cycling and inducing the mitochondrial ATP generation. Hence, *C. salsa* is effective on protecting the myocardial ischemia [54].

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Chapter II

Materials and methods

II.1. Materials

II.1.1. Plant materials

Cistanche Phelypaea (*C. tinctoria*) is a parasitic plant like all members of the Orobanchaceae family, it is chlorophyll-free, obligate parasitic. The flowers are bright yellow and distributed throughout the plant in brown tones, the leaves are lanceolate, reduced to brownish scales **[1]**.

Figure II.1. Picture real of *C. phelypeae*

II.1.1.1. Geographical distribution

Local: Common on the high plateaus and northern Algerian Sahara; rarer in the western and central Sahara.

Regional: North Africa.

Global: North Africa [2]

II.1.1.2. The taxonomy of the species

This classification refers to the botanical classification **(tabII.1)** [3].

Table II.1. Botanical classification of the studied species

REGION	TYPE
DOMAIN	Biota
REIGN	Plantae
SUB-REIGN	*Viridaeplantae*
INFRA-REIGN	*Streptophyta*
CLASS	*Equisetopsida*
CLADE	*Tracheophyta*
CLADE	*Spermatophyta*
SUB-CLASS	*Magnoliidae*
SUPER-ORDRE	*Asteranae*
ORDRE	*Lamiales*
FAMILY	*Orobanchaceae*
GENRE	*Cistanche*
SPECIE	*Cistanche phelypaea*

In this study, we used the whole plant *C. Phelypaea* (*C. tinctoria*), which have been collected during the flowering period 4thJanuary 2020 and 10th February 2020 from Abalessa region (**figure II.1**) (22°53'17.7"N; 4°51'44.5"E); that affiliated regionally to Tamanrasset state and it's located about 80 km toward the N55A road.

Figure II.2 the place of collecting the plant C.Phelypaea

II.1.2. Materials and methods used in preparing the plant materials

In order to prepare the plant sample, we followed the steps in the table below

Table II.2 Method and materials uses

	Used methods	*Materials*
Collecting	the plant has been collected during the flowering period	• Scissors • Plastic bags
Drying	The collected plant material was airdried at room temperature for 3 to 4 weeks	• Clean cloth
Grinding	After drying, the plant material is cut by scissors to get a little part	• Scissors • Mortar

Other materials, solvents and chemical reagents we used in the laboratory are listed as follows: Soxhlet apparatus, Clevenger apparatus, rotavapor apparatus, separated funnel, beaker, volumetric flask, chloroform ($CHCl_3$), ethanol (C_2H_5OH) and distilled water (H_2O).

II.2. methods and Phytochemical study

II.2.1. Extraction procedure

Essential oils are plant-based volatile products with strong aromatic components that are made up of different chemical compounds. For example, alcohols, hydrocarbons, phenols, aldehydes, esters and ketones are some of the major components of essential oil **[4].** Essential oils found in many different plants, especially the aromatic plants, vary in odor and flavor, which are governed by the types and amount of constituents present in oils **[5]**.

II.2.1.1. Hydrodistillation method (Clevenger)

Hydrodistillation is one of the oldest and easiest methods to extract essential oils from aromatic and medicinal plants **[6]**. By heating water which contains the plant material, vapors can extract essential oils. The setup comprises also a condenser and a decanter to collect the condensate and to separate essential oils from water (**Fig II.3**) **[7]**.

