République Algérienne Démocratique et Populaire
Ministère de l'Enseignement Supérieure et de la Recherche Scientifique
Université de Bejaia

FACULTÉ DES SCIENCES DE LA NATURE ET DE LA VIE
DÉPARTEMENT DE BIOLOGIE PHYSICO-CHIMIQUE
Laboratoire de Biotechnologie Végétale et Ethnobotanique

THÈSE

Présentée par
Nassim BELKACEM

Pour l'obtention du grade de

DOCTEUR EN SCIENCES

Filière: Biologie
Option: Biochimie

Thème

Activité antioxydante, antibactérienne et cytotoxique d'extraits de *Cedrus atlantica* et effet des ultrasons sur leurs propriétés physicochimiques

Soutenue le: 08 Juillet 2021 Devant le Jury composé de:

Président	Mme. BEDJOU Fatiha	Professeur	Univ. de Bejaia
Rapporteur	Mme. KHETTAL Bachra	Professeur	Univ. de Bejaia
Examinateur	Mme. OUKIL Naima	MCA	Univ. de Bejaia
Examinateur	Mme. BOUDJELAL Amel	Professeur	Univ. de M'Sila
Examinateur	M. BENKHALED Abderrahim	MCA	Univ. de M'Sila
Examinateur	M. BASLI Abdelkader	MCA	Univ. de Skikda

Année universitaire 2020-2021

The people's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research
University of Bejaia

جامعة بجاية
Tasdawit n Bgayet
Université de Béjaïa

FACULTY OF NATURAL AND LIFE SCIENCES
DEPARTMENT OF PHYSICAL AND CHEMICAL BIOLOGY
Laboratory of Vegetal Biotechnologies and Ethnobotany

THESIS

PRESENTED BY
Mr. Nassim BELKACEM

In partial fulfillment of the requirements for the degree of

Doctor of Science

Domain: Biology
Option: Biochemistry

Theme

Antioxidant, antibacterial and cytotoxic activities of *Cedrus atlantica* extracts and the effect of ultrasound on their physicochemical properties

Defended on: July 8th, 2021

Board of Examiners:

Chairman	Mrs. BEDJOU Fatiha	Professeur	Univ. of Bejaia
Supervisor	Mrs. KHETTAL Bachra	Professeur	Univ. of Bejaia
Member	Mrs. OUKIL Naima	MCA	Univ. of Bejaia
Member	Mrs. BOUDJELAL Amel	Professeur	Univ. of M'Sila
Member	Mr. BENKHALED Abderrahim	MCA	Univ. of M'Sila
Member	Mr. BASLI Abdelkader	MCA	Univ. of Skikda

Academic year 2020-2021

DEDICATION

To

‘‘My Father’’

To

‘‘My Mother’’

To

My brothers and sisters

ACKNOWLEDGMENTS

First, all the thanks belong to God.

I would like to thank my supervisor Professor Khettal Bachra for accepting to advise me and for her help, support and guidance during this work as well as for providing an environment conducive for research. I am deeply grateful to all members of the jury, Professor F. Bedjou, Professor A. Boudjellal, Dr. R. Benkhaled, Dr. N. Oukil, and Dr. A. Basli, for carefull reading of the manuscript and agreeing to participate in the defense of this thesis.

I would also like to extend my sincere appreciation to Dr. Hatim AlKhatib for his help. There has been a significant contribution of Dr. Chiraz Soumia M. Amrine, Pr. Yasser Bustanji, Pr. Mohammad Hudaib, Mr. Harfi Tsoufik, Ms. Iman Amrani, Ms. Bashaer Abu-Irmaileh, Ms. Saida Mallouh, and Mr. Ismail Abaza to the evolution of this thesis. Without them, this dissertation would not have been realized. I wish to express my gratitude to them for their kind interest and helpful suggestions.

Thanks also for Naima and Saida, engineers of the physical and chemical biology laboratories. Many thanks to all of my friends and colleagues.

Thank you for you all.

Nassim Belkacem

Abbreviations

- AAPH: 2 2'-Azo-bis-(2-AmidinoPropane) di-Hydrochloride
- ABTS: 2,2-Azino-bis-(3-ethyl BenzoThiazoline-6-Sulfonic acid)
- AchE: Acetylcholinesterase
- ATCC: American Type Culture Collection
- ATP: Adenosine triphosphate
- Aβ1–42: Amyloid β peptide
- BchE: Butyrylcholinesterase
- BDFD: 3,4-bis(3,4-dimethoxy- phenyl)furan-2,5-dione
- BHA: Butylhydroxyanisol
- CFU: Colony Forming Unit
- CHIKV: Chikungunya virus
- CNS: Central Nervous System
- COX-2: Cyclooxygenase-2
- cP: Centipoise
- DMEM: Dulbecco's Modified Eagle Medium
- DMPD: N,N-dimethyl-ρ-phenylenediamine
- DMSO : Dimethyl sulfoxide
- DPPH: Diphenyl picrylhydrazyl
- EC50 : Median effective concentration
- EDTA: Ethylenediamine tetraacetic acid
- ELISA: Enzyme-linked immunosorbent assay
- EPM: Elevated plus maze
- EPS: Extracellular polymeric substances
- FAAH: Fatty acid amide hydrolase
- MAGL: Monoacylglycerol lipase
- FBS: Fetal bovine serum
- FRAP: Ferric reducing antioxidant power
- GABA: Gamma-aminobutyric acid
- GC/MS: Gas chromatography–mass spectrometry
- GSH: Reduced Glutathione
- GSH-Px: Glutathione peroxidase
- HSV-1: Herpes simplex virus type 1
- LDM: Light–dark model
- LOX: Lipoxygenase
- LPS: Lipopolysaccharide
- MBC : Minimum bactericidal concentration
- MDA: Malondialdehyde
- MHB : Muller-Hinton Broth
- MIC: Minimum inhibitory concentration
- mRNA: Messenger ribonucleic acid
- MSG: Monosodium glutamate

- MSSA: Methicillin-sensitive Staphylococcus aureus
- mTOR: Mammalian target of rapamycin
- MTT: 3(4,5-dimethyl-thiazoyl-2-yl)2,5 diphenyl-tetrazolium bromide
- MWM: Morris water maze
- NCTC: National Collection of Type Cultures
- NF –κB: Nuclear factor-κB
- NMDA: N-methyl-D-aspartic acid
- OECD: Organisation for Economic Co-operation and Development
- OFT: Open- field test
- OxHLIA: Oxidative haemolysis inhibition assay
- PBS: Phosphate-buffer saline
- PIS: Plantar incision surgery
- PTZ: Pentylenetetrazole
- RI: Retention Index
- ROS: Reactive Oxygen Species
- RPMI: Roswell Park Memorial Institute medium
- SOD: Superoxide Dismutase
- SPME: Solid-phase microextraction
- TAC: Total Antioxidant Capacity
- TBARS: Thiobarbituric Acid Reactive Substance
- TNF-α: Tumor necrosis factor alpha
- TPTZ: 2,4,6-Tris(2-pyridyl)-s-triazine
- WHO: World Health Organization

List of figures

List of tables

Table of contents

Introdution

Introduction

According to World Health Organization (WHO), a medicinal plant is defined as any plant containing in one or more of its parts substances of therapeutic interest or which are precursors in the synthesis of drugs (Kuete 2014). It is estimated that around 80% of the world's population is treated primarily with medicinal plants (Upton et al. 2011). Most of these traditional medications require the use of plant extracts and / or their active ingredients. Medicinal plants play an important role in Africa and in developing countries where the health system and the availability of drugs are limited (Yuan et al. 2016). Some plant extracts and by-products were used in Human health, example of resveratrol and quercetin in capsule and tablet dosage forms as food complements.

Plants are a potential source of several important active substances of therapeutic interest because of their ability to produce different active chemical entities through secondary metabolism during the growth stages of the physiological development or during periods of stress due to lack of nutrients or attack of microorganisms (Naik and Al-Khayri 2016). The secondary metabolites have a wide variety of chemical structures and can be simply classified into three main groups: Terpenoids (dominant in essential oils, composed almost entirely of carbon and hydrogen atoms), Phenolics (made from simple sugar, with benzene rings, hydrogen and oxygen) and compounds containing nitrogen and/or sulphur (Chinou 2008).

There is an increasing interest to find new plant-derived medicines as antioxidant, antibacterial and anticancer agents (Al-Dabbas et al. 2006). Several investigations support that oxidative stress is responsible for damage to vital cellular molecules such as DNA, proteins and lipids, thus playing a key role in the development of numerous pathologies including carcinogenesis (Al-Dabbas et al. 2006; Basli et al. 2017; Kumar et al. 2014). Phenolic compounds and essential oils may inhibit the production of reactive oxygen species (ROS) by different mechanisms, including radical scavenging by hydrogen donation, chelating metals responsible for the formation of free radicals and inhibition of enzymes like xanthine oxidase implicated in superoxide ion production (Fantini et al. 2015). Antioxidant agents may show potential anticancer effects (Kumar et al. 2014). Furthermore, this continuous need for new

entities of therapeutic interest is also due to multidrug resistance bacteria emergence, commercial drugs adverse effects and economic convenience (Rahman and Islam 2013).

Cancer is one of the world's leading causes of morbidity. According to GLOBOCAN 2020, 19.3 million new cancer cases were diagnosed worldwide, with approximately 10 million deaths in 2020 (Hyuna et al 2021). According to Amin et al. (2009), roughly half of drugs used are of plant origin; either directly derived from plants, or chemically modified natural entities. Many researchers are concentrating their efforts on developing new cancer-prevention strategies, one of which was that described by Sporn (1976) as the use of natural, synthetic, or biological agents to reverse, suppress, or prevent either the early stages of carcinogenesis or the progression of premalignant cells to invasive disease (Rather and Bhagat 2018). However, cancer prevention is one of the well-documented biological properties of plant extracts (Basli et al. 2017). In fact, polyphenols protect against human cancer cell lines and reduce the number or growth of tumors (Yang et al. 2001). In addition, essential oils have been shown to have anticancer properties via different mechanisms (Blowman et al. 2018)

The *Cedrus* genus belongs to the family of Pinaceae. It comprises four endemic species which are *C. deodara, C. brevifolia, C. libani, and C. atlantica*. These species had several traditional uses in their native countries. Herein, *C. atlantica* is originated from North Africa (Algeria and Morocco) (Fidah et al. 2016), well known, in part, for its Cedarwood oil and for its high wood quality (Dakir et al. 2005). The Cedarwood oil was investigated in previous studies for several biological activities. It demonstrated promising antioxidant (Inaam et al. 2015), antimicrobial (Benouaklil et al. 2017; Zrira and Ghanmi 2016), antifungal (Aberchane et al. 2003), antiviral (Chao et al. 2000), anti-inflammatory (Baylac and Racine 2003), analgesic (Emer et al. 2018; Martins et al. 2015), anticancer (Saab et al. 2012 a and b), and anticholinesterase activities. Furthermore, the essential oil of *C. atlantica* showed to have mollucidal (Lahlou 2003), larvicidal (Zoubi et al. 2017), acaricidal (Gene Lim et al. 2011), insecticidal (Ainane et al. 2019; Choi et al. 2003) and repellent properties (Martynov et al. 2019), as well as an activity against phytopathogenic agents (Popović et al. 2018).

Literature shows a lack of data on phenolic compounds from *C. atlantica* (Fadel et al. 2016; Hofmann et al. 2020). Yet, to the best of our knowledge, there was no study published about phenolic compounds extracted from *C. atlantica* branches.

The present study aims to contribute into investigating for the first time, the chemical composition of the essential oil of the Algerian *C. atlantica* cones. We also profiled the phenolic contents of the organic extracts from the branches. Furthermore, we evaluated the antioxidant and antibacterial activities *in vitro*, as well as their cytotoxic effects against MCF-7 breast cancer cell line. Moreover, an acute toxicity study has been conducted on the crude extract from branches according to the OECD recommendations (OECD 2001). Finally, the ultrasonic power has been applied on the methanolic extract in the objective to enhance the physicochemichal properties.

Part I

Review on Cedrus genus

I.1. *Cedrus* genus description

The genus *Cedrus* comprises four true cedars from the pinaceae family, with geographically distinct distribution (Quézel and Santa 1962, 1963; Toth et al. 2005). It includes one Himalayan species, *Cedrus deodara* G. Don native to Afghanistan, Nepal and India; as well as three Mediterranean mountain species, including *Cedrus atlantica* (Endl.) Manetti, from Morocco and Algeria, *Cedrus libani* A. Rich, from Lebanon, Syria and Turkey; and *Cedrus brevifolia* Henry, from Cyprus Island (**Fig. 1**).

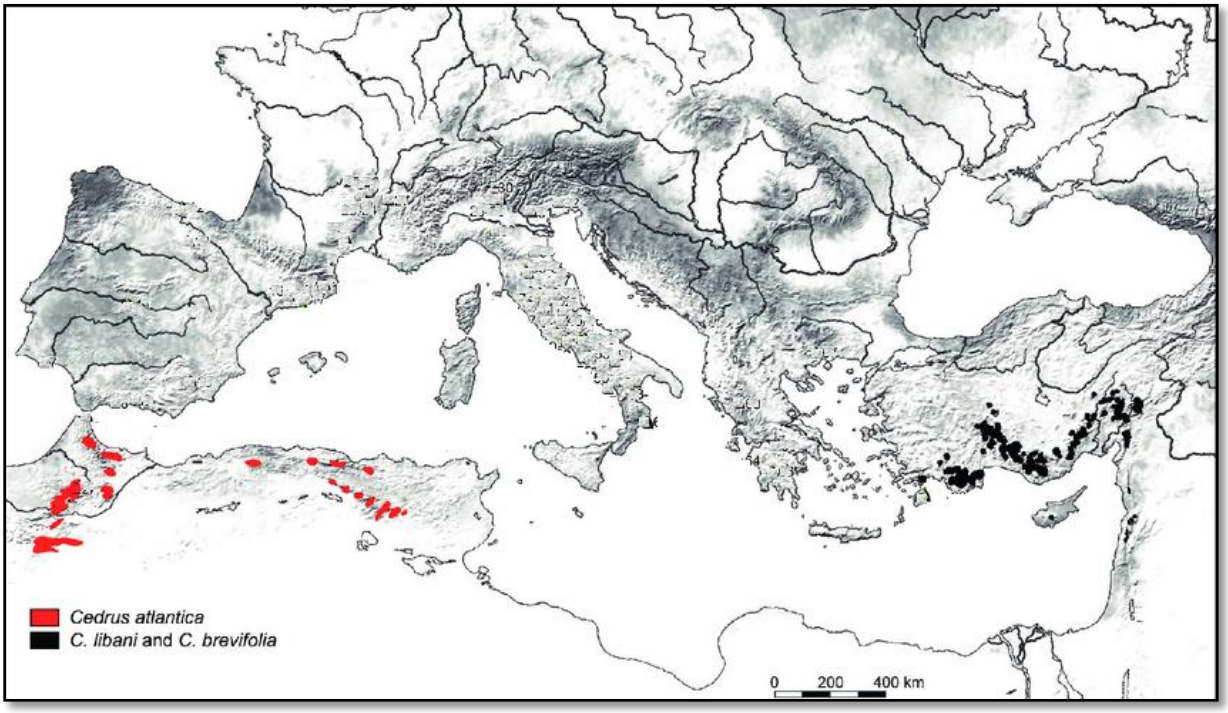

Figure 1. Geographic location of Cedar species native to the Mediterranean basin (Adapted from Magri 2012)

There was no genetic marker found to distinguish between the four cedar species (Pijut 2000; Savill and Wilson 2015). According to Qiao et al. (2007) and Dagher-Kharrat et al. (2007), *C. deodara* was the first to diverge, and *C. atlantica* descended from the common ancestor of *C. libani* and *C. brevifolia*, which share a strong genetic similarity. Some taxonomic studies using genetic markers have classified these latter species as one (Fady et al. 2000; Karam et al. 2019; Sabatier et al. 2003; Scaltsoyiannes 1999). Furthermore, using biochemical markers, *C. atlantica* and *C. libani* were found to be poorly differentiated from each other (Panetsos et al. 1992). Various characteristics distinguish these four species,

including needle size, cone length and diameter, seed length, pollination period, and maturity duration (Farjon 1990; Toth et al. 2005).

I.2. Biological activities of *Cedrus* species other than *C. atlantica*

I.2.1. C. deodara

C. deodara was the most investigated cedar species for the biological activities of its extracts. In fact, several studies on the antioxidant, antimicrobial, antitumor, anti-inflammatory, analgesic, immunomodulatory, anti-diabetic, anti-hyperlipidemic, and anti-ulcer activities have been published. In addition, anxiolytic, anti-depressant, anti-epileptic, and memory enhancing effects on the central nervous system (CNS) were investigated. Furthermore, there were also reports of thrombolytic, spasmolytic, anti-urolithiatic, macrofilaricidal, larvicidal, antileshmanial, and acaricidal activities.