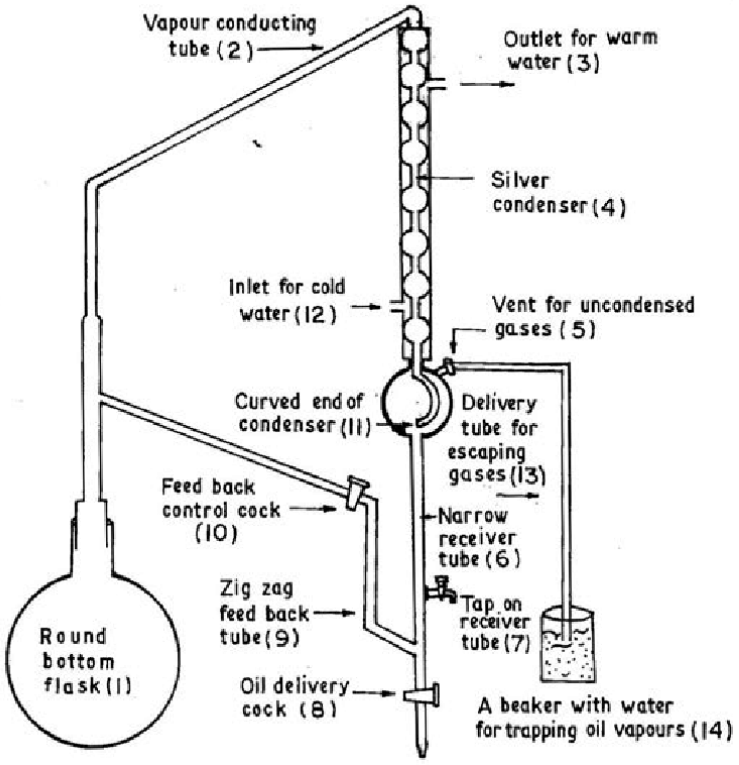

Figure II.3. The schematic subsidize apparatus for hydrodistillation (Clevenger)

A mass of 100 g of *C. phelypeae* was introduced into a glass balloon of 1000 ml containing a sufficient amount of distilled water. By using balloon heater, the mixture was brought to the boil point, the vapors loaded with essential oils pass through the vertical tube then into the condenser, where the condensation will take place. The droplets thus produced accumulate in the tube filled with distilled water beforehand. Due to the deference of density, the essential oil floats on the surface of the water. The hydrodistillation process lasts 4 hours. The essential oils obtained are collected in a bottle protected from light and stored at (4–6°C).

Figure II.4. The essential oils extraction of *C. phelypeae* by Clevenger

II.2.1.2. Soxhlet Extraction

A Soxhlet extractor is a piece of laboratory apparatus [8] invented in 1879 by **Franz von Soxhlet**. It was originally designed for the extraction of a lipid from a solid material [9].

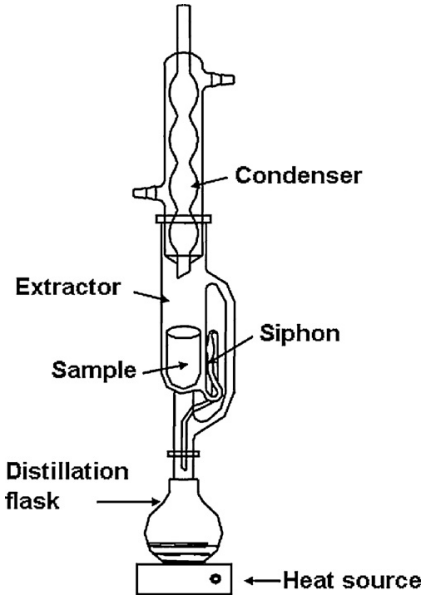

Figure II.5. Conventional Soxhlet extractor

44.24 g of the plant was prepared in 2 soxhlet cartridges. The extraction was performed using a soxhlet apparatus with a 1 L balloon which contains 400 ml of chloroform in a balloon heater set at 38°C. The extraction process took 3 days to exhaust the plant material. The resulting solution was vaporized using the rotary evaporator at 38°C to obtain the chloroform extract. We repeat the experiment by choosing an ethanol solution with the same mass.

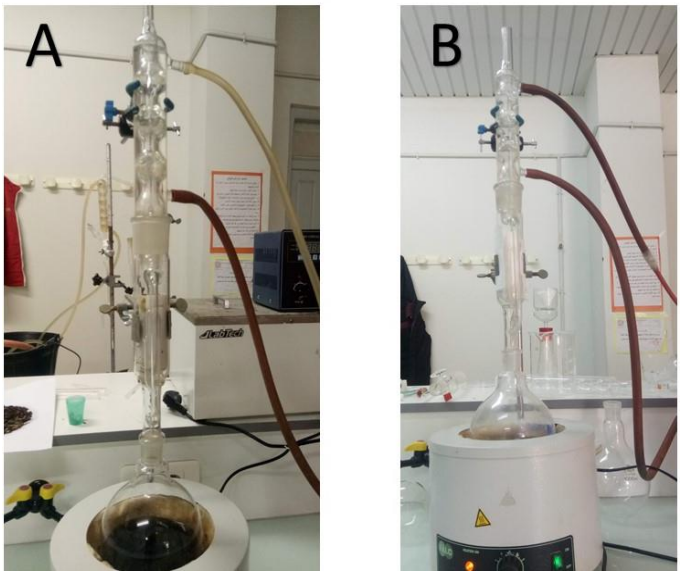

Figure II.6. Soxhlet extraction apparatus (A: with ethanol, B: with chloroform)

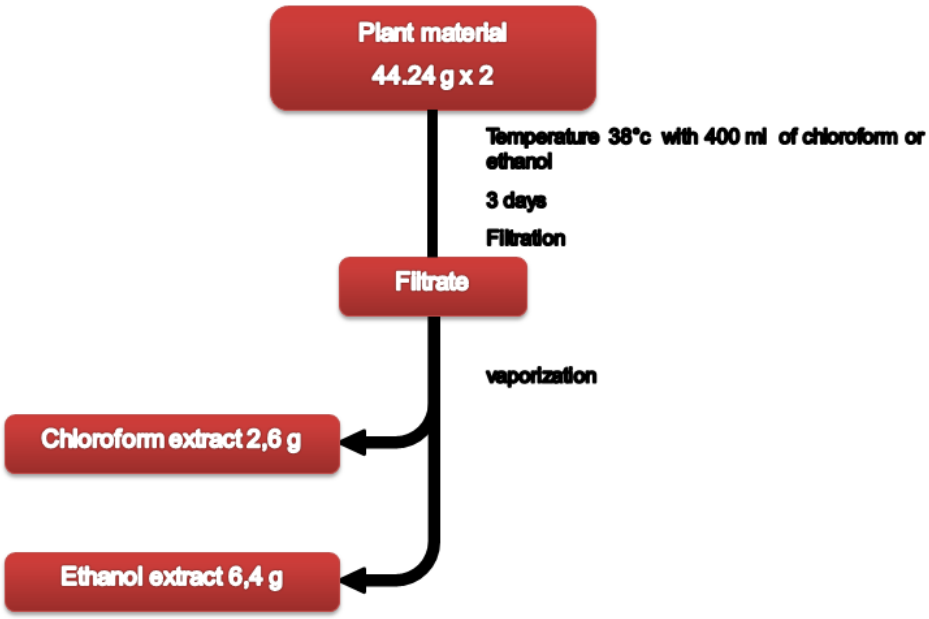

Figure II.7. Schema of the plant extraction withe MeOH and $CHCl_3$

II.2.1.3. Separation withe the rotary evaporation

A rotary evaporator is a specially designed instrument for the evaporation of solvent (single-stage or straight distillation) under vacuum. The evaporator consists of a heating

bath with a rotating flask, in which the liquid is distributed as a thin film over the hot wall surfaces and can evaporate easily. The evaporation rate is regulated by the heating bath temperature, the size of flask, the pressure of distillation and the speed of rotation **[10]**.