I.2.1.1. Antioxidant activity

Several studies on the antioxidant activities of *C. deodara* extracts have been reported in literature (**Table I**). The essential oils hydrodistilled from needles demonstrated a strong scavenging effect against $ABTS^{\bullet+}$, $DPPH^{\bullet}$, superoxyde, and hydroxyl free radicals, as well as a strong lipid peroxydation reducing power with an IC_{50} value of 0.79±0.75 µg/mL (Zaman et al. 2018; Zeng et al. 2012). Similarly, essential oil extracted from the rhizome demonstrated significant free radical scavenging activity against $DPPH^{\bullet}$ (Chen et al. 2020). Furthermore, the essential oil hydrodistilled from the stem significantly increased the glutathion (GSH) level in the brain (Chaudhary et al. 2014). Also, the organic extracts of wood and needles demonstrated a potential antioxidant activity against, $DPPH^{\bullet}$, $ABTS^{\bullet+}$, and superoxide anion free radicals (Jain et al. 2015; Kadam et al. 2021; Liang et al. 2014; Qian-Da et al. 2020; Yu et al. 2019). Yasmeen et al. (2015) demonstrated that extracts from stem and needles using polar solvents enclosed potential antioxidants. The hydro-methanolic extract from needles had a potential oxidative stress down-regulator effect by increasing the activities of superoxide dismutase (SOD), catalase (CAT), and glutathion peroxydase (GSH-Px), and decreasing the malondialdehyde (MDA) level in liver (Talluri et al. 2018; Wu et al. 2015). According to Chaudhary et al. (2014), the choloroform extract had the best antioxidant activity by lowering MDA levels.

Table I: Antioxidant activity.

Plant part	Extract	Extraction method	Major compounds	Methods used / Targets	Properties / Effects	Refrences
Needles	Essential oil	Hydro-distillation	*α*-terpineol, linalool, limonene, anethole and caryophyllene.	Scavenging free radicals: $ABTS^{\bullet+}$, $DPPH^{\bullet}$, Superoxyde and Hydroxyl. Lipid peroxidation. Reducing power assays. Metal chelating activity	IC_{50}: 0.36 ± 0.28, 0.53 ± 0.21, 0.69 ± 0.35 and 1.29 ± 0.16 *μ*g/ml IC_{50}: 0.79±0.75 *μ*g/ml IC_{50} : 28.16 μg/ml	(Zeng et al. 2012b) (Zaman et al. 2018)
	Ethanolic extract	Maceration	Methyloconiferin, ferulic acid-β-D-glucoside and wikstromol	$DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radical-scavenging assays. Reactive oxygene from hydroxyl radica and hydrogene peroxyde. Lipide peroxydation inhibition. Reduce ferric ion.	IC_{50}: 32.40±0.76, 0.48±0.01, 1.73±0.01, 89.48±0.54 and 9.85±0.14 μg/ml, respectively	(Qian-Da et al. 2020).
	Methanolic extract	Maceration	Protocatechuic acid, *2R*,*3R*-dihydromyricetin, massonianoside B and myricetin-3-*O*-β-D-glucopyranoside	$DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radical-scavenging assays	IC_{50} values of 24.33 and 35.94 μg/ml, respectively.	(Yu et al. 2019)
				Oxidative haemolysis inhibition assay (OxHLIA) Protective effect against CCl4-induced lipid peroxidation in mice	The extracts improved the activities of SOD, CAT and GSH-Px, and decreased the MDA.	(Wu et al. 2015)
Wood	Organic extracts	Maceration	Flavonoids and tannins	$DPPH^{\bullet}$,$ABTS^{\bullet+}$ free radicals, and superoxide anion scavenging assays.	IC_{50} from 61.89 to 122.42 μg/ml.	(Jain et al. 2015)

(Continued)

	Chloroform extract	Maceration	Atlantone, himaphenolone, atlantolone, deodardione and atlantone-2,3-diol	Total antioxidant capacity (TAC) Reducing power ability	TAC value of 187.67 ± 11.78 mg AAE/g	(Chaudhary et al. 2015)
	Aqueous extract	Maceration	ND	Estimation of Reduced Glutathione Levels-GSH Estimation of Glutathione-s-Transferase Levels-GST Estimation of Superoxide Dismutase (SOD) Levels Estimation Level of Thiobarbituric Acid Reactive Substance Levels (TBARS) Estimation of Catalase Levels (CAT) Estimation of Protein Levels	The aqueous extract significantly reduced the oxidative stress induced by alloxan.	(Jain et al. 2014)
Stem	Organic extracts	Soxhlet	Flavonoids, terpenoids and tannins.	Estimation of Malondialdehyde MDA Estimation of glutathione (GSH)	Chloroform extract had the best antioxidant activity. MDA levels decreased. GSH significantly increased in brain.	(Chaudhary et al. 2014)
	Essential oil	Hydro-distillation	Terpenoids			
Various parts	Aqueous and hydro-methanolic extracts	Maceration	ND	Levels of thiobarbituric acid reactive species Levels of superoxide dismutase Levels of catalase activity Levels of reduced glutathione	The extracts had potential oxidative stress down-regulator effect.	(Talluri et al. 2018)

ND : Not determined.

The polysaccharides extracted from needles via aqueous extraction demonstrated remarkable antioxidant activity (Zeng et al. 2014). Also, the aqueous extract of wood significantly reduced the oxidative stress caused by alloxan (Jain et al. 2014).

I.2.1.2. Antimicrobial activity

Several studies on the antimicrobial potential of *C. deodara* extracts have been published (**Table II**). The essential oils were found to have promising antibacterial and antifungal properties. In fact, the essential oil hydrodistilled from the needles had a strong bactericidal effect against typical food-borne microorganisms, which could be attributed to the induction of cytoplasmic outflow and plasmolysis mechanisms observed using transmission electron microscopy (Zeng et al. 2012b). According to Wu et al. (2016)**,** a phenolic compound isolated from *C. deodara* needles damaged the cytoplasmic membrane of *S. aureus*, resulting in significant membrane hyperpolarization and loss of membrane integrity, and acted as a potential bacterial biofilm inhibitor by affecting the attachment phase of biofilm formation through targeting sortase A (Wu et al. 2019). The essential oil hydrodistilled from wood demonstrated antibacterial activity against food and plant pathogens (Ramadass et al. 2019; Truchan et al. 2019). Several studies revealed that the essential oil had antifungal activity against all tested strains (Kumar et al. 2020; Mohd et al. 2015). However, the root oil showed zone of inhibition against *A. fumigatus* at a concentration of 150 µg/disc, but no antifungal activity against *C. albicans* at the same concentration (Parveen et al. 2010). Chaudhary et al. (2012b) and Verma et al. (2011) found low antifungal activity against the tested strains. According to Tarranum et al. (2014), *C. deodara* essential oils had no antifungal activity against *Aspergillus niger* MTCC281, and *Candida albicans* MTCC183.

The hydro-ethanolic extracts from needles inhibited the extracellular polysaccharides (EPS) of the *S. mutans* biofilm and had a MIC value of 6.25 µg/µl (Zhang et al. 2020). *S. aureus* was the most sensitive strain to the hydro-methanolic extracts of needles, which inhibited biofilm formation and disintegrated the complex biofilm architecture (Wu et al. 2018a; Yu et al. 2019). Similarly, dihydromyricetin from needles significantly reduced *S. aureus* biofilm biomass and biofilm cell metabolic activity (Wu et al. 2018b). The organic extracts of wood demonstrated effective antibacterial activity against *E. coli* (Jain et al. 2019), and *S. aureus* (Yasmeen et al. 2015). In contrast, Kumar et al. (2014a) demonstrated that ethanolic and chloroformic extracts of wood had no inhibition against tested strains.

Table II: Antimicrobial activity.

Plant part	Extract	Extraction method	Major compounds	Antimicrobial activity	Properties / Effects	References
Needles	Essential oil	Hydro-distillation	3-p-trans-coumaroyl-2-hydroxyquinic acid	Antibacterial activity	MIC: 2.5 mg/ml (*B. cereus*)	(Wu et al. 2016)
				Antibiofilm inhibition activity	Inhibited *S.aureus* and *E.coli* biofilm formation	(Wu et al. 2019; Zaman et al. 2018)
			α-terpineol, linalool, limonene, anethole and caryophyllene.	Antibacterial activity	Induction of cytoplasmic outflow and plasmolysis.	(Zeng et al. 2012b)
				Antifungal activity	*R. oryzae* the most susceptible strain.	
	Ethanolic extract	Maceration	Methyloconiferin, ferulic acid-β-D-glucoside and wikstromol	Antibacterial activity	MIC: 6.25 µg/µl (*S.mutans*)	(Zhang et al. 2020)
	Methanolic extract	Maceration	2R,3R-Dihydromyricetin	Biofilm inhibition activity	Inhibited and disintegrated the complex biofilm architecture.	(Wu et al. 2018a)
				Antibacterial activity	Decreased the intracellular ATP of *S. aureus* cells.	(Wu et al. 2018b)
	Aqueous extract	Maceration	ND	Antibacterial activity Antifungal activity	*S. aureus* most sensitive bacterial strain *C. albican*s and *A.niger* were found resistant.	(Arshan et al. 2020)

(Continued)

Stem	Essential oil	Hydro-distillation	Terpenoids, and phenols	Antibacterial activity	EO and chloroform extracts showed the highest antibacterial activities.	(Chaudhary et al. 2012b)
	Organic extracts	Soxhlet	Flavonoids and tannins	Antifungal activity	Less antifungal activity was observed.	(Chaudhary et al. 2012a)
Wood	Essential oil	Hydro-distillation	cedrol, widdrol, thujic acid and β- thujaplicin	Antibacterial activity	The greatest inhibition zone of 23.8 mm against MSSA.	(Truchan et al. 2019)
	Organic extracts	ND	Phenols, tannins, phytosterols, flavonoids and terpenoids.	Antibacterial activity	Efficient against *E.coli*	(Jain et al. 2019)
Sawdust	Hexane	Maceration	Atlantones	Antifungal activity	Effective against *Aspergillus flavus, A. niger, A. ochracoeus, A. parasiticus,* and *A. sydowii.*	(Chaudhary et al. 2012a)
Root	Essential oil	ND	Trans-atlantone and allo-himachalol	Antifungal activity	Effective against *A. fumigatus.* Not active against *C.albicans*	(Parveen et al. 2010)
Bark	Aqueous extract	Maceration	ND	Antiviral activity	CHIKV inhibition in the plaque reduction assay format.	(Raghavendhar et al. 2019)

ND : Not determined.

The aqueous extract from needles demonstrated the greatest zone of inhibition against *E. coli* and *S. aureus* (Arshan and Gul 2020; Ramzan et al. 2021). Shikimic acid isolated from needles was found to be effective against *S. aureus* through interactions with membrane proteins and lipids (Bai et al. 2015). The hexane extract from sawdust showed an antifungal activity against *A. flavus*, *A. niger*, *A. ochracoeus*, *A. parasiticus*, and *A. sydowii* (Chaudhary et al. 2012a). Likewise, the ethanolic and methanolic extracts from needles exhibited antifungal activity (Joshi et al. 2018; Metreveli et al. 2020) with a strongest fungicidal effect recorded for *P. infestans* (Metreveli et al. 2020). However, Chaudhary et al. (2012) reported that the organic extracts from the stem had less antifungal activity. On the other hand, in the plaque redution assay format, the aqueous extract from the bark demonstrated antiviral activity by inhibiting chikungunya virus (CHIKV) (Raghavendhar et al. 2019).

I.2.1.3. Antitumor activity

The antitumor studies of *C. deodara* samples were summarized in **Table III**. The essential oils hydrodistilled from the bark induced apoptosis in human colon cancer cell lines (HCT-116 ans SW-620) by inhibiting nuclear factor kappa B (Bhagat et al. 2020). The wood oil was cytotoxic to K562 human chronic myelogenous leukemia cells, with an IC_{50} value of 37.09±1.4 mg/ml (Saab et al. 2012b). The ethanolic extract from the root exhibited a cytotoxic activity against a panel of cancer cell lines, with an IC_{50} value of 157.5 µg/ml against MCF-7 breast cancer cells (Suryavanshi et al. 2014). Similarly, the IC_{50} values for hydro-ethanolic extracts of needles and wood were 38.82±1.74 µg/ml, 114.12 µg/ml, and 20 µg/mL, respectively, against A549, Hep G2, and MCF-7 cancer cell lines (Gaidhani et al. 2013; Shi et al. 2016; Shi et al. 2019). Furthermore, a chloroform extract from wood was found highly cytotoxic to a panel of breast, CNS, cevix, colon, liver, and prostate cancer cells (Singh et al. 2007).

I.2.1.4. Anti-inflammatory, analgesic and immunomodulatory activities

The anti-inflammatory, analgesic, and immunomodulatory activities of *C. deodara* samples were shown in **Table IV**. The essential oils inhibited the production of inflammatory cytokines in TPA-induced ear oedema by inhibiting COX-2/TNF-α/NF- κβ activation (Chen et al. 2020), and had a lipoxygenase inhibitory effect with an IC_{50} value of 16.5±1.6 µM (Baylac and Racine 2003).

Table III: Antitumor activity.

Plant part	Extract	Extraction method	Major compounds	Methods used / Targets	Properties / Effects	References
Needles	Ethanolic extract	Ethanol hot refluxing	Lignans	A549, HeLa, MKN 45, HepG2 and HT-29 cells	A549 cells were found the most sensitive with IC_{50}: 39.82±1.74 µg/ml	(Shi et al. 2019)
		Maceration	Myricetin,quercetin, kaempferol and isorhamnetin	HepG2, HeLa, MKN28, SHG-44 and A549 cells	HepG2 were the most sensitive with IC_{50} value of 114.12 µg/ml	(Shi et al. 2016)
Bark	Essential oil	Hydro-distillation	2-(tert-Buyl)-6-methyl-3-(2-(trifluoromethyl) benzyl)imidazo [1,2-a]pyridine; 9- Octadecenoic acid and Copaene	HCT-116 and SW-620 cells.	Induces Apoptosis in by Inhibiting Nuclear Factor kappa B.	(Bhagat et al. 2020)
Root	Ethanolic extract	Maceration	Wikstromol, matairesinol and dibenzyl butyrolactol	MCF-7, MDA MB-231, HEK- 293, and HaCaT cells	IC_{50}: 157.5 µg/ml (MCF-7).	(Suryavanshi et al. 2014)
Wood	Essential oil	Hydro-distillation	ND	K562 cells	IC_{50}: 37.09±1.4 mg/ml	(Saab et al. 2012b)

(Continued)

	Hydro-ethanolic extract	Maceration	ND	MCF-7, Colo-205, Hop-62, HT-29, SiHa, DWD, T24, PC3, A-549, ZR-75-1, A-2780, DU-145, and K562	IC_{50}: 20 µg/ml (MCF-7)	(Gaidhani et al. 2013)
	Chloroform extract	Soxhlet	Wikstromol, matairesinol and dibenzyl butyrolactolignan	**Breast:** MCF-7, T-47 D **CNS:** SF-539 SKNMC, IMR-32, SKNSH SNB-78; **Cervix:** Hela, SiHa ; **Colon:** COLO-205, HCT-15, HT-29, SW-620; **Liver:** HEP-G2 **Prostate:** DU-145, PC-3.	 IC_{50}: 15.6 µg/ml IC_{50}: 9.78 µg/ml IC_{50}: 29.09 µg/ml IC_{50}: 16.4 ng/ml IC_{50}: 52.74 µg/ml IC_{50}: 41.67 µg/ml IC_{50}: 28.35 µg/ml IC_{50}: 39 µg/ml IC_{50}: 8.3 µg/ml IC_{50}: 5.4 µg/ml IC_{50}: 21.05 µg/ml IC_{50}: 12.24 µg/ml IC_{50}: 40.9 µg/ml IC_{50}: 116.03 µg/ml IC_{50}: 41.2 µg/ml IC_{50}: 3.52 µg/ml	(Singh et al. 2007)
ND	Extracts	ND	ND	BHK-21 cells	Cell growth inhibition	(Chauhan and Joshi 2018)

Table IV: Anti-inflammatory, analgesic and immunomodulatory activities.

Plant part	Extract	Extraction method	Major compounds	Methods used / Targets	Properties / Effects	References
Needles	Organic extracts	Soxhlet	ND	**Immunomodulatory activity** *-Nitric oxide (NO) produced by mammalian mononuclear cells*	Showed potential immuno-modulatory effect.	(Narayan et al. 2017)
Wood	Essential oil	Hydro-distillation	ND	**Immunomodulatory activity** *-Neutrophil adhesion test in rats Arthus reaction in mice.* *-SRBC-induced delayed type hypersensitivity and hemagglutination anti-body titer in mice.* *-Oxazolone-induced contact hyersensitivity in mice.*	Inhibited humoral and cell-mediated immune responses.	(Shinde et al. 1999b)
				Anti-inflammatory activity *-Effect on compound 48/80 induced pedal oedema in rats and degranulation of isolated rat peritoneal mast cells.* *-Effect on lipoygenase enzyme activity.*	Significant inhibition at 200 µg/ml	(Shinde et al. 1999a)
				-Carrageenan-induced pedal oedema in rats *-Adjuvant induced arthritis in rats*	Significant inhibition at 50 and 100 mg/kg	(KT et al. 1998; Shinde et al. 1999c)

(Continued)

				Analgesic activity *-Acetic acid induced writhing response in mice* *-Hot plate reaction time method in mice* *-Tail clip method*	Centrally and peripherally mediated activity	
Stem	Ethanol Methanol Aqueous	Soxhlet	Polyphenols	**Anti-inflammatory activity** *-Carrageenan-induced paw oedema model*	Hydroalcoholic extracts showed better inhibition than the aqueous extract.	(Pandey 2018)
Bark *(Ayurvedic formulation)*	Aqueous extract	Decoction	ND	**Anti-inflammatory activity** *-Effect of MRQ on carrageenan-induced paw oedema* *-Effect on heat-induced haemolysis* *-5-Lipoxygenase inhibition assay* **Analgesic activity** *-Determination of analgesic effects in rats* *-Effects on RA patients*	Possess significant anti-inflammatory and analgesic activities.	(Thabrew et al. 2003)
Rhizome	Essential oil	Steam distillation	Thujopsene, α-cedrene, cedrol and (+)-cuparene	**Anti-inflammatory activity** *-TPA-induced ear oedema by inhibiting COX-2/TNF-α/NF-κB activation.*	Inhibited the production of inflammatory cytokines	(Chen et al. 2020)
ND	Essential oil	ND	ND	**Anti-inflammatory activity** *Lipoxygenase inhibitory effect*	IC_{50} value of 5±0.5 ppm (16.5±1.6 µM)	(Baylac and Racine 2003)

ND : Not determined.