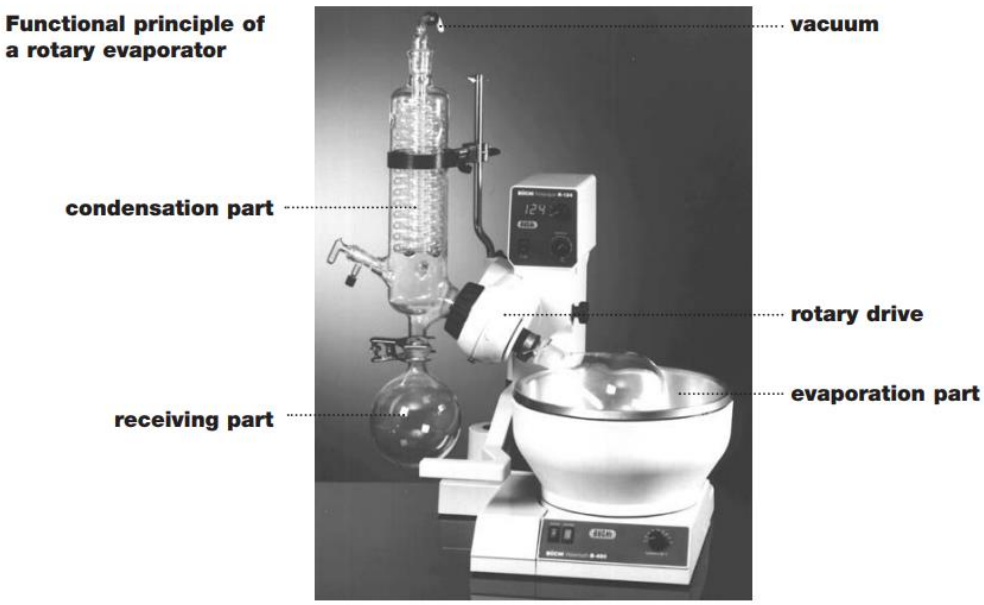

Figure II.8. Rotary evaporator apparatus

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Chapter III

Results and discussion

III.1. Extraction yield

The percentage yield (%) is defined as the ratio of the mass of the extract to that of the dry powdered plant. It is calculated by the following formula:

$$R\% = M/M_0 \times 100$$

M: mass of crude extract
M_0: mass of dried plant

III.1.1. Essential oil yield

After the hydrodistillation extraction, we noted that *Cistanche phelypaea* provides low yields of essential oil (few drops).

III1.2. Soxhlet extraction yield of chloroform and ethanol extracts

The use of 44.24 g of the plant material in soxhlet extraction with chloroform yield 2.6 g of the extract which mean that the yield is: R% = 2.6/44.24 x 100 = **5.877%**

The extraction of 44.24 g of the plant material soxhlet and ethanol as solvent led to the obtaining of 6.4 g of the extract with a percentage yield calculated as follow: R% = 6.4/44.24 x 100 = **14.46%**

The obtained results indicates that *C. phelypaea* contains more polar components than the nonpolar ones, which is reflected on the extract amount obtained from the Soxhlet extraction, and the essential oil.

III.2. Analysis and the chemical composition of essential oils

In this study, we couldn't investigate the chemical composition of our *C. phelypaea* essential oil because of the covid-19 confinement, which orient us to base on the previous reported data about, from PhD thesis and master memories on the Algerian *Cistanche* genus.