The essential oil hydrodistilled from wood inhibited carrageannan-induced pedal oedema and adjuvant-induced arthritis in rats with a significant inhibition at 50 and 100 mg/kg body weight (KT et al. 1998, Shinde et al. 1999c); and a significant inhibition at 200 µg/mL in compound 48/80 induced either pedal oedema in rats or degranulation of isolated rat peritoneal mast cells (Shinde et al. 1999a). In the carrageenan-induced paw edema model, hydro-alcoholic extracts from the stem inhibited paw oedema better than the aqueous extract (Pandey 2018). Shinde et al. (1999c) found that the essential oil hydrodistilled from wood had centrally and peripherally mediated analgesic activity in the acetic acid induced writhing response and hot plate reaction time method in mice; on the other hand, KT et al. (1998) found that the oil had no significant analgesic effect in the acetic acid induced writhing syndrome in mice and in the tail clip method. The aqueous extract from an Ayurvedic formulation containing *C. deodara*, had a significant anti-inflammatory effect on rheumatoid arthritis patients (Thabrew et al. 2003).

The wood oil exhibited immunomodulatory properties, inhibiting both humoral and cell-mediated immune responses (Shinde et al. 1999b). Similarly, organic extracts of needles demonstrated a potential immunomodulatory effect by lowering the nitric oxide (NO) produced by mammalian mononuclear cells (Narayan et al. 2017).

I.2.1.5. Effects on the central nervous system

The *C. deodara* samples exhibited several central nervous system activities (**Table V**). In fact, an ethanolic extract of wood demonstrated anticonvulsant activity via GABAminergique transmission inhibition (Dhayabaran et al. 2014). At doses of 100 mg/kg and 200 mg/kg, the organic extracts demonstrated anticonvulsant activity in maximal electron hock induced convulsion and by estimating GABA levels in the rat brain (Dhayabaran et al. 2012). In contrast, the chemoshok test revealed that the essential oil hydrodistilled from wood lacked anticonvulsant activity (KT et al. 1998). Tanwar et al. (2019) demonstrated antiepileptic activity of the essential oil. Kumar et al. (2014) showed that an ethanolic extract from wood had an anti-depressant effect by significantly reducing immobility time at 100 mg/kg i.p, in tail suspension and fored swim tests. At 100 mg/kg and 200 mg/kg, organic extracts from wood demonstrated promising anxiolytic activity in elevated plus maze (EPM) and light-dark models in mice (Dhayabaran et al. 2010; Dhayabaran et al. 2012). In addition, the chloroform extracted from the stem had the best memory-enhancing activity in the Morris water maze (MWM) behavioural test (Chaudhary et al. 2014).

Table V: Central nervous system effects.

Plant part	Extract	Extraction method	Major compounds	Biological activity / Target	Properties / Effects	References
Needles	Essential oil	Solid-phase microextra-ction (SPME)	β-myrcene, D-limonene, α,β- pinene and β-caryophyllene	**Effects on human physiology and psychology** *-Tests of human physiological indicators* *-Test of human psychological indicators*	Smelling the essential oil produced relaxing effects.	(Song et al. 2016)
	Alcoholic extract	Maceration	Cedrin	**Anti-neurotoxicity activity** *-Protection of PC12 cells against neurotoxicity induced by Aβ1–42*	Inhibit apoptosis induced by $A\beta_{1-42}$ in PC12 cells.	(Zhao et al. 2018)
Wood	Essential oil	Hydro-distillation	Pyrrolone-fused benzosuberene compounds	**Antiepilepsy activity** *-Effect on PTZ-induced clonic seizures.* *-Effect on mRNA levels (PI3K/AKT/mTOR pathway).*	Pyrrolone-fused benzosuberene had potential antiepileptic activity.	(Tanwar et al. 2019)
	Ethanolic extract	Maceration	3,4-bis(3,4-dimethoxy-phenyl)furan-2,5-dione (BDFD)	**Anti-depressant effect** *-Tail suspension test* *-Forced Swim test* *-Estimation of brain monoamine after BDFD treatment*	Showed a significant decrease in immobility time at 100 mg/kg**,** *i.p.*	(Kumar et al. 2014b)

(Continued)

				<u>Anticonvulsant activity</u> *-N-methyl-D-aspartic acid (NMDA)-induced lethality test* *-Estimation of brain gamma-aminobutyric acid (GABA).*	Anticonvulsant activity produced through inhibitory GABAminergic transmission.	(Dhayabaran et al. 2014)
				<u>Anxiolytic activity</u> *-Elevated plus maze (EPM),* *-Open- field test (OFT) in mice.* *-Light–dark model (LDM) in mice*	BDFD showed promising anxiolytic activity.	(Dhayabaran et al. 2012)
	Organic extracts	Soxhlet	Tannins and phenolic compounds.	**<u>Anxiolytic activity</u>** *-Elevated plus maze model* *-Light-dark model* *-Locomotor activity* **<u>Anticonvulsant activity</u>** *-Maximal electroshock induced convulsion* *-Estimation of GABA levels in rat brain*	Doses of 100 mg/kg and 200 mg/kg exhibited anxiolytic and anticonvulsant activity.	(Dhayabaran et al. 2010)
Stem	Organic extracts	Soxhlet	Flavonoids, terpenoids and tannins.	**<u>Memory-enhancing activity</u>** *-Behavioral testing: Morris water maze (MWM)*	The chloroform extract had the best memory-enhancing activity.	(Chaudhary et al. 2014)
	Essential oil	Hydro-distillation	Terpenoids and phenols.			

I.2.1.6. Anti-diabetic, anti-obesity and anti-hyperlipidemic activities

The anti-diabetic, anti-obesity and anti-hyperlipidemic activities of *C. deodara* samples were shown in **Table VI**. The essential oil hydrodistilled from the cones inhibited α-amylase with an IC_{50} value of 34.47±0.54 μg/ml (Xu et al. 2017). Organic wood extracts reduced blood glucose levels and demonstrated promising anti-hyperglycemic activity (Devmurari et al. 2010; Jain et al. 2014; Singh et al. 2013). Taxifolin isolated from *C. deodara* restored caveolin 1/NF-κβ signaling-related mRNA and proteins in rats with streptootocin (STZ) induced diabetic nephropathy (Zhao et al. 2018).

The petroleum ether extract from wood significantly reduced body weight at 200 mg/kg and 400 mg/kg (Pradhan et al. 2016). Similarly, needle polysaccharides inhibited fat accumulation, resulting in an anti-obesity effect (Liu et al. 2018). Organic wood extracts also had an anti-hyperlipidemic effect (Patil et al. 2011).

I.2.1.7. Anti-ulcer activity

C. deodara demonstrated an anti-ulcer activity (**Table VII**). In fact, the essential oils hydrodistilled from wood decreased the volume of gastric fluid and increased the pH (Kumar et al. 2011). The essential oil derived from the root possessed anti-ulcer properties while having no effect on kidney or liver tissues (Mashaal et al. 2020).

Table VI: Anti-diabetic, anti-obesity and anti-hyperlipidemic activities.

Plant part	Extract	Extraction method	Major compounds	Biological activity / Target	Properties / Effects	References
Cones	Essential oil	Hydro-distillation	Cyclofenchene, β-pinene, β-myrcene. and D-limonene.	**Anti-diabetic activity** *α-amylase inhibition*	IC_{50}: 34.47 ± 0.54 μg/ml	(Xu et al. 2017)
Needles	ND	ND	Pine needle polysaccharides	**Anti-obesity activity** *Effects on cell differentiation and fat metabolism in 3T3-L1 cells*	Fat accumulation inhibition.	(Liu et al. 2018)
Wood	Ethanolic and aqueous extract	Maceration	Phenolics, tannins and flavanoids.	**Anti-diabetic activity** *-Antihyperglycemic action in alloxan induced rats.*	Blood glucose significantly reduced.	(Jain et al. 2014)
	Organic extracts	Soxhlet	Flavonoids and tannins.	**Anti-hyperlipidemic effect** *-Induction of MSG-induced obesity -Biochemical Parameters*	Biochemical parameters significantly decreased.	(Patil et al. 2011)
	Petroleum ether extract	Soxhlet	ND	**Anti-obesity activity** *-Reduces body weight in alloxan -induced diabetic rats*	Effective at 200 mg/kg and 400 mg/kg.	(Pradhan et al. 2016)
Bark	Organic extracts	Soxhlet	Polyphenols	**Anti-diabetic activity** *Oral Glucose Tolerance Test.*	Effective at 500 mg/kg	(Singh et al. 2013)
ND	ND	ND	Taxifolin	**Anti-diabetic activity** *Effects on streptozotocin -induced diabetic nephropathy in rats*	Restored the levels of Caveolin-1/NF-κB signaling-related mRNA and proteins	(Zhao et al. 2018)

Table VII: Anti-ulcer activity.

Plant part	Extract	Extraction method	Major compounds	Methods used / Targets	Properties / Effects	References
Wood	Eseential oil	Hydro-distillation	Terpenoids, phenols, alcohol and ketone.	-Gastric secretion in pylorus-ligated rats -Gastric lesions induced by ethanol -Histopathological evaluation	The volume of gastric fluid decreased. The pH of gastric fluid increased. The number of ulcer, ulcer score and ulcer index decreased.	(Kumar et al. 2011)
Roots	Eseential oil	ND	ND	-Histopathological effects in ethanol induced ulcer on rats (Wistar Strain).	The essential oil had anti-ulcerproperties without effecting kidney and liver tissues.	(Mashaal et al. 2020)

ND : Not determined.

I.2.1.8. Other biological activities

C. deodara samples exhibited several other biological activities (**Table VIII**). The thrombolytic activities of the essential oils hydrodistilled from stem and needles ranged from 22.86±0.7% to 32.64±0.5% (Zaman et al. 2018). The petroleum ether extract from wood was found to be effective in preventing sodium oxalate (NaOx)-induced nephrolithiasis (Ramesh et al. 2010). In addition, the essential oil derived from roots demonstrated a nephroprotective effect in a rat model of cyclophosphamide-induced nephrotoxicity (Kazi 2017). In antagonizing epinephrine-induced contraction of the guinea pig seminal vesicle, the petroleum ether extract from wood demonstrated more potent *in-vivo* spasmolytic activity than papaverine (Kar et al. 1975). Organic extracts of needles demonstrated potent antileishmanial activity at doses ranging from 25 µg/ml to 200 µg/ml (Narayan et al. 2017). The essential oil demonstrated promising activity against the Malaria vector (*Anopheles culicifacies*) (Kala et al. 2020), *Tenebrio molitor* (LC_{50} value of 3.41 percent) (Buneri et al. 2019), *Tetranychus urticae* Koch (LC_{50} value of 113.44 mg/l) (Reddy and Dolma 2018), and *Plutella xylostella* (L.) (LC_{50} value of 1.08 mg m/l) (Reddy et al. 2016). The methanolic extract of wood demonstrated promising macrofilaricidal activity (Nisha et al. 2007). In addition, the ethanolic extract of needles demonstrated significant protistocidal activity against *Paramecium caudatum* (Metreveli et al. 2020).

I.2.2. C. libani

C. libani samples were investigated for a number of activities. Several studies on the antioxidant, antimicrobial, antitumor, anti-inflammatory, wound healing, anti-diabetic and anti-ulcer activities have been published. Furthermore, cholinesterase inhibitory, antiparasitic, larvicidal, and insecticidal activities have also been evaluated.

I.2.2.1. Antioxidant activity

The studies on the antioxidant activity of *C. libani* samples were shown in **Table IX**. The essential oil hydrodistilled from wood demonstrated significant anti- $DPPH^{\bullet}$ free radical activity (Venditti et al. 2020). Likewise, cone methanolic extract scavenged the free radical $DPPH^{\bullet}$ with IC_{50} values ranging from 0.35 to 17.21 µg/ml (Semerci et al. 2020). With a metal chelation capacity of 58.04±0.70 percent, the shoot ethyl acetate extract was the most effective (Senol et al. 2015).

Table VIII: Other biological activities.

Plant part	Extract	Extraction method	Major compounds	Biological activity / Target	Properties / Effects	References
Needles	Essential oil	Hydro-distillation	ND	**Thrombolytic Activity** Clot lysis observation	Ranged from: 22.86±0.7 to 32.64±0.5 %.	(Zaman et al. 2018)
				Acaricidal activity *Tetranychus urticae* Koch	LC_{50}: 113.44 mg/l	(Reddy and Dolma 2018)
	Organic extracts	Soxhlet	ND	**Antileishmanial activity** *Leishmania donovani*	Active dose: 25-200 µg/ml	(Narayan et al. 2017)
	Ethanolic extract	Maceration	ND	**Protistocidal activity** *Paramecium caudatum*	Strong protistocidal activity	(Metreveli et al. 2020)
	Methanolic extract			**Larvicidal activity** *Anopheles stephensi* (Antimalarial activity)	LC_{50}: 81.89 ppm	(Khanavi et al. 2013)
	Aqueous extracts			**Preservative effect** Thiobarbituric acid reacting substances (TBARS) value	The lipid oxidative stability was improved.	(Mahajan et al. 2016)
Wood	Essential oil	Hydro-distillation	Himachalenes, atlantones, himachalene oxide and himachalol.	**Insecticidal activity** *Plutella xylostella* L.	LC_{50}: 815 µg/ml and 1.08 mg/ml	(Chaudhary et al. 2011; Reddy and Dolma 2018)

(Continued)

				<u>Larvicidal activity</u> Malaria vector, *Anopheles culicifacies*	Had promising larvicidal activity.	(Kala et al. 2020)
	Methanolic extract	Soxhlet	ND	**<u>Macrofilaricidal activity</u>** *-Worm motility assay* *-MTT-formazan colorimetric assay*	Promising macrofilaricidal activity.	(Nisha et al. 2007)
	Petroleum ether	Maceration	Himachalol	***<u>In Vivo</u>* <u>Spasmolytic Activity</u>** *-GI propulsion of charcoal suspension in Rats.* *-Effect on intestinal movements in-Cats isolated Guinea Pig auricle*	More potent than papaverine in antagonizing epinephrine-induced contraction.	(Kar et al. 1975)
		Soxhlet	Triterpenes, saponins, phytosterols and fixed oils.	**<u>Diuretic activity</u>** **<u>Anti-urolithiatic activity</u>** *-Sodium oxalate–induced urolithiatic model in rat*	Preventive effect against NaOx induced nephroliathiasis.	(Ramesh et al. 2010)
Root	Essential oil	ND	ND	**<u>Anti-nephrotoxic activity</u>** -Prevention effect in: cyclophosphamide induced nephrotoxicity in rat model	Nephroprotective effect linked to the antixoidant activity.	(Kazi 2017)
ND	Essential oil	ND	ND	**<u>Larvicidal activity</u>** *Tenebrio molitor*	LC_{50} : 3.41%	(Buneri et al. 2019)

ND: Not determined.

Table IX: Antioxidant activity.

Plant part	Extracts	Extraction method	Major compounds	Methods used / Targets	Properties / Effects	References
Cones	Methanolic extract	Soxhlet	Polyphenols	-DPPH$^{\bullet}$ radical scavenging assay	IC_{50} ranging from 0.35 to 17.21 µg/ml.	(Semerci et al. 2020)
Needles Shoots	Organic extracts	Maceration	Polyphenols and flavonoids	-DMPD and DPPH$^{\bullet}$ radical scavenging assays. -Fe^{2+} ferrozine test system for metal-chelation. -Ferric-reducing antioxidant power (FRAP) assay. -Phosphomolibdenum-reducing antioxidant power (PRAP) assay.	The shoot-EtOAc extract had the highest metal chelation capacity (58.04±0.70%).	(Senol et al. 2015)
Wood	Essential oil	Hydro-distillation	Himachalenes isomers, (E)- and (Z)-α-atlantones and α-acorenol	-DPPH$^{\bullet}$ radical scavenging assay	*C. libani* wood oil was the most active.	(Venditti et al. 2020)
	Cedar tar	Thermal conversion of biomass in the absence of oxygen.	Polyphenols and flavonoids	-DPPH$^{\bullet}$ radical scavenging assay	Cedar tar did not show any antioxidant activity	(Takci et al. 2019)

I.2.2.2. Antimicrobial activity

The antimicrobial activities of *C. libani* samples were shown in **Table X**. The essential oils hydrodistilled from wood (Venditti et al. 2020), needles (Demirci et al. 2020), and cones (Fahed et al. 2017) were found to have antibacterial properties. The methanolic extract of cones had high antibacterial activity against *S. epidermidis*, *S. aureus*, and *B. subtilis* (Semerci et al. 2020). Cedar wood tar demonstrated antibacterial activity against *E. coli* and *S. haemolyticus*, with a MIC value of 5% (Takci et al. 2019). *B. subtilis* was found to be the most sensitive strain to organic extracts of cones and needles (Dığrak et al. 1999). These, however, had no antifungal effect. The wood oil had remarkable activity against *C. albicans* (Venditti et al. 2020). Similarly, the essential oil hydrodistilled from the cones demonstrated strong antifungal activity against dermatophytes species, with MIC values ranging from 32 µg/ml to 64 µg/ml (Fahed et al. 2017).