These studies were performed using gas chromatography (GC) and / or gas chromatography coupled with electron impact mass spectrometry Analysis (GC-EIMS). The identification of the profiles components was based on retention times, linear retention indices (kovat index) and on a commercial database and mass spectra library constructed from known substances and literature data on the MS **[1, 2]**. It has been reported that the extracted essential oil of *C. phelypaea* was about approximately ≥ 0.007 %; (in accordance with our results). The obtained oil has a very dark color, almost black with an specific intense odor **[2]**. Twenty compounds were identified which represent a percentage of 93.4 % of the total composition of the volatile oil (see tab. 2). The results show that the oxygenated sesquiterpenes are the major components with a very high percentage of 68.2%, these compounds are mainly represented by (E,E)-methyl farnesoate with a percentage of 44.8 %, (E)-nerolidyl acetate (9.6 %) and ϒ-eudesmol (9.4 %). The other identified compounds, which represent a percentage of 14.6 %, are mainly 1-tetradecanol (6.8 %), and (E)-2-heptadecene (05 %). Oxygenated monoterpenes represent 4.8 % of the oil composition, while sesquiterpene hydrocarbons represent only 1 %. Limonene is the only monoterpenic hydrocarbon identified with a percentage of 3.2 % **[2]**.

Table III.1. Chemical composition of the essential oil of *Cistanche phelypaea*.

constituents	**%**
Limonene	3.2
Fenchone	1.0
linalool	0.6
4-terpineol	0.6
Endo- fenchyl acetate	1.6
Carvone	0.5
Oxide de piperitenone	0.5
(*E*)-β-damascene	0.5
(*E*)-geranyl acetone	1.1

B-selinene	0.5
n-pentadecane	0.5
δ-cadinene	2.3
(E)-nerolidol	2.0
1-Hexadecane	0.5
ϒ-eudesmol	9.4
1-tetradecanol	6.8
(E)-2-heptadecene	5.0
(E)-nerolidyl acetate	9.6
(E,E)-α-farnesal	2.4
(E,E) methyl farnesoat	44.8
Hydrocarbures monoterpene	3.2
Monoterpene oxygens	4.8
Hydrocarburus sesquiterpene	1.0
Sesquiterpenes oxygens	68.2
Apocarotenoids	1.6
unknown compounds	14.6
Total identify	**93.4**

In other research, the major volatile compounds of *C. tinctoria* are terpenoids, aldehydes, ketones, alcohols, and aromatic derivatives **[3]**. The chemical content and percentage of essential oils may vary in different species, at different harvesting times, and at specific growing locations and microenvironments **[3]**. The reported qualitative and quantitative data about the fatty acids composition indicates the presence of 18 fatty acids where oleic acid was the major one with a percentage of 28.1 %, followed by palmitic acid (25.0 %) and linoleic acid (16.6 %). mentioned that the nutritional value of linoleic acid is due to its metabolism at tissue levels which produces the hormone-like prostaglandins **[3]**.

Table III.2. Relative percentages of fatty acids in *Cistanche*

Fatty acids	%
C 10:0 Capric acid	3.82 ± 0.08
C 12:0 Lauric acid	0.63 ± 0.02
C 14:0 Myristic acid	3.94 ± 0.06
C 15:0 Pentadecanoic acid	0.60 ± 0.02
C 16:0 Palmitic acid	25.0 ± 0.35
C 16:1n-7 Palmetoleic acid	2.24 ± 0.02
C 17:0 Margaric acid	0.58 ± 0.02
C 18:0 Stearic acid	3.55 ± 0.09
C 18:1n-9 Oleic acid	28.1 ± 0.22
C 18:2n-6,9 Linoleic acid	16.6 ± 0.16
C 18:3n-3,6,9 Linolenic acid	1.53 ± 0.03
C 22:0 Arachidic acid	0.82 ± 0.02
C 22:2 Docosadienoic acid	1.34 ± 0.02
C 23:0 Tricosanoic acid	2.03 ± 0.04
C 24:1 Teracosenoic acid	0.47 ± 0.01
C 26:0 Cerotic acid	1.66 ± 0.03
C 30:0 Melissic acid	0.49 ± 0.01
Total unknown compounds	**6.35 ± 0.02**

The analysis of the free sterols previously reported, provides rich information about the quality and the identity of the investigated oil. In fixed oils, neither cultivation of new breeding lines nor environmental factors have been found to alter content and composition of free sterols significantly in contrast to the fatty acid composition, which has been changed dramatically by breeding programs**[4]**.