On the other hand, ethanolic extracts from wood, cones, and leaves demonstrated antiviral activity against herpes simplex virus type 1 (HSV-1) with IC_{50} values of 0.44 mg/ml, 0.50 mg/ml, and 0.66 mg/ml, respectively (Loizzo et al. 2008).

I.2.2.3. Antitumor activity

Several antitumor activities were observed in *C. libani* samples (**Table XI**). The essential oil hydrodistilled from wood exhibited a cytotoxic effect on a panel of cancer cell lines (Venditti et al. 2020), with an IC_{50} value of 23.38±1.7 mg/ml against K562 human chronic myelogenous leukaemia cells (Saab et al. 2012b), and IC_{50} values ranging from 29.46 µg/ml to 61.54 µg/ml against human CCRF‑CEM leukemia cells, Drug-sensitive CCRF‑CEM, and multidrug-resistant P-glycoprotein-expressing CEM/ADR5000 leukemia cells (Saab et al. 2012a).

The ethanolic extract from seeds had an IC_{50} value of 40.57±1.16 µg/ml towards K562 cells (Saab et al. 2011). The hexane extract of stem demonstrated high activity against DMBA/TPA skin carcinogenesis while being less toxic than commonly used drugs (Daher et al. 2016). In addition, hexane extract from wood had an IC_{50} value of 8.8 µg/ml against B16-F10 murine melanoma cells (Shebaby et al. 2020), as well as IC_{50} values of 8.1 µg/ml, 10.1 µg/ml, and 9.9 µg/ml against SF-268 brain cancer, HT-29 colon cancer, and CaCo-2 colon cancer cell lines, respectively (Elias et al. 2019).

Table X: Antimicrobial activity.

Plant part	Extract	Extraction method	Major compounds	Antimicrobial activity	Properties / Effects	References
Cones	Essential oil	Hydro-distillation	α-pinene, β-pinene, limonene and β-caryophyllene	**Antibacterial activity**	MIC values ranged from 32-64 µg/ml)	(Fahed et al. 2017)
				Antiviral activity: herpes simplex virus type 1 (HSV-1)	IC_{50}: 0.50 mg/ml.	(Loizzo et al. 2008)
	Methanolic extract	Soxhlet	Polyphenols	**Antibacterial activity**	High activity against *S. epidermidis, S. aureus*, and *B. subtilis.*	(Dığrak et al. 1999 ; Semerci et al. 2020)
Needles	Essential oil	Hydro-distillation	Germacrene D, 1-epi-cubenol, trans a-bisabolene and β -Caryophyllene	**Antiviral activity:** herpes simplex virus type 1 (HSV-1)	IC_{50} value of 0.66 mg/ml	(Loizzo et al. 2008)
Wood	Essential oil	Hydro-distillation	Himachalol, himachalenes isomers, (E)- and (Z)-α-atlantones and α-acorenol	**Antifungal activity**	Remarkable activity against the yeast *C. albicans.*	(Venditti et al. 2020)
			β-himachalene, α-himachalene and γ -himachalene	**Antiviral activity:** herpes simplex virus type 1 (HSV-1)	IC_{50} value of 0.44 mg/ml.	(Loizzo et al. 2008)
Resins	Ethanolic extract	Maceration	ND		Effective at 80 µg/ml (*Bacilus* strains)	(Kizil et al. 2002)

ND: Not determined.

Table XI: Antitumor activity.

Plant part	Extract	Extraction method	Major compounds	Methods used / Target	Properties / Effects	References
Wood	Essential oil	Hydro-distillation	β-Himachalene, α-himachalene, γ-himachalene, himachalol, α-acorenc and γ-(Z)-atlantone	Human CCRF‑CEM leukemia cells. A375, MDA-MB 231, HCT116, and K562 cells.	IC_{50} values from 29.46 to 61.54 µg/ml (CCRF‑CEM cells) IC_{50}: 23.38±1.7 mg/ml (K562)	(Saab et al. 2012a) (Saab et al. 2012b)
	Hexane extract	Maceration	Himachalol	B16-F10 murine melanoma cells	IC_{50}: 8.8 µg/ml and 7.3 µg/ml at 24 and 48 h, respectively	(Shebaby et al. 2020)
				Brain cancer cell line SF-268	IC_{50}: 8.1 µg/ml	(Elias et al. 2019)
				Colon cancer cell lines: HT-29; Caco-2.	IC_{50}: 10.1 µg/ml IC_{50}: 9.9 µg/ml	
				Ovarian cancer cell line (Sk-OV-3)	IC_{50} > 50 µg/ml	
Stem xylem	Hexane extract	Maceration	2-Himachalen-7-ol	DMBA/TPA skin carcinogenesis	High activity with relatively lower toxicity than commonly used drugs.	(Daher et al. 2016)
Seeds	Ethanolic extract	Ultrasound assisted maceration	Oleic acid and neo-abietol.	K562 cells bioactivity assays : *-Antiproliferative activity.* *-Erythroid differentiation induction.*	IC_{50}: 40.57±1.16 µg/ml	(Saab et al. 2011)

I.2.2.4. Anti-inflammatory and wound healing activities

The anti-inflammatory and wound healing properties of *C. libani* samples were presented in **Table XII**. The essential oil hydrodistilled from cones demonstrated a potential anti-inflammatory effect in an acetic acid-induced increase in capillary permeability (Tumen et al. 2011). In addition, hexane extract from wood exhibited significant anti-inflammatory effects in formalin-induced paw oedema in rats, as well as inhibition of LPS-induced COX-2 protein expression in isolated rat monocytes (Elias et al. 2019).

I.2.2.5. Anti-ulcer activity

C. libani samples presented anti-ulcer activity. In fact, aqueous extracts of needles demonstrated significant anti-ulcerogenic activity in rats (Yeşilada et al. 1993). The cone methanolic extract fractions inhibited *Helicobacter pylori* NCTC 11637 with MIC values ranging from 1.95 mg/mL to 250 mg/mL (Yeşilada et al. 1999).

I.2.2.6. Other biological activities

C. libani samples exhibited other biological activities (**Table XIII**). The essential oil hydrodistilled from wood, in fact, demonstrated an α-amylase inhibition effect with an IC_{50} value of 0.14 mg/ml (Loizzo et al. 2007). The wood oil was highly active against *Dermestes maculatus* (Abdel-Maksoud et al. 2019). The essential oil of seeds had larvicidal activity against *Culex pipiens* with LC_{50} values ranging from 47.8 ppm to 116.0 ppm (Cetin et al. 2009). Furthermore, the methanolic extract of needles inhibited cholinesterase with an IC_{50} value of 1.25 mg/ml (Senol et al. 2015).

Table XII: Anti-inflammatory and wound healing activities.

Plant part	Extract	Extraction method	Major compounds	Methods used / Target	Properties / Effects	References
Cones	Essential oil	Hydro-distillation	ND	**<u>Wound healing activity:</u>** *Linear incision wound model.* *Circular excision wound model.* **<u>Anti-inflammatory activity:</u>** *Acetic acid-induced increase in capillary permeability.*	Displayed remarkable wound healing and anti-inflammatory activities.	(Tumen et al. 2011)
Wood	Hexane extract	Maceration	2-Himachalen-7-ol	**<u>Anti-inflammatory activity</u>** *Formalin-induced paw edema in rats.* *Inhibition of LPS-induced COX-2 protein expression in isolated rat monocytes*	Exhibited significant anti-inflammatory effects.	(Elias et al. 2019)

ND : Not determined.

Table XIII: Other biological activities.

Plant part	Extract	Extraction method	Major compounds	Methods used / Targets	Properties / Effects	References
Needles	Essential oïl	Hydro-distillation	Germacrene D, 1-epi-cubenol, trans-a-bisabolene and β –Caryophyllene	**Anti-diabetes activity** α-amylase inhibition	IC_{50} value of 0.14 mg/ml	(Loizzo et al. 2007)
	Organic extracts	Maceration	Polyphenols and flavonoids	**Cholinesterase inhibitory** AChE and BChE inhibitory effect	The methanolic extract had an IC_{50} value of 1.25 mg/ml	(Senol et al. 2015)
Wood	Essential oil	ND	α-pinene, β-myrcene and limonene.	**Insecticidal activity** *Dermestes maculatus*	*C. libani* wood oil showed the highest activity.	(Abdel-Maksoud et al. 2019)
Seeds	Essential oïl	Hydro-distillation	ND	**Larvicidal activity** Mosquito : *Culex pipiens*	LC_{50} values from 47.8 to 116.0 ppm	(Cetin et al. 2009)

ND : Not determined.

I.2.3. C. brevifolia

C. brevifolia is the least investigated of the *Cedrus* species (**Table XIV**). In fact, only four studies on antioxidant and antimicrobial activities were found in literature. At 50 mg/ml, the essential oil hydrodistilled from needles exhibited inhibition percentages of 56%, 31%, and 17% in the $DPPH^{\bullet}$ radical scavenging, AAPH induced lipid peroxydation, and soybean LOX assays, respectively (Boutos et al. 2020). The hydro-methanolic extract of bark demonstrated strong reducing effect, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging activities with EC_{50} values of 9.1 ± 0.1 µg/ml, 13.9 ± 0.3 µg/ml, and 2.3 ± 0.0 µg/ml, respectively (Cretu et al. 2014). Cretu et al. (2013) found that the crude methanolic extract of the bark had the highest superoxide anion radical scavenging activity, while the ethyl acetate and n-butanol fractions were the most active in 15-Lipoxygenase inhibition and hydroxyl radical scavenging assays. Moreover, trans-p-coumaric acid and taxifolin isolated from the organic extracts of needles were found to be the most active ingredients (Douros et al. 2018). The essential oil of needles demonstrated an antibacterial effect with a MIC value of 0.018±0.0007 mg/ml against *E. coli*; and an antifungal effect with a MIC value of 0.025±0.0002 mg/ml against *A. fumigatus*, higher than the pure compounds (Boutos et al. 2020).

Table XIV: Biological activities of *C. brevifolia* samples.

Plant part	Extract	Extraction method	Major compounds	Methods used / Target	Properties / Effects	References
				Antimicrobial activity		
Needles	Essential oil	Hydro-distillation	α-pinene Limonene	Four Gram-negative and four Gram-positive bacteria. Eight fungal strains.	MIC: 0.018±0.0007 mg/ml (*E. coli*), and 0.025±0.0002 mg/ml (*A. fumigatus*).	(Boutos, Tomou et al. 2020)
				Antioxidant activity		
Bark	Hydro-methanolic extract and its fractions	Maceration	Taxifolin, catechin, Epicatechin and procyanidin	$DPPH^{\bullet}$ radical scavenging activity. AAPH induced linoleic acid lipid peroxidation assay Soybean LOX Inhibition	<u>At 50mg/ml, inhibition of:</u> 56% 31% 17%	(Boutos et al. 2020; Douros et al. 2018)
				$DPPH^{\bullet}$ radical scavenging. $ABTS^{\bullet+}$ radical scavenging. Reducing power assay.	EC_{50}: 13.9 ± 0.3 μg/ml EC_{50}: 2.3 ± 0.0 μg/ml EC_{50}: 9.1 ± 0.1 μg/ml	(Cretu et al. 2014)
				Superoxide anion radical scavenging assay 15-Lipoxygenase inhibition assay. Hydroxyl radical scavenging assay.	The crude extract showed the highest activity. Ethyl acetate and n-butanol fractions were the most	(Cretu et al. 2013)

I.3. Botanical caracteristics and classification of *Cedrus atlantica*

The Atlas cedar (*Cedrus atlantica* (Endl.) Manetti ex Carriere) is an endemic species that originated from the North African mountains (Algeria and Morocco). It is the only species of the genus *Cedrus* occurring in North Africa. It is a large tree, often exceeding 50 m (Brunetti et al. 2001). *Cedrus atlantica*'s taxonomic position belongs to the phylum ¨Spermaphyta¨, subphylum ¨Gymnospermae¨, class ¨Pinopsida¨, Order ¨Pinales¨, and family of pinaceae (Quézel and Santa 1962, 1963).

C. atlantica is distinguished by different characteristics. The root system is developed, but it rarely rotates, and the shaft's stability is well assured. The bark when young is smooth and brown, but as it ages, it becomes sinuous with crevices. Typically, the circumference of the trunk is 1 to 2 m. Atlas cedar is a monoecious species that blooms in autumn; male flowers are upright cylindrical catkins that are greenish yellow; female flowers are erect ovoid catkins (cones). The cones are cylindrical in shape. Their maturity lasts two years after flowering, and they are purplish brown in color, with a diameter of 5-8 cm and a maximum height of 10 cm. At the top, the needles are grouped in small clusters and carried by short twigs. Their color ranges from light to dark green or glaucous to blue and they are quite rigid, measuring 1 to 2 cm long. The seeds are triangular in shape, large, 10-15 mm long, reddish brown in color, and have a broad wing at the end. The tree appearance: When young, it has a pyramidal shape with a straight shaft, a regular and pointed crown with a curved arrow, and a tabular shape with age (Brunetti et al. 2001; Debazac 1964; M'hirit and Benzyane 2006; Toth et al. 2005).

I.3.1. Geographical distribution of Cedrus atlantica

The Atlas cedar is organized into seven blocks (Mhirit 1999), in North Africa, including four in the Moroccan mountains and three in the Algerian mountains (**Fig. 2**).

In Algeria, cedar covers an area of approximately 33,000 ha and is divided into two natural areas: The humid cedar groves are found on the well-watered coastal mountains (Babors, Djurdjura, Blideen Atlas, and Ouarsenis), while the dry cedar groves are found on the southern continental mountains of the Saharan Atlas. The latter are represented in the east by the Aures and Belezma cedar forests, which cover approximately 17,000 ha (Oudjehih 1999).

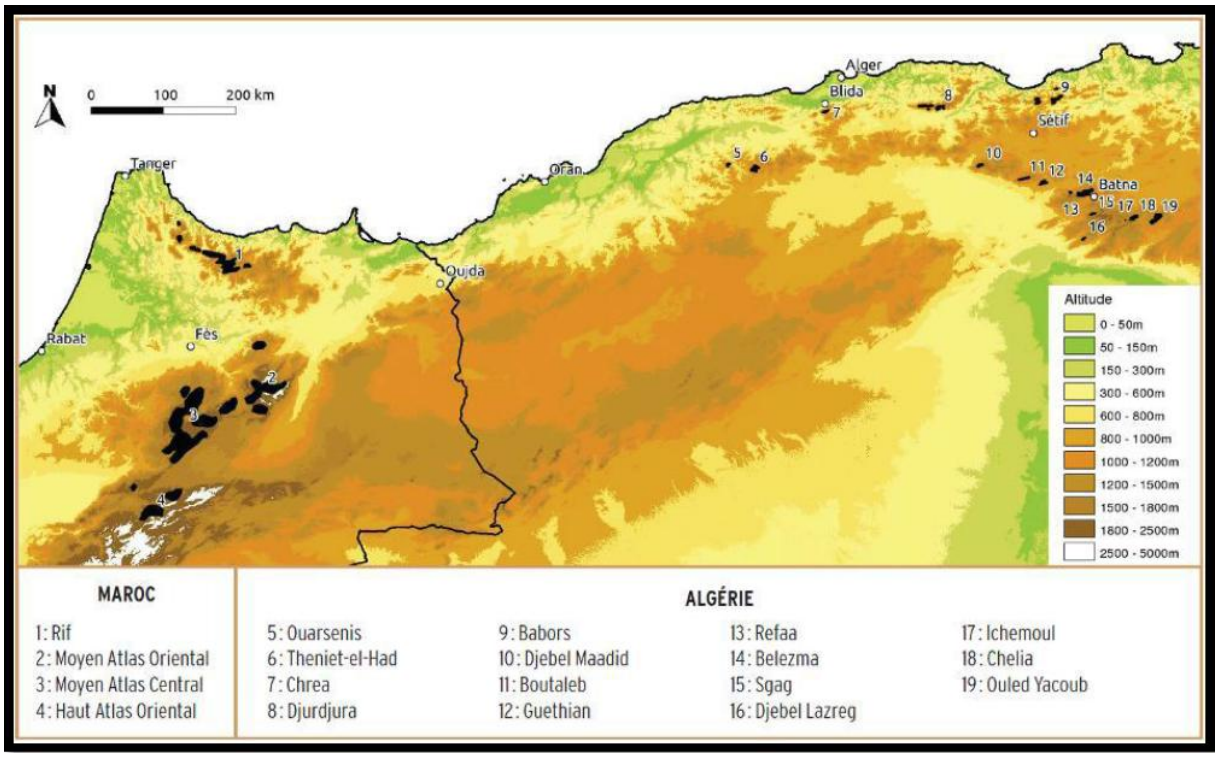

Figure 2. Geographic distribution of *C. atlantica* (Lefèvre et al. 2016)

As an ornamental and reforestation species, cedar has been successfully introduced to many countries outside of its natural distribution. According to Panetsos et al. (1992), it was introduced in several European countries (France 1862, Italy 1866, Bulgaria 1890), as well as the United States and Russia, since the previous centenary. Introductions made in various countries show that cedar can grow vigorously in climatic conditions that differ from its native area.

I.3.2. Traditional uses

Cedrus atlantica essential oil had anti-inflammatory properties (Baylac and Racine 2003) and antimicrobials (Hammer et al. 1999) explaining its utilisation in traditional skin acne treatments.

It is also useful in the treatment of hair loss in a combination aromatherapy oils (Ormerod et al. 2000), also cellulose and its derivatives extracted from the bark are used in the treatment of bronchitis, cough and indigestion (Perrot et al. 1971). Several other traditional applications have been reported on various websites.

I.3.3. Biological activities

C. atlantica samples were investigated for a variety of activities. Several studies on the antioxidant, antimicrobial, antitumor, anti-allergic, acetylcholinesterase inhibitory, anti-inflammatory, and analgesic activities have been published. In addition, acaricidal, molluscicidal, larvicidal, and insecticidal activities have also been evaluated.

I.3.3.1. Antioxidant activity

The antioxidant activities of *C. atlantica* samples were summarized in **Table XV**. The essential oil of the branches had an IC_{50} value of 315.85±0.97 mg/ml against $DPPH^{\bullet}$ free radical (Inaam et al. 2015). The essential oil of the cones, on the other hand, demonstrated a 45% inhibition (Paun et al. 2013). The hydro-ethanolic extract macerated from the aerial parts exhibited significant antioxidant activity in scavenging the free radical $DPPH^{\bullet}$ (Fadel et al. 2016). The hydro-methanolic extract of cones showed comparable results (Hofmann et al. 2020). The ethanolic extract from seeds, however, demonstrated lower $DPPH^{\bullet}$ free radical scavenging activity with an IC_{50} value of 0.4 mg/ml (Naimi et al. 2015). The tar methanolic extract had a TAC value of 262.75±14.43 mg Eq AA/g (Skanderi and Chouitah 2020).

I.3.3.2. Antimicrobial activity

Several studies on the antimicrobial activities of *C. atlantica* extracts have been reported in literature (**Table XVI**). Indeed, diterpene alcohols isolated from the neutral hexane extract of the cones using soxhlet had significant activity against *B. cereus*, *Streptococcus* C and *E. faecalis* (Dakir et al. 2005). The hydromethanolic extract macerated from the cones had the most antibacterial effect against the multi-resistant *E. faecalis* with an MIC value of 15.1 µg/ml (Maya et al. 2017). The essential oil hydrodistilled from sawdust demonstrated potential antibacterial effects with MIC values of 0.4 µl/ml against *E. coli* and *B. cereus*, and 0.2 µl/ml against *B. subtilis* (Zrira and Ghanmi 2016). Similarly, Bennouna et al. (2019) demonstrated that sawdust essential oil had an antibacterial activity against *B. safensis* and *B. subtilis* with MIC values of 2% v/v and 1% v/v, respectively; and MBC value of 8% v/v. In addition, the wood essential oil was found effective against *A. baumanii, A. sobria, E. faecalis, K. pneumoniae, P. aeruginosa, S. typhimurium, S. marcescens, M. luteus,* and *S. mutans* (Benouaklil et al. 2017; Chaudhari et al. 2012; Hammer et al. 1999).

Table XV: Antioxidant activity.

Plant part	Extract	Extraction method	Major compounds	Methods used / Target	Properties / Effects	References
Cones	Essential oil	Hydro-distillation	α ,β-himachalène, α-longipinene, β-chamigrene, and longifolene (V4).	DPPH• radical-scavenging assay.	Inhibition of 45%.	(Paun et al. 2013)
	Alocoholic extract	Ultrasonic extraction	Polyphenols	DPPH• radical-scavenging assay. FRAP: Ferric-reducing antioxidant power	IC_{50}:14.91 ± 2.00µg /ml FRAP value of 24.19 ± 0.45 mg AAE/g dw	(Hofmann et al. 2020)
Seeds	Ethanolic extract	Maceration	Flavonoids	DPPH• radical-scavenging assay.	IC_{50} value of 0.4 mg/ml	(Naimi et al. 2015)
Branches	Essential oil	Hydro-distillation	α-pinene, menthyl acetate, 1-tetradecene,and caryophyllene	DPPH• radical-scavenging assay.	IC_{50}: 315.85±0.97 mg/ml	(Inaam et al. 2015)
Tar (from wood)	Methanolic extract	Dissolution	Himachalene, α -atlantone, α-calacorene, (z) nuciferol and ar-turmerone	Phosphomolybdenum method. Ferric-reducing antioxidant power	TAC: 262.75±14,43 mg Eq AA /g tar EC_{50}: 0.075 ± 0.00028 mg /ml	(Skanderi and Chouitah 2020)
Aerial parts	Ethanolic extract	Maceration	Flavonoids	DPPH• radical-scavenging assay.	IC_{50} value of 8.9 µg/ml	(Fadel et al. 2016)

Table XVI: Antimicrobial activity.

Plant part	Extract	Extraction method	Major compounds	Antimicrobial activity	Properties / Effects	References
Cones	Essential oil	Hydro-distillation	β-himachalène, α-longipinene, β-chamigrene, longifolene, α, β -pinene, β- farnesene, bornyl acetate, and α-terpineol.	Antibacterial activity	MIC>1% vv against *E.coli* and *S. aureus*	(Paun et al. 2013)
	Methanolic extract	Maceration	γ-tocotrienolic acid δ-(E)- deoxy-amplexichromanol daglesioside IV and (+) taxifolin		Most potent against *E. faecalis* (MIC:15.1 µg/ml)	(Maya et al. 2017)
Needles	Essential oil	Hydro-distillation	α, β-pinene, α-himachalene, β-himachalene, myrcene, limonene, longifolene, δ –cadinene and cis-α-atlantone.	Antibacterial activity	MIC value of 0.25 mg/ml (*E. coli*)	(Derwich et al. 2010)
				Antifungal activity	Effective at 150 ppm against *Phytophthora citrophthora.*	(Chebli et al. 2004 ; Bouchra et al. 2003)
					MICs of 100 and 200 µl/ml (*T.asahii* and *T.cutaneum*)	(Uniyal et al. 2013)
Wood	Essential oil	Hydro-distillation	Alpha-cedrene, cedrol, and cis-thujopsene, α-himachalene, α longipinene, hamachalol, cuprenene and E-a-atlantone .	Antibacterial activity	EO (30 :1) exhibited 15 mm of inhibition zone against MRSA	(Chao et al. 2008)
					E. faecalis most sensitive (MIC 0.5% (v/v))	(Hammer et al. 1999)

.

(Continued)

Sawdust	Essential oil	Hydro-distillation	γ-himachalane, β-himachalane, γ-calamenene, δ-cadinen, E-*γ*-atlantone, E-*α*-atlantone, 5-isocedranol and 9-iso-thujopsanone.	Antibacterial activity	MICs ranging from 1% to 2%.	(Bennouna et al. 2019)
					MICs of 0,4 µl/ml for *E. coli* and *B. cereus*.	(Zrira and Ghanmi 2016)
				Antifungal activity	MICs ranging from 0.5% to 1%.	(Bennouna et al. 2019)
					MICs ranging from 1/1000 to 1/400 v/v.	(Fidah et al. 2019)
					Gloeophyllum trabeum inhibited at 1/1000 v/v.	(Fidah et al. 2016)
Bark	Essential oil	Hydro-distillation	α-pinene, 1-tétradécène, menthyle acetate and caryophyllène	Antifungal activity	Inhibition activity against *Fusarium culmorum*	(Uwineza et al. 2018a)

Also, the wood oil (30:1) exhibited 15 mm of inhibition zone against methicillin-resistant *S. aureus* (MRSA) (Chao et al. 2008). MIC values ranged from 0.25 mg/ml to 1.62 mg/ml were recorded for leaves' essential oil against seven bacterial strains (Derwich et al. 2010). The essential oil and the hydromethanolic extract from cones had antifungal activity against several tested strains (Bennouna et al. 2019; Fidah et al. 2019; Maya et al. 2017; Rhafouri et al. 2014).

I.3.3.3. Antitumor activity

The antitumor activities of *C. atlantica* samples were presented in **Table XVII**. The essential oil hydrodistilled from wood was found to be cytotoxic against K562 human chronic myelogenous leukemia cells with an IC_{50} value of 59.37±2.6 mg/ml (Saab et al. 2012b). In addition, the oil steam distilled from bark inhibited cell growth in HL-60, K562, Jurkat, P338D1, and RAW264.7 cells, while also arresting the cell cycle in the G0/G1 phase with apoptosis induction, resulting in leukemia cell death (Hung et al. 2020). The bark essential oil inhibited the growth of human hepatocellular carcinoma cells both *in vitro* and *in vivo*, by inducing apoptosis through caspase-dependent and independent apoptosis pathways (Huang et al. 2020). Hexane extract from the cones had an antitumor effect on a panel of cancer cells with IC_{50} values greater than 5 µg/ml, including A-549 (human lung carcinoma), H-116 (human colon carcinoma), PSN1 (human pancreatic adenocarcinoma), T98G (human Caucasian gioblastoma), and SKBR3 (human breast carcinoma) (Barrero et al. 2005).

I.3.3.4. Analgesic and anti-inflammatory activities

The anti-inflammatory and analgesic activities of *C. atlantica* samples were presented in **Table XVIII**. The essential oil inhibited lipoxygenase with IC_{50} values ranging from 31 ppm to 50 ppm (Baylac and Racine 2003). The essential oil hydrodistilled from wood alleviates acute post-operative pain by activating the descending pain modulation pathway (Martins et al. 2015). In addition, the *C. atlantica* essential oil had an antihyperalgesic effect by either releasing or inhibiting endocannabinoid degradation (Emer et al. 2018).

Table XVII: Antitumor activity.

Plant part	Extract	Extraction method	Major compounds	Methods used / Target	Properties / Effects	References
Cones	n-hexane	Soxhlet	Abietane diterpenoids	A-549, H- 116, PSN1, T98G, and SKBR3.	IC_{50} values higher than 5 µg/ml	(Barrero et al. 2005)
Wood	Essential oil	Hydro-distillation	ND	K562 cells	IC_{50}: 59.37±2.6 mg/ml	(Saab et al. 2012b)
Bark	Essential oil	Steam-distillation	Thujopsene, alpha-cedrene, alpha-cadinene, cedrol, and isolongipholene	HepG2, Mahlavu, Huh7 and J5 cells.	IC_{50}: 27.09±1.83 µg/ml, 33.57±2.84 µg/ml, 32.83 ± 4.31 µg/ml, and 6.09±3.28 µg/ml, respectively.	(Huang et al. 2020)
				HL-60, K562, Jurkat, P338D1, and RAW264.7	Reduced cell growth. Cell cycle arrest in the G0/G1 phase. Induced apoptosis.	(Hung et al. 2020)

ND : Not determined.

Table XVIII: Analgesic and anti-inflammatory activities.

Plant part	Extract	Extraction method	Major compounds	Methods used / Target	Properties / Effects	References
Analgesic activity						
Wood	Essential oil	Hydro-distillation	α-himachalene, γ-himachalene and β-himachalene	-Plantar incision surgery (PIS) -Evaluation of locomotor activity -Mechanical hypersensitivity	Alleviates acute post-operative pain by activating the descending pain modulation pathway.	(Martins et al. 2015)
ND	Essential oil	ND	α-himachalene, γ-himachalene and β-himachalene	-Plantar incision surgery (PIS) -Evaluation of mechanical hyperalgesia -Investigation of the ECB signaling via CB1R and CB2R -Effects of combined administration of sub-effective dose of FAAH or MAGL inhibitor and EO inhalation	Antihyperalgesic effect by releasing, or inhibiting the degradation of endo-cannabinoid.	(Emer et al. 2018)
Anti-inflammatory activity						
				Inhibition of 5-Lipoxygenase	31 ppm<IC_{50} ≤50 ppm	(Baylac and Racine 2003)

ND : Not determined.

I.3.3.5. Other biological activities

Other biological activities of *C. atlantica* samples have been reported (**Table XIX**). The acetylcholinesterase inhibitory activity of the essential oil hydrodistilled from wood was 14.40±3.94 % (Phrompittayarat et al. 2014). In addition, the aerial parts and wood essential oils were insecticidal against *Tribolium confusum*, *Culex pipiens*, *Tenebrio molitor*, and *Toxoptera aurantii* (Zoubi et al. 2017, Ainane et al. 2019, Kaoutar et al. 2019, Orchard et al. 2019). Molluscicidal activity was also observed against *Bulinus truncatus*, with an LC_{50} value of 0.47 ppm. The Cedrol-loaded nanostructured lipid carrier demonstrated a promising effect in the prevention of anaphylactic reactions (Chakraborty et al. 2017).

I.3.4. Phytochemistry of C. atlantica

I.3.4.1. Chemical composition of C. atlantica essential oils

Several studies have been conducted on the phytochemistry of the essential oils obtained from different parts (cones, seeds, needles, wood, and sawdust) of *C. atlantica* harvested from various geographical areas.

a) Monoterpene hydrocarbons

The major compound in the essential oil derived from cones, seeds, and needles was α-pinene (Boudarene et al. 2004a; Boudarene et al. 2004b). β-pinene was revealed in the composition of essential oils hydrodistilled from various plant parts. Other monoterpene hydrocarbones found in the essential oil composition of *C. atlantica* include myrcene, p-cymene (Lahlou 2003), camphene, α-phellandrene (Yassaa et al. 2000), β-phellandrene (Derwich et al. 2010), limonene, and ocimene (Lamiri et al. 2001) (**Fig. 3**).

Table XIX: Other biological activities.

Plant part	Extract	Extraction method	Major compounds	Methods used / Target	Properties / Effects	References
Needles	Essential oil	Hydro-distillation	α-pinene, β-pinene and β-myrcene	**Molluscicidal activity** *Bulinus truncatus*	LC_{50} value of 0.47 ppm	(Lahlou 2003)
Wood	Essential oil	Hydro-distillation	α-pinene, α -himachalene and β-himachalene	-**Acetylcholinesterase inhibitory activity** -**Antipest activity** *Sitophilus granarius* and *Tenebrio molitor*. *Tribolium confusum*	Inhibition: 14.40±3.94% Active against *T. molitor* larvae Active on *T. confusum*.	(Phrompittayarat et al. 2014) (Orchard et al. 2019 ; Martynov et al. 2019)
Aerial parts	Essential oil	Hydro-distillation	α-himachalene, β-himachalene, γ-himachalene, cedrol, isocedranol and α-pinene	**Insecticidal activity** *Tribolium confusum* *Culex pipiens* (Diptera: Culicidae)	LC_{50} and LC_{90} of 782.43 ppm and 1253..93 ppm against *Culex pipiens*	(Zoubi et al. 2017 ; Ainane et al. 2019)
Branches	Essential oil	Hydro-distillation	β-himachalene, α-himachalene and atlantol.	**Aphicide activity** *Toxoptera aurantii*	LC_{50} estimated at 6.80 ml/l	(Kaoutar et al. 2019)
			Cedrol (loaded nanostructured lipid carrier)	**Anti-allergic effect** Trypan blue exclusion assay of mast cell viability	Promising effect in protection of anaphylactic reactions.	(Chakraborty et al. 2017)

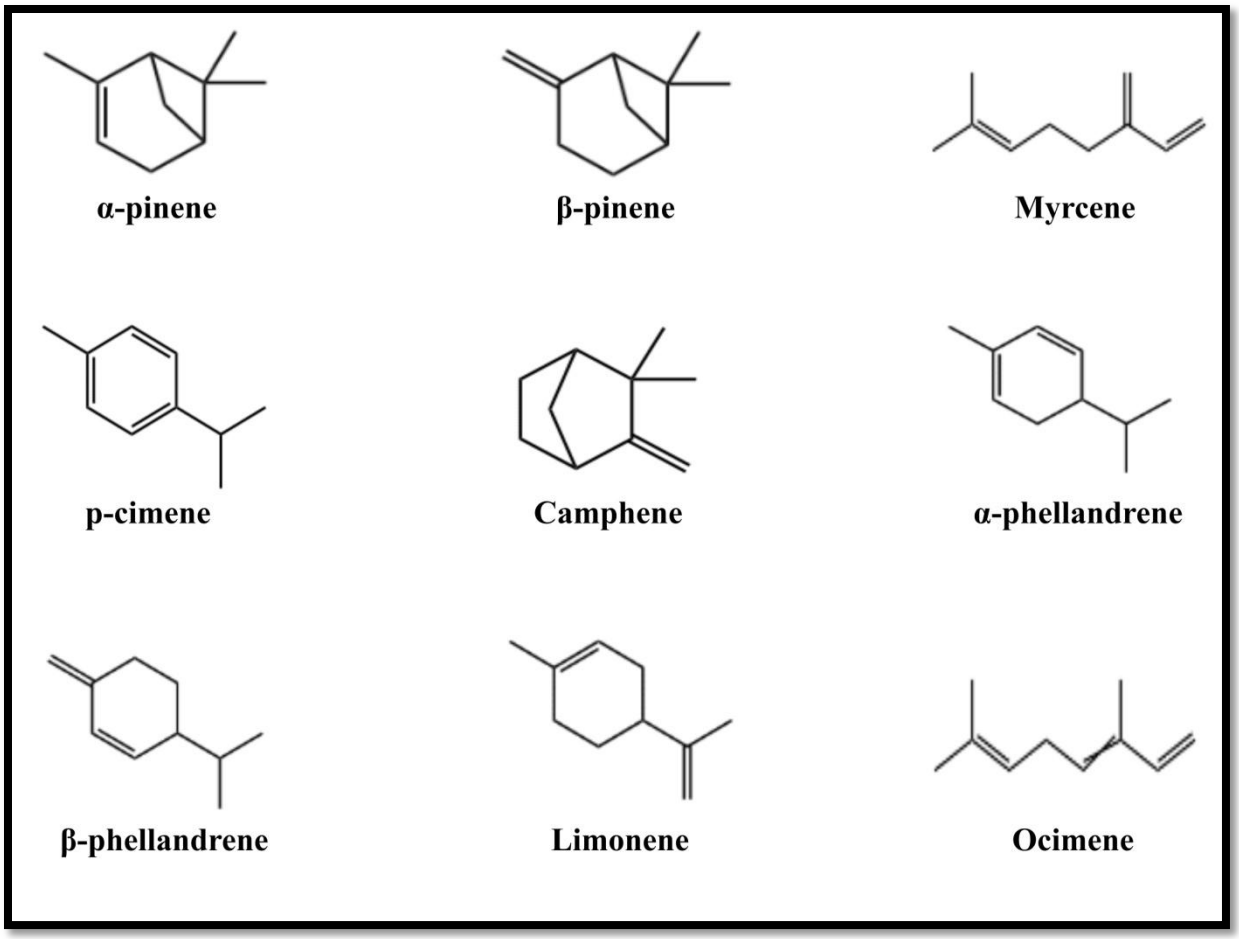

Figure 3. *C. atlantica* monoterpene hydrocarbons (Saab et al. 2018).

a) Sesquiterpene hydrocarbons

α-himachalene, β-himachalene, and γ-himachalene have been identified as major components in the wood oil (Aberchane et al. 2004, Satrani et al. 2006, Derwich et al. 2010). Other sesquiterpene hydrocarbons isolated in the essential oil composition of *C. atlantica* include β-caryophyllene (Boudarene et al. 2004b), longifolene, β-calacoren, cuparene (Aberchane et al. 2004), germacrene D, α-cadinene, humulen, copaen (Derwich et al. 2010), α-murolene (Lahlou 2003), and β-farnesene (Paoli et al. 2011) (**Fig. 4**).