The essential oil content of *C. phelypaea* rich in sterols is presented in **Table III.3**. Remarkable levels were estimated in the oil, which made up 29.4 g/kg oil. The main component was *β*-sitosterol which represented ca. 77.4 % of the total sterol content. Other

components, e.g. stigmasterol, D7-avenasterol and D5-avenasterol, were present at approximately equal amounts (6–9 % of total sterols) [4].

Table III.3. Sterol composition (g/kg) of *C.e phelypaea* oil

Compound	(g/kg)
Stigmasterol	2.10 ± 0.04
β-Sitosterol	22.8 ± 0.19
Δ^5-Avenasterol	1.89 ± 0.03
Δ^7-Avenasterol	2.65 ± 0.05

Previous papers reported the investigation of *C. phelypaea* hydrocarbons profile using GC presented in **Table III.4**. The main identified hydrocarbons were C_{21}, C_{26} and C_{32} which represent together about 61.2 % of total identified hydrocarbons. Low amounts of C_{12}, C_{18} and C_{22} were also detected.

Table III.4. Relative percentage of hydrocarbons in *Cistanche*

Hydrocarbons	%
Dodecane (C_{12})	0.43 ± 0.06
Pentadecane (C_{15})	5.37 ± 0.09
Octadecane (C_{18})	0.62 ± 0.03
Eicosane (C_{20})	4.11 ± 0.07
Uneicosane (C_{21})	19.3 ± 0.20
Doeicosane (C_{22})	0.69 ± 0.03
Hexaeicosane (C_{26})	23.8 ± 0.33
Octaeicosane (C_{28})	2.61 ± 0.12
Triacontane (C_{30})	3.04 ± 0.03
Dotriacontane (C_{32})	18.0 ± 0.25
Tetratriacontane (C_{34})	1.20 ± 0.03

Hexatriacontane (C_{36})	2.44 ± 0.07
Total unknown compounds	18.2 ± 0.19

Nutritionally important components such as tocopherols (vitamin E) improve the stability of oils. Reported Data about the qualitative and quantitative composition of vitamin E is summarized in **Table III.5**. NP-HPLC technique was used to eliminate column contamination problems and allow the use of a general lipid extraction for tocopherols separation **[4]**. High levels of vitamin E were observed in the oil (3.35 g/kg). *β*-Tocopherol was the major component followed by α-tocopherol. Both tocopherols isomers comprised more than 87 % of total vitamin E content in the oil. *Y* and *δ*-tocopherols were detected in lower amounts accounting 14–16 % of the total vitamin E content.

Table III.5. Tocopherol composition (g/kg) of *C. phelypaea*

Compound	**(g/kg)**
α-Tocopherol	0.75 ± 0.02
β-Tocopherol	2.19 ± 0.07
ϒ-Tocopherol	0.14 ± 0.01
δ-Tocopherol	0.27 ± 0.01

References

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Conclusion

Conclusion

In the recent years, there has been increasing interest in the valuation of wild plants, especially local ones. So we conducted this phytochemical study which aims to valuing one of the Saharan plants which is *C. phelypaea* that grows in our local environment: Tamanrasset region.

Traditionally, *C. phelypaea* has been used as a medicinal herb with multiple biological activities. The resulted data *C. phelypaea* summarized up in this review have not only taught us its poly-phytochemical composition, with particularly high content of flavonoids, and multiple biological properties, but also prompted us to note that detailed and in depth biochemistry and molecular biology studies of *C. phelypaea.*

The chemical constituents of *Cistanche* species and their analysis methods have been described. Among the analyzed ones, the majority has been concentrated on PhGs which are known for various biological effects.

In this work we have extracted essential oil in lower yield, together with chloroform and ethanol extracts using the clevenger and soxhlet method, respectively. Further studies are