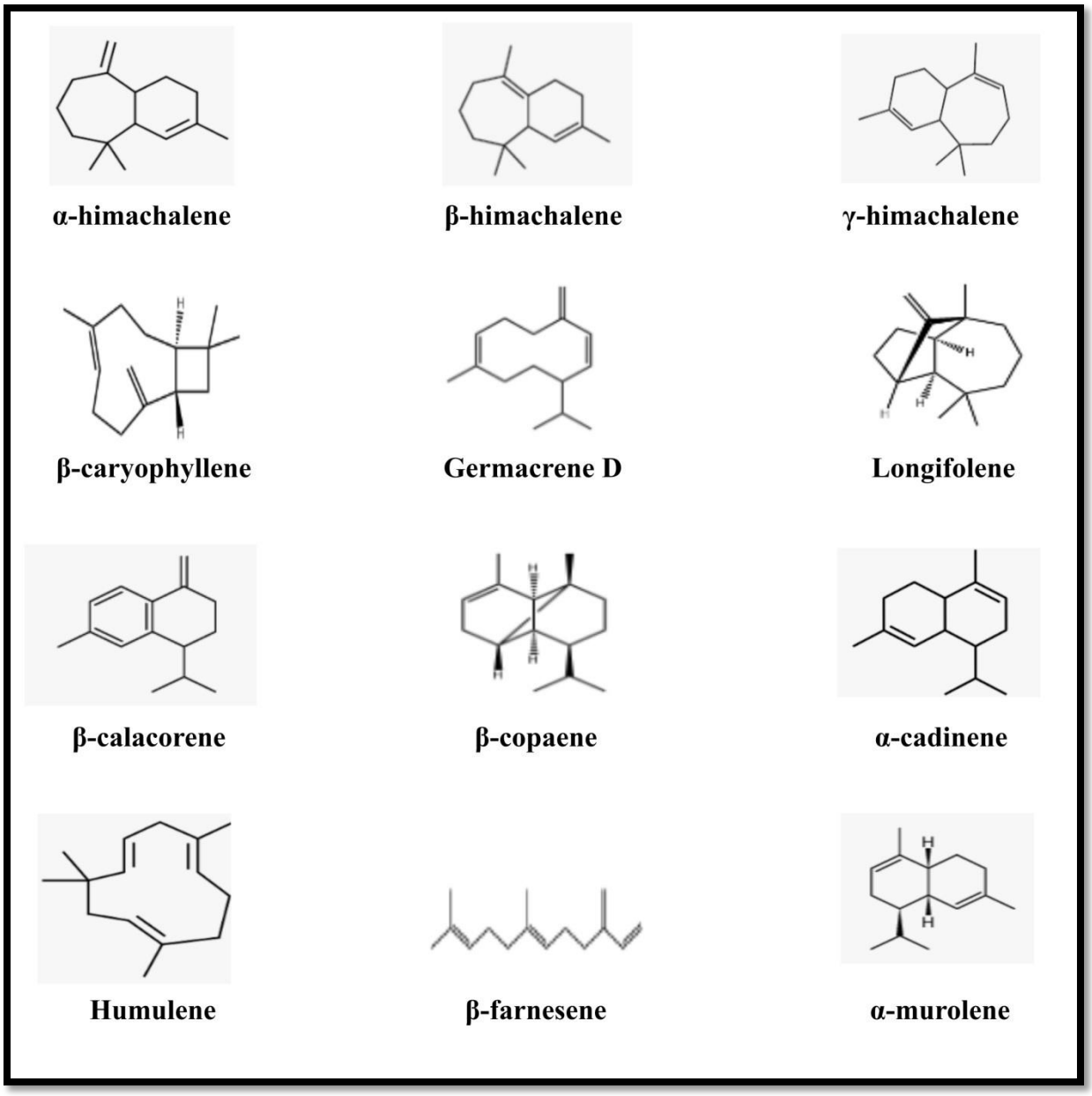

Figure 4. *C. atlantica* sesquiterpene hydrocarbons (Saab et al. 2018).

b) Oxides

The wood oil has yielded cedroxide (Fidah et al. 2019), caryophyllene oxide and himachalene oxide (Aberchane et al. 2004; Boudarene et al. 2004b). Manoyle oxide, an oxygenated diterpene was highly expressed in the essential oil of *C. atlantica* seeds (Boudarene et al. 2004a).

c) Ketones

The main sesquiterpene ketones found in the *C. atlantica* essential oil were α-atlantone; γ-atlantone (Chalchat et al. 1994, Saab et al. 2005, Satrani et al. 2006) and

deodarone (Chalchat et al. 1994, Aberchane et al. 2004, Satrani et al. 2006). The wood oil contained also cedranone, camphor and thujopsanone (Fidah et al. 2019).

d) Alcohols

Several sesquiterpene alcohols were observed in the wood oil, including cedrol, tumerol, himachalol, cedranol, β-santalol, E-Z-farnesol (Fidah et al. 2019), and 1-epicubenol (Satrani et al. 2006, Paoli et al. 2011). Monoterpene alcohols such as linalool (Yassaa et al. 2000), terpineol (Boudarene et al. 2004a), and verbenol (Boudarene et al. 2004b) have also been reported.

e) Esters and aldehydes

C. atlantica wood oil contained hexyl isobutyrate, benzyl benzoate, and Z-β-santalol acetate (Fidah et al. 2019). Bornyl acetate was also found in the essential oil of *C. atlantica* (Lahlou 2003).The aldehyde 4-acetyl-1-methylcyclohexene was hydrodistilled from *C. atlantica* wood oil (Aberchane et al. 2004).

I.3.4.2. Chemical composition of C. atlantica extracts

Few studies on the phytochemistry of *C. atlantica* organic extracts have been conducted. Tocotrionelic acid derivative and O-acylated flavonol glycoside have been isolated from hydromethanolic extract of the *C. atlantica* cones (Maya et al. 2017). It has been reported that the ether diethylic extract from cones contains resinic acids such as sandaracopimaric, abietic, isopimaric, levopimaric, palustic, dehdroabietic and neobietic acids (Norin and Winell 1971). Barrero et al. (2005) demonstrated that the hexane extract of cones contained five abietane diterpenes. Furthermore, abietane diterpenes and lignans were identified in *C. atlantica* resins (Nam et al. 2011).

Part II

Materials and methods

In this study, the essential oil was hydrodistilled from *C. atlantica* cones and organic extracts derived from its branches. The essential oil was analyzed using gas chromatography-mass spectrometry and the phenolic components levels in the organic extracts were determined. Both samples were tested for different biological activities. An acute toxicity study was carried out on female wistar mice. Finaly, the effect of the ultrasonic power on the methanolic extracts was assessed.

II.1. Plant material

Cedrus atlantica branches and cones were harvested in Akfadou forest (36°41'49.9"N, 4°36'07.7"E) at an altitude of 1600 m in May 2015 (**Fig. 5**). Botanical identification was carried out in the Laboratory of Plant Biotechnology and Ethnobotany at Bejaia University by Dr. F. Bekdouche using Quezel and Santa flora (Quézel and Santa 1962, 1963). The plant specimen was deposited in the School of Pharmacy at the University of Jordan under a voucher number (Cea-2018-5-42). Branches were dried at room temperature in the shade and subsequently converted into a powder of less than 250 µm in diameter. The cones were dried at room temperature in the shade.

Figure 5. Original image of *Cedrus atlantica* in Akfadou forest (Adekar) (source of harvested cones and branches)

II.2. Extraction and characterization of *C. atlantica* essential oil and extracts

II.2.1. Essential oil extraction

C. atlantica cones dried at room temperature were cut into small pieces and then subjected to hydrodistillation for 2.5 h using Clevenger-type apparatus (Boudaren et al. 2004a). Anhydrous sodium sulphate was added to the extracted transparent essential oil in order to eliminate water contamination after that stored at 4°C in hermetic sealed vial until being used.

$$\text{Yield (\%)} = (V_o/W_i).100$$

Where, V_o and W_i are the obtained volume of essential oil and initial weight of cones, respectively.

II.2.2. Organic extracts and fractions' preparation

The organic extraction was performed using soxhlet apparatus (Reihenheizgerät 4, Germany). An amount of 21 g of dry plant powder was subjected to organic extraction with 200 mL of different solvents (Methanol, ethanol and acetone). The obtained extract solutions were filtered and then evaporated using a rotavapor (Heidolph, Germany).

The organic extracts were subjected to a typical partitioning protocol (Rostagno and Prado 2013) (**Fig. 6**). An amount of 10 g of the extract was dissolved in 200 ml of methanol 5% and placed in a separating funnel. A volume of 150 ml of solvents with increased polarity (Hexane: H, chloroform: Chl, ethyl acetate: EtOAc and n-butanol: But) were successively added. The obtained solutions of corresponding fractions were filtered and then the solvents evaporated. The dry extracts and fractions were conserved at 4°C in darkness until usage after being weighed and the percentage yield was calculated using the following formula:

$$\text{Yield (\%)} = (W_o/W_i).100$$

Where, W_o and W_i are the obtained extract (or fraction) and initial weights, respectively.

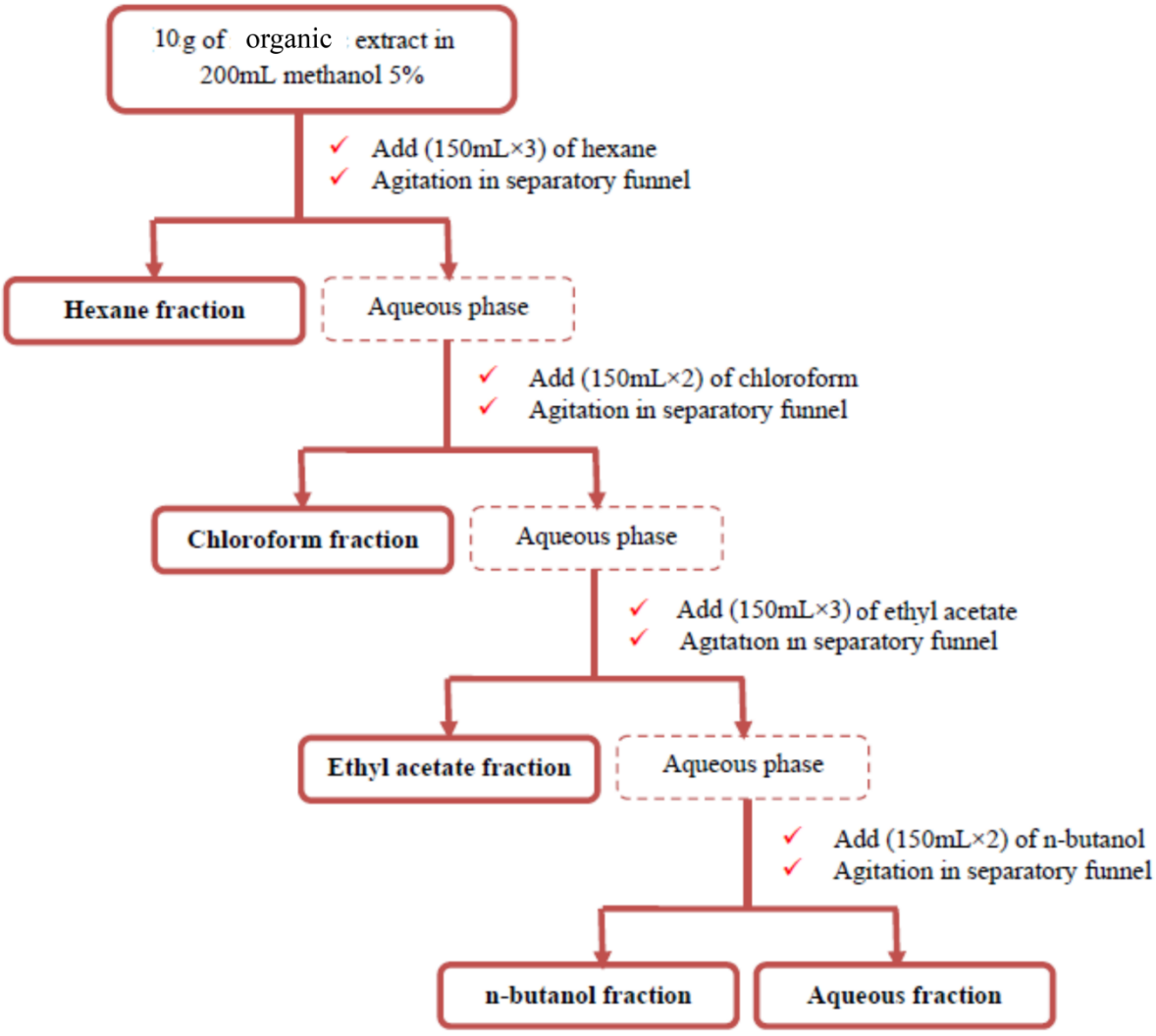

Figure 6. Partitioning protocol scheme (Rostagno and Prado 2013).

II.2.3. Gas chromatography-mass spectrometry analysis of C. atlantica cones essential oil

An approximate sample volume of 1 µl of essential oil, appropriately diluted in GC-grade n-hexane, was analyzed using a Varian Chrompack CP-3800 GC/MS/MS-200 (Saturn, The Netherlands) equipped with an automatic injector in the split mode and a flame ionization detector. A DB-5 GC capillary column was used (Dimensions: 30 m × 0.25 mm; 0.25 µm film thickness) consisting of 95% dimethyl polysiloxane and 5% diphenyl. Helium was used as the carrier gas at a flow rate of 1ml/min. A linear temperature program was applied starting from the initial column temperature of 60^{o}C (hold time: 1 min) and raised to 250^{o}C (hold time 3 min) at a heating rate of 3^{o}C/min.

The identification of the essential oil separated chemical entities was assessed by comparing their arithmetic retention indices (Kovat's Index) with the reported values in literature (mainly Adma's library) and also by matching their corresponding mass spectra with those of the data bank of the instrument software (Terpene ThermoQuest, General purpose and NIST libraries). A mixture of n-alkane hydrocarbon (C8-C20) was subjected to GC/MS analysis under the same chromatographic conditions (**Fig. 7**) as described above and the corresponding retention times were recorded and used to calculate the arithmetic Kovat's index for each essential oil component according to Van den Dool and Kratz equation:

$$RI_x = 100\,n + 100\,(t_x - t_n) / (t_{n+1} - t_n)$$

- $\mathbf{t_{n+1}}$ and $\mathbf{t_n}$ retention times of the reference n-alkane hydrocarbons eluting immediately before and after compound "**X**"
- $\mathbf{t_x}$ retention time of compound "**X**"

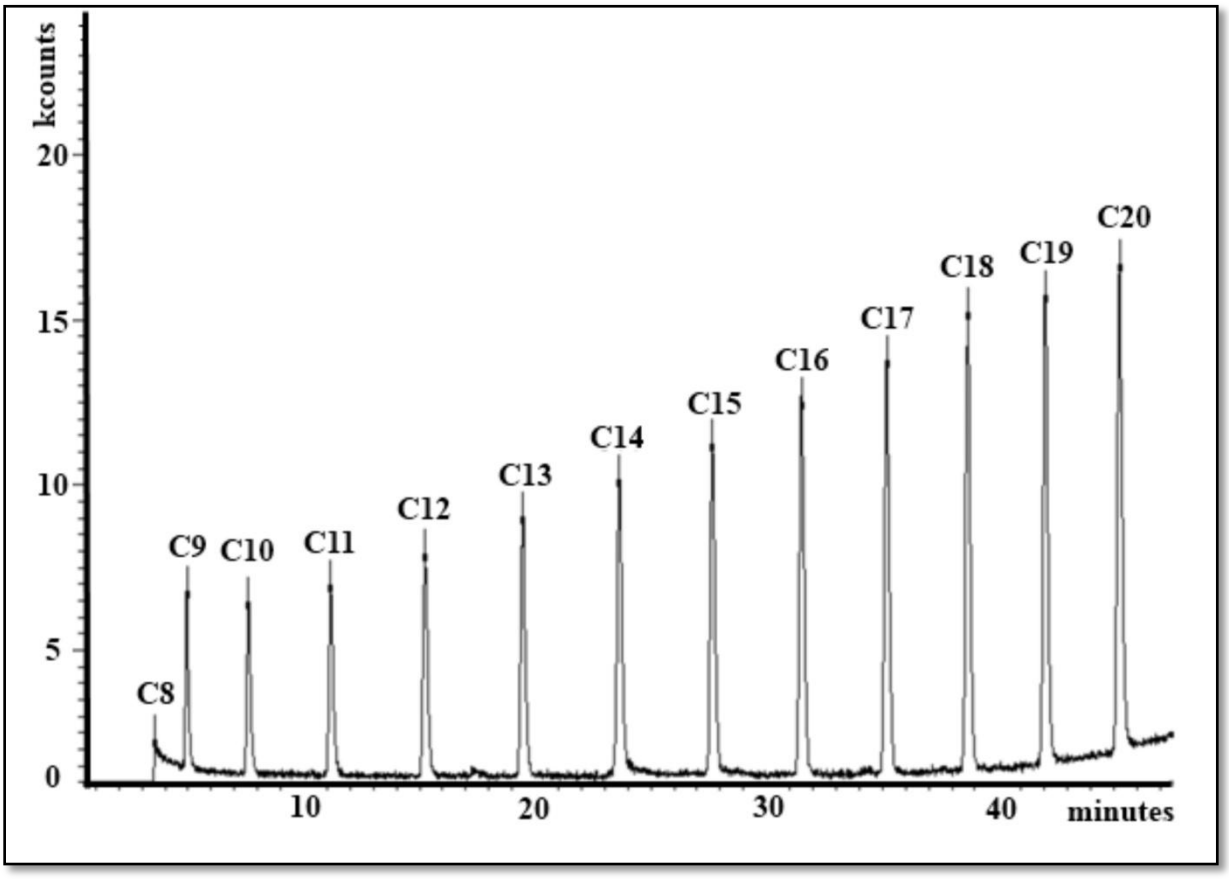

Figure 7. Gas chromatography-mass spectrometry chromatogram of n-alkane hydrocarbons (C8-C20).

The percentage content of each oil compound was obtained by integrating each peak surface area, assuming a unity response by all compounds (Adams 2007).

II.2.4. Total polyphenol content determination

The determination of total polyphenol content was performed by the Folin-Ciocalteu method (Wong et al. 2006). A sample volume of 200 µl (Extracts or fractions of the methanolic extract: Chl, EtOAc, But, and Aqueous (Aq)) was poured in 1 ml of Folin-Ciocalteu reagent (1/10 dilution), then 800 µl of sodium carbonate (75 g/l) were added after 4 min. The absorbance was measured at 765 nm using a spectrophotometer (UV-9200, Biotech, Germany) after 60 min incubation at room temperature in darkness. The results were expressed in melligram equivalent gallic acid per gram of dry sample (mg Eq GA/g) obtained from a standard curve plotted using gallic acid with a concentration range from 25 to 100 µg/ml.

II.2.5. Flavonoid content determination

The flavonoid content was determined using the aluminium chloride method (Quettier-Deleu et al. 2000). A sample volume of 1 ml (methanolic extract or its fractions: Chl, EtOAc, But, and Aq) was added to 1 ml of $AlCl_3$ 2%. The absorbance was measured at 430 nm after 10 min incubation at room temperature. The results were expressed in melligram equivalent quercetin per gram of dry sample (mg Eq Q/g) obtained from a standard curve plotted using quercetin with a concentration range from 1.625 to 30 µg/ml.

II.2.6. Condensed tannin content determination

The amount of condensed tannin was estimated using the vanillin method (Ba et al. 2010). A sample volume of 500 µl (methanolic extract or its fractions: Chl, EtOAc, But, and Aq) was mixed with 3 ml of vanillin 4% in methanol and 1.5 ml of HCl 37%. The absorbance was measured at 500 nm after 20 min incubation at 30^{o}C. The results were expressed in melligram equivalent catechin per gram of dry sample (mg Eq C/g) obtained from a standard curve plotted using catechin with a concentration range from 25 to 300 µg/ml).

II.3. Biological activities evaluation

II.3.1. Antioxidant activity evaluation

II.3.1.1. $DPPH^{\bullet}$ radical scavenging assay

Diphenyl picrylhydrazyl radical ($DPPH^{\bullet}$) scavenging activity was carried out using the protocol described by Shirwaikar et al. (2006). A sample volume of 1 ml (methanolic extract or its fractions: Chl, EtOAc, But, and Aq) at different concentrations (3.125, 6.25, 12.5, 25, 40 and 50 µg/ml) was mixed with 1 ml of $DPPH^{\bullet}$ methanolic solution (0.1 mM). The essential oil was tested at the concentration range of 3.125 mg/ml to 40 mg/ml. The absorbance was measured at 517 nm after 30 min incubation in darkness. Butylhydroxyanisol (BHA) and vitamin C (Vit C) were used as standards at the same concentrations in the same conditions. The $DPPH^{\bullet}$ radical scavenging activity was calculated using the following formula:

$$\%\ \text{radical scavenging activity} = [(A_c - A_s)/A_c] \cdot 100$$

Where, A_c and A_s are the absorbance of the control (the sample was replaced by 1 ml of methanol) and the sample, respectively.

II.3.1.2. $ABTS^{\bullet+}$ radical scavenging assay

$ABTS^{\bullet+}$ radical scavenging activity was carried out using the protocol described by Le et al. (2007). The radical $ABTS^{\bullet+}$ was formed by mixing aqueous ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) solution (7 mM) and aqueous potassium persulfate solution (2.45 mM) with a ratio 1:1 then incubated 16 h in darkness at room temperature. A sample volume of 100 µl (methanolic extract or its fractions: Chl, EtOAc, But, and Aq) at different concentrations (12.5, 25, 50, 100, 150 and 200 µg/ml) was mixed with 1.9 ml of $ABTS^{\bullet+}$ radical solution already diluted with ethanol until obtaining the absorbance of 0.7 ± 0.02 at 734 nm. The essential oil was tested at the concentration range of 12.5 mg/ml to 200 mg/ml. The absorbance was measured at 734 nm after 7 min incubation in darkness at room temperature. BHA and Vit C were used as standards at the same concentrations in the same conditions. The results were expressed in mmol equivalent Trolox per gram of dry sample (mmol Eq T/g) obtained from a standard curve plotted using Trolox with the concentrations of 0.025, 0.05, 0.1, 0.2 and 0.3 mM.

The $ABTS^{\bullet+}$ radical scavenging activity was calculated using the following formula:

$$\% \text{ radical scavenging activity} = [(A_c - A_s)/A_c] \cdot 100$$

Where, A_c and A_s are the absorbance of the control (the sample was replaced by 100 µl of methanol) and the sample, respectively.

II.3.1.3. Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) was estimated using the protocol described by Thaipong et al. (2006). A sample volume of 150 µl (Essential oil, methanolic extract or its fractions: Chl, EtOAc, But, and Aq) at a determined concentration was added to 2850 µl of FRAP solution freshly prepared by mixing sodium acetate buffer (300 mM, pH=3.6), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ, 10 mM in 40 mM HCL) and ferric chloride (20 mM) in darkness at 37°C with a ratio of 10:1:1 (Benzie and Strain 1996). The absorbance was measured at 593 nm after 15 min incubation in darkness at 37°C. The results were expressed in milligram equivalent vitamin C per gram of dry sample (mg Eq Vit C/g) obtained from a standard curve plotted using Vit C with the concentrations of 3.125, 6.25, 12.5, 25 and 50 µg/ml.

II.3.2. Antibacterial activity evaluation

II.3.2.1. Bacterial strains

Antibacterial activity experiments were carried out against the following bacteria obtained from the American type culture collection (ATCC): Gram-positive strains (*Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 11778); and Gram-negative strain (*Escherichia coli* ATCC 25921), and clinical isolates (Gram-positive strain (*Listeria innocua*); and Gram-negative strains (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*)) provided by the microbiology laboratory of the University Hospital of Tizi Ouzou:.

II.3.2.2. Preparation of bacterial inocula

Bacterial suspensions of each strain were prepared by diluting few colonies in sterile aqueous saline solution (NaCl 0.85%) scraped from an overnight culture in agar plates incubated at 37°C. The turbidity was adjusted to 0.5 McFarland which corresponds to approximately 10^8 CFU/ml (Ferraro 2009). This was performed by comparison to a prepared

standard consisting of 50 µl of anhydrous barium chloride ($BaCl_2$ 1.175%) added to 9.95 ml of sulphuric acid (H_2SO_4 1%). The mixture had an optical density comprising between 0.08 and 0.13 at 625 nm (Abu-Lafi et al. 2017)**.** Bacterial suspensions were then diluted in Muller-Hinton Broth (MHB) to yield approximately 5 x 10^6 CFU/ml for being used in the antibacterial activity tests within 15 min.

II.3.2.3. Disc diffusion assay

Disc diffusion method was carried out in accordance with the Manual of clinical microbiology of the American Society for Microbiology (Jorgensen and Turnidge 2015). Prepared filter paper discs (6 mm of diameter), impregnated with a specific sterile single concentration of the methanolic fractions (Chl, EtOAc, But and Aq), have been deposited with a sterile forceps onto the surface of the Mueller-Hinton agar medium that has been inoculated by the test bacteria using a sterile cotton swab. Plates were inverted and left for 15 min in ambiant air before incubation at 37°C for 18-24 h. DMSO, with 1% concentration, was used as negative control. Inhibition zone diameters were measured with calipers. Tests were performed once for each fraction.

II.3.2.4. Determination of minimum inhibitory and minimum bactericidal concentrations

Minimum inhibitory concentrations (MIC) were determined using a broth microdilution method (Abu-Lafi et al. 2017). Stock solutions of each plant sample (Essential oil and fractions of the methanolic extract: EtOAc, But, and Aq) were prepared in dimethyl sulfoxide (DMSO) and sterilized by filtration through a 0.22 µm syringe filter. A volume of 100 µl of diluted solutions for each sample was transferred into the first well of 96-well microplate then subjected to serial twofold dilution to get a concentration range from 31.25 to 1000 µg/ml for the fractions and from 0.03 to 1% for the essential oil. Subsequently, each well was filled with 100 µl of each bacterial suspension. The microplates were then incubated at 37°C for 18-24 h. Gentamicin antibiotic was used as a positive control to determine the sensitivity of each tested strain; MHB with DMSO was used as negative control and MHB without bacteria was used as sterility control. The DMSO final concentration was 1% in all wells. The lowest concentration with no visible bacterial growth was recorded as the minimum inhibitory concentration (MIC) (Jorgensen and Turnidge 2015). Subsequently, 50 µl from these wells were subcultured onto agar plates and incubated at 37°C for 18-24 h. The

lowest concentration that exhibited no bacterial growth was recorded as minimum bactericidal concentration (MBC). All measurements were carried out in triplicate.

II.3.3. Cytotoxic activity evaluation

II.3.3.1. Cell culture

Breast cancer cell-lines (MCF-7) obtained from the American type culture collection were cultured in RPMI 1640 with L-Glutamine (EuroClone, Italy) complemented with 10% fetal bovine serum (FBS) (GE Healthcare, USA), 10 mM HEPES buffer (pH 7.3) (Caisson, USA), 100 U/mL of penicillin (EuroClone, Italy) and 100 U/ml of Streptomycin (EuroClone, Italy) and incubated at 37^{o}C in a humidified atmosphere of 5% CO_2. Media was changed when its colour became clear each 48 to 72 h. When cells reached confluence, the media was withdrawn from the 75 cm^2 flask, washed with phosphate-buffer saline (PBS) (EuroClone, Italy) and 1.5 ml of Trypsin-EDTA without phenol red, calcium and magnesium (EuroClone, Italy) was added and incubated at 37^{o}C in a humidified atmosphere of 5% CO_2 for 5 min to detach the cells. Then, 3 ml of the media was added to stop trypsin effect and subcultured by pipetting into new flasks. Cell count after trypsinization was performed with a haemocytometer using trypan blue dye (Promega Corporation, USA) exclusion assay (Yousef et al. 2018).

II.3.3.2. Cytotoxicity and MTT assays

In the cytotoxicity assay, active cells' mitochondrial dehydrogenase enzymes reduce the MTT to blue formazan indicating cell viability (Van de Loosdrecht et al. 1994). Cytotoxicity of each plant sample was assessed by MTT assay (Yousef et al. 2018). A volume of 100 µl of MCF-7 cells was seeded at a density of 7000 cells/well in 96-well microplates and allowed to attach overnight. A same volume of each plant extract (Essential oil and fractions of the methanolic extract: EtOAc, But, and Aq) already dissolved in DMSO was tested in triplicate at different concentrations diluted in the prepared RPMI media. The final concentration of DMSO was 1%. Media with 1% DMSO was tested as negative control, while doxorubicin was tested as positive control at different concentrations ranging from 0.006 to 100 µM. After 24 h incubation, the media was removed from each well and replaced by 100 µl of fresh media and then 15 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Promega Corporation, USA) was added in umber conditions followed by incubation for 3 h at 37^{o}C in a humidified atmosphere of 5% CO_2, then 100 µl solubilization

stop solution mix (Promega Corporation, USA) was added to each well to solubilize blue formazan crystals and left for 24 h. Optical densities (OD) were measured using an ELIZA microplate reader (model Elx 808, BioTek instruments, USA) at 570 nm. Cytotoxicity effect (C(%)) was calculated by the following formula:

$$C\ (\%) = [(OD_C - OD_T) / OD_C] * 100$$

Where, OD_T and OD_C are the optical density of treated cells and the negative control, respectively.

Similar experiments were achieved on fibroblast normal cell lines seeded at a density of 10^5 cells/well using DMEM low glucose (EuroClone, Italy) as culture media in all experimental steps. IC_{50} values, representing the concentrations of each tested sample that demonstrate 50% cytotoxicity, were calculated as the average of three replicates.

II.4. Acute toxicity study

II.4.1. Animals

Nine female wistar mice chosen at random were tested for the acute toxicity study. The animals were marked with numbers to allow their identification. They were kept one week in their cages for acclimatization purpose under laboratory conditions before the experiments. The animals had free access to diet and water. All procedures were performed according to the ethical principles in animal research (Arts et al. 2014).

II.4.2. Determination of the median lethal dose (LD50)

The acute toxicity evaluation of the crude extract from branches was determined according to the OECD Guideline for testing of chemicals (OECD 2001). A volume of 1 ml of the extract solubilized in aqueous solution was administered orally in a single dose by gavage, starting with 2000 mg/kg as described in **Figure 8**.

The dose limit was 5000 mg/kg. Each mouse was treated at an interval of 48 h. The animals were fasting 4 h for diet (except water) prior extract administration and 2 h after treatment. The toxicity signs in each mouse have been recorded in the first 4 hours, one week and two weeks after dosing.

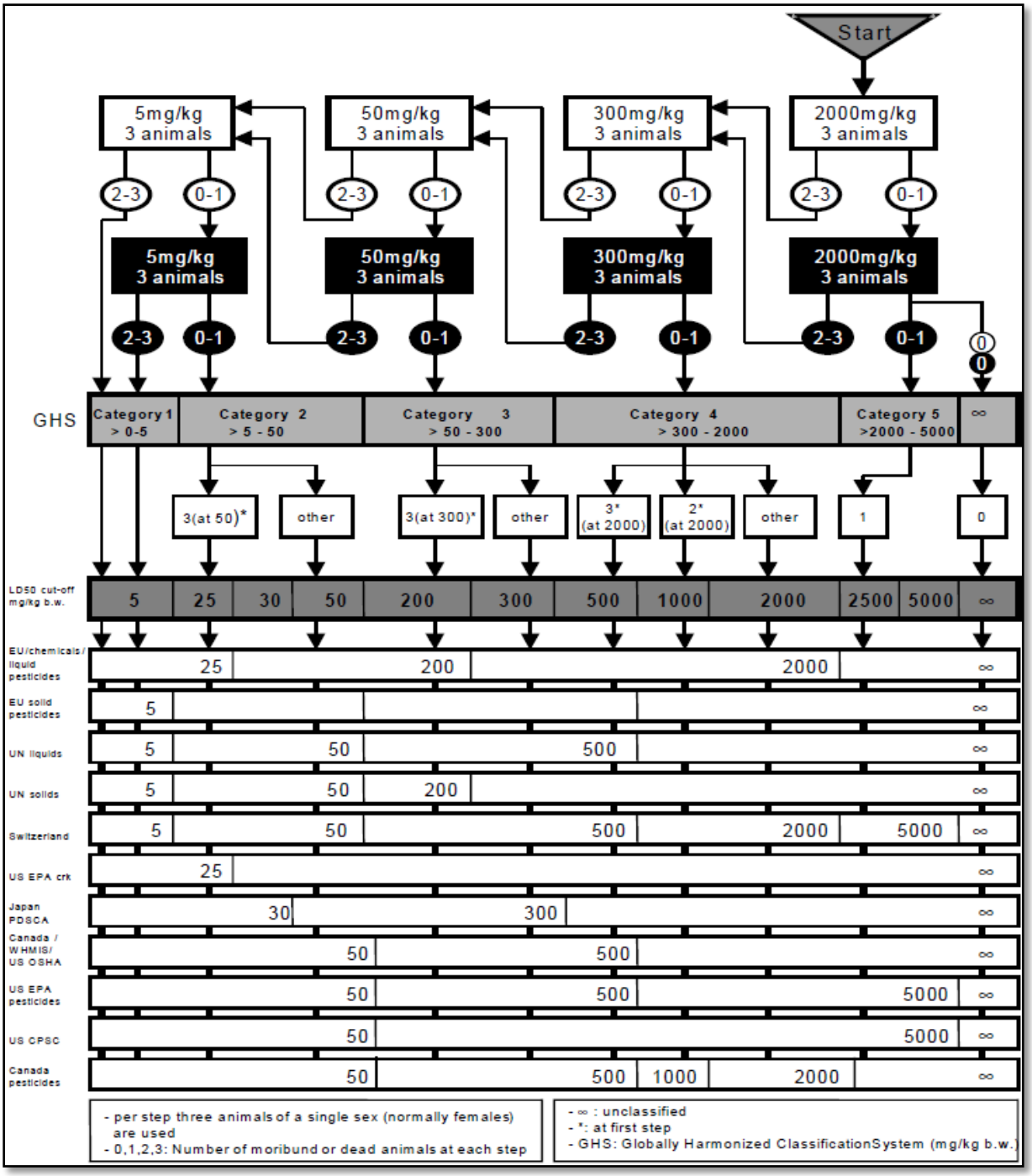

Figure 8. Procedure for determining the LD_{50} for an initial dose of 2000 mg/Kg (OCDE 2014)

The value of the LD_{50} will give information on the toxicity of the methanolic extract according to the classification reported by Viau and Tardif (2003) as follows:

- LD_{50}< 5 mg/kg: *Extremely toxic.*
- 5 mg/kg <LD_{50}< 50 mg/kg: *Very toxic*

- 50 mg/kg < LD_{50} < 500 mg/kg: *Toxic*
- 500 mg/kg < LD_{50} < 5000 mg/kg: *Little toxic*
- LD_{50} > 5000 mg/kg. *Very little toxic or non-toxic*

II.5. Effect of ultrasound on the physico-chemical properties of *C. atlantica* methanolic extracts

II.5.1. Viscosity measurement

The viscosity was meseaured using a viscometer (Viscometer Viscotech VR 3000, Spain) for the methanolic extract subjected to ultrasonic power (ultrasonic cleaner, Brasonic 2510E-DTH, USA) at different sonication times (10, 20, and 30 min), and 42 KHz frequency.

II.5.2. Solubility determination

An excess amount of the untreated extract and that sonicated at different times (10, 20, and 30 min) was added to a predetermined volume of methanol. The mixtures were left for 24 h, then the solubility determined.

II.5.3. $DPPH^{\bullet}$ radical scavenging

The antioxidant ability to scavenge the free radical $DPPH^{\bullet}$ by the untreated extract and that subjected to ultrasonic waves at different durations (10, 20, and 30 min) has been assessed by the above-described protocol.

II.6. Statistical analysis

All experiments were carried out in triplicate. The results were expressed as mean ± standard deviation (SD). The data were subjected to statistical analysis (t-test) using GraphPadPrism statistical software (version 5). Statistical differences yielding $P \leq 0.05$ were considered significant.

Part III

Results & discussion

III.1. Extraction and characterization of *C. atlantica* essential oil and extracts

III.1.1. Extraction yield

The essential oil extracted from *C. atlantica* cones yielded 0.41 percent v/w. The yield is lower than that obtained from cones collected in the Ifrane region of Morocco (0.62 percent v/w) (Paun et al. 2013) and from branches (0.6-0.7 percent v/w) (Kaoutar et al. 2019). Other studies on the needles revealed a higher amount of hydro-distilled essential oils (1.82 percent v/w) (Derwich et al. 2010). Furthermore, seeds and wood yielded the highest levels ranging from 2.1 to 9.19 percent v/w) (Nam et al. 2015, Fidah et al. 2016, Zrira and Ghanmi 2016, Benouaklil et al. 2017). Indeed, the extraction yield of essential oils has been reported to vary depending on the harvest period, plant part and age, vegetative cycle as well as to the geographical location (Ainane et al. 2019).

The results of extraction rates using soxhlet as a method of extraction with different solvents were presented in **Figure 9A**. The extraction yield was found to be related to the nature of the solvent ($P < 0.05$). Methanol had the highest percentage at 19.70±2.68%, followed by ethanol at 11.54±1.17%; whereas, the lowest yield at 9.69±1.07% was obtained with acetone. This is because the plant sample contains components with varing polarities and solubilities.

The organic extracts were fractionated using solvents of increasing polarities, and the results were presented in **Figure 9B, C, and D**. But and Aq fractions had the highest percentages in the methanolic extract (**Fig. 9B**), at 35.39±1.22% and 36.46±1.91% ($P > 0.05$), respectively; followed by H at 12.55±3.02%, EtOAc at 9.29±0.28%, and Chl at 3.64 ±0.72% ($P < 0.05$). Similarly, the But and Aq fractions of the ethanoic extract produced the highest yields at 33.57±2.2% and 26.03±2.54% ($P > 0.05$), respectively; followed by H at 16.65±1.48% ($P < 0.05$), Chl and EtOAc at 10.90±0.91% and 8.56±0.98% ($P > 0.05$), respectively (**Fig. 9C**). However, acetone fractions showed a little different trend with But fraction exhibiting the highest rate at 38.95±1.98% ($P < 0.05$), followed by H at 28.93±3.2% ($P < 0.05$), then Chl and EtOAc at 17.43±3.15% and 14.99±2.35% ($P > 0.05$), respectively. The Aq fraction, on the other hand, had the lowest yield of 6.94±1.93% ($P < 0.05$) (**Fig. 9D**).

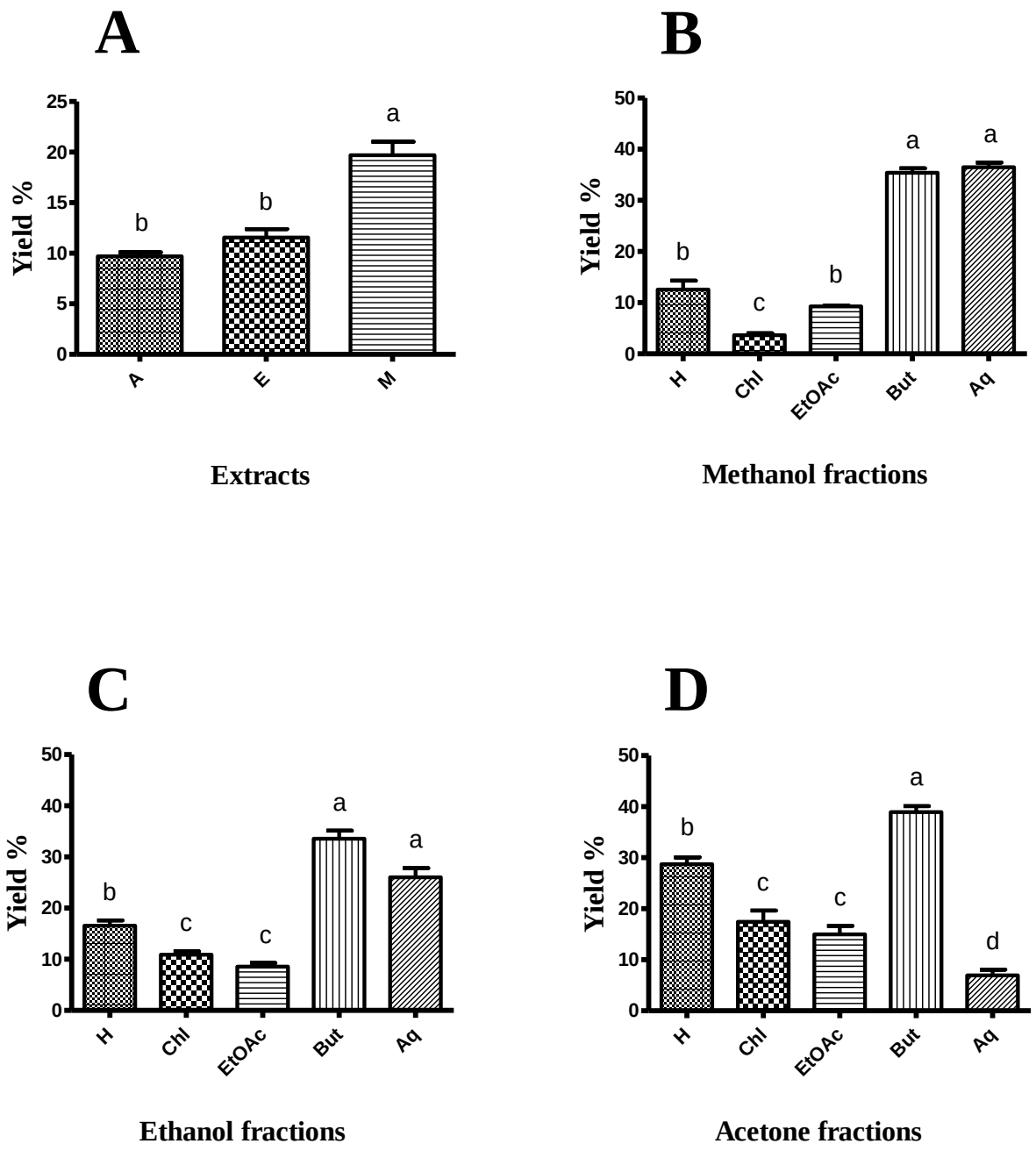

Figure 9. *C. atlantica* extracts and fractions yields.

A: Acetone, E: Ethanol, M: Methanol, H: Hexane, Chl: Chloroform, EtOAc: Ethyl acetate, But: n-Butanol, Aq: Aqueous. For each graph, values followed by different letter were significantly different ($P<0.05$). Values were expressed as mean ± SD (n=3).

Approximately 71.86% and 59.6% of the constituents were isolated in the most polar fractions (But and Aq) of methanolic and ethanolic extracts, respectively. However, acetone extracted a lower percentage (approximately 45.89%). According to these findings, the components extracted with methanol from *C. atlantica* branches are mainly polar. In fact, Reichardt and Welton (2011) reported that the normalized values of solvent polarities of methanol, ethanol and acetone were 0.762, 0.654 and 0.355, respectively. Several studies have demonstrated that the nature of the solvent influences the extraction yield, phenolic content and consequently their biological activities (Do et al. 2014; Hadzri et al. 2014; Kamarudin et al. 2016).

III.1.2. Gas chromatography-mass spectrometry analysis of cones essential oil

Terpenic composition of the *C. atlantica* essential oil hydrodistilled from its cones, as determined by GC-MS, was shown in **Figure 10**.

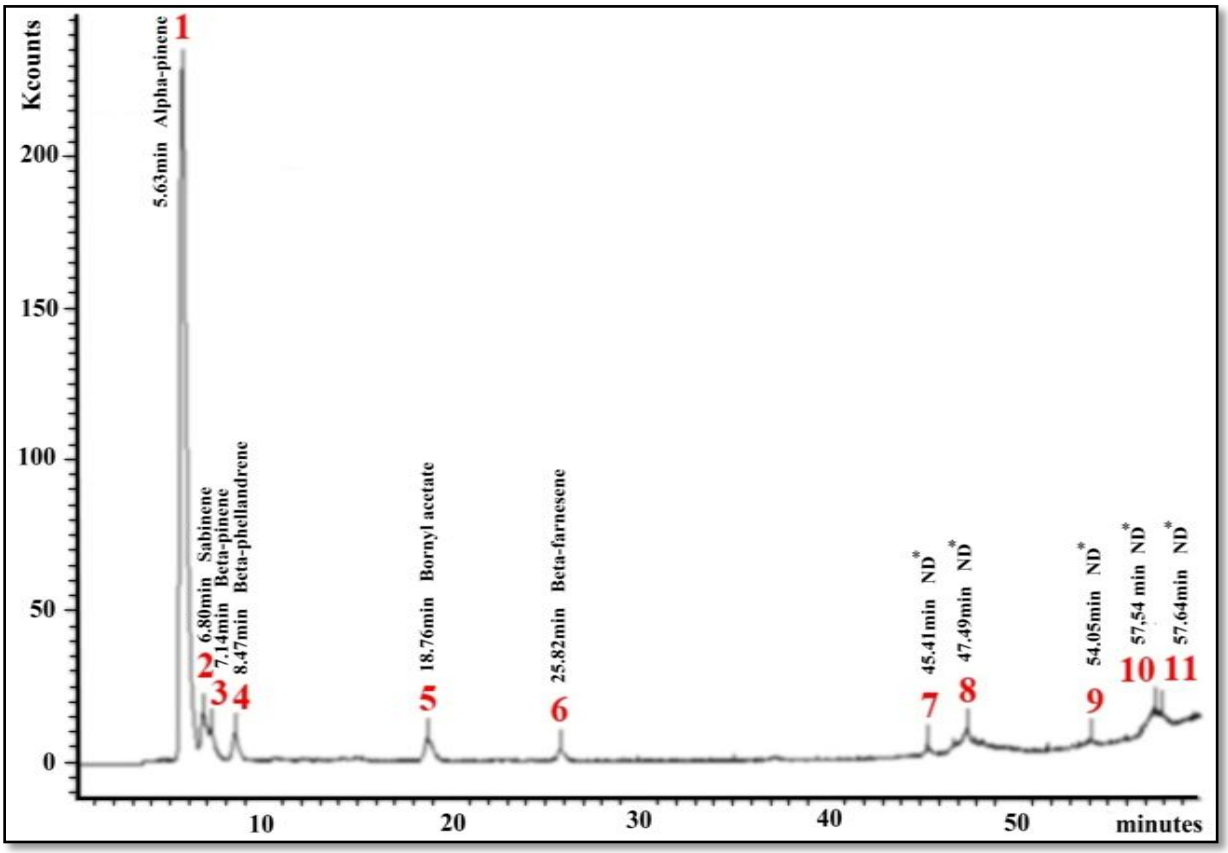

Figure 10. Gas chromatography-mass spectrometry chromatogram of *C. atlantica* cone essential oil (Belkacem et al. 2021).

*ND: Not determined.

Six compounds were successfully separated and identified using their retention index and mass spectral matching, accounting for more than 93 percent of the total oil components **(Table XX)**. The *C. atlantica* essential oil was characterized by dominant levels of monoterpene hydrocarbons representing 89.18% and a lower content of sesquiterpene hydrocarbons represented by traces of β-farnesene (0.96%). Alpha-pinene was the most abundant monoterpene hydrocarbon component (81.49%), followed by sabinene (3.21%), β-phellandrene (2.53%) and β-pinene (1.95%). The essential oil also contained a small amount of bornyl acetate (2.96%) with miscellaneous structure. This tested essential oil did not contain any diterpens, oxygenated monoterpenes, or sesquiterpenes.

Table XX
Essential oil composition of *C. atlantica* cones.

Peak N°	RT (min)	RI	Component	Area	Percentage %
1	5.63	926	Alpha-pinene	6191000	81.49
2	6.80	969	Sabinene	243959	3.21
3	7.14	982	Beta-pinene	148445	1.95
4	8.47	1024	Beta-phellandrene	192346	2.53
5	18.76	1283	Bornyl acetate	225345	2.96
6	25.82	1454	Beta-farnesene	73505	0.96
7	45.41	-	UT*	59307	0.78
8	47.49	-	UT*	160155	2.10
9	54.05	-	UT*	55854	0.73
10	57.54	-	UT*	152775	2.01
11	57.64	-	UT*	93850	1.23
				7596541	93.13

UT*: Unidentified traces	6.85%
Monoterpene hydrocarbons	89.18%
Sesquiterpene hydrocarbons	0.96%
Miscellaneous	2.96%
Totalidentified	93.13%

The mass spectra (MS) and chemical structures of the volatile components isolated from the essential oil of *C. atlantica* cones were presented in **Figure 11**. From our meticulous investigation, no GC-MS study on the hydrodistilled essential oil from Algerian *C. atlantica* cones has been reported in the literature. However, the study carried out by Boudarene et al. (2004a) on the essential oil of *C. atlantica* seeds harvested in two different areas [Ould Yakoub (OY) and Tala Guilef (TG)] revealed that the oils contained forty-four and twenty-eight components, respectively, with the following main components: α-pinene (37.1-5.5%), β-pinene (8.6-1.9%), myrcene (3.6-0.6%), limonene (2.5-0.6%), bornyl acetate (5.4-4%), β-farnesene (6.8-1.9%) and manool (8.3-20.7%).

In comparison to our findings, the essential oil of *C. atlantica* cones harvested in the Moroccan region of Ifrane had a different composition. β-himachalene (29.4%), α-longipinene (20.75%), β-chamigrene (14.39%), and longifolene (V4) (11.61%) were the main components (Paun et al. 2013). However, variations in essential oil composition may be due to a variety of known factors such as plant part, harvesting period, geographical origin and genetic parameters (Inan et al. 2011; Telci et al. 2009).

III.1.3. Total polyphenol content

Polyphenols are important non-enzymatic antioxidants found in plants. The TPC in *C. atlantica* samples was measured using the Foilin-Ciocalteu method, as shown in **Figure 12**. TPC values were calculated using the following equation from a standard curve plotted with Gallic acid: Abs=0.011 [AG] +0.035, R^2=0.996. Polyphenolic extraction was significantly influenced by solvent nature ($P < 0.05$) (**Fig. 12A**). The acetonic extract had the highest TPC at 314.09±4.47 mg Eq GA/g extract, followed by the methanolic extract at 257.03±4.36 mg Eq GA/g extract; corresponding to 30.43±0.43 mg Eq GA/g dry powder and 50.63±0.86 mg Eq GA/g dry powder ($P < 0.05$); respectively. The ethanolic extract had the lowest value, with 236.09±7.81 mg Eq GA/g extract corresponding to 27.24±0.90 mg Eq GA/g dry powder ($P < 0.05$). As a result, methanol had the highest level of polyphenols extracted from branch powder, whereas acetonic extract had the highest concentration. This suggests that methanol dissolved more plant components, such as polyphenols and other compounds like polysaccharides, than the other solvents. The extraction yield of polyphenols increases as the polarity of the solvent increases. Methanol was shown to have potential extractive abilities for phenolic compounds from other plant samples, either alone or in aqueous combinations (Hayet et al. 2009; Lu and Foo 2001; Pinelo et al. 2004).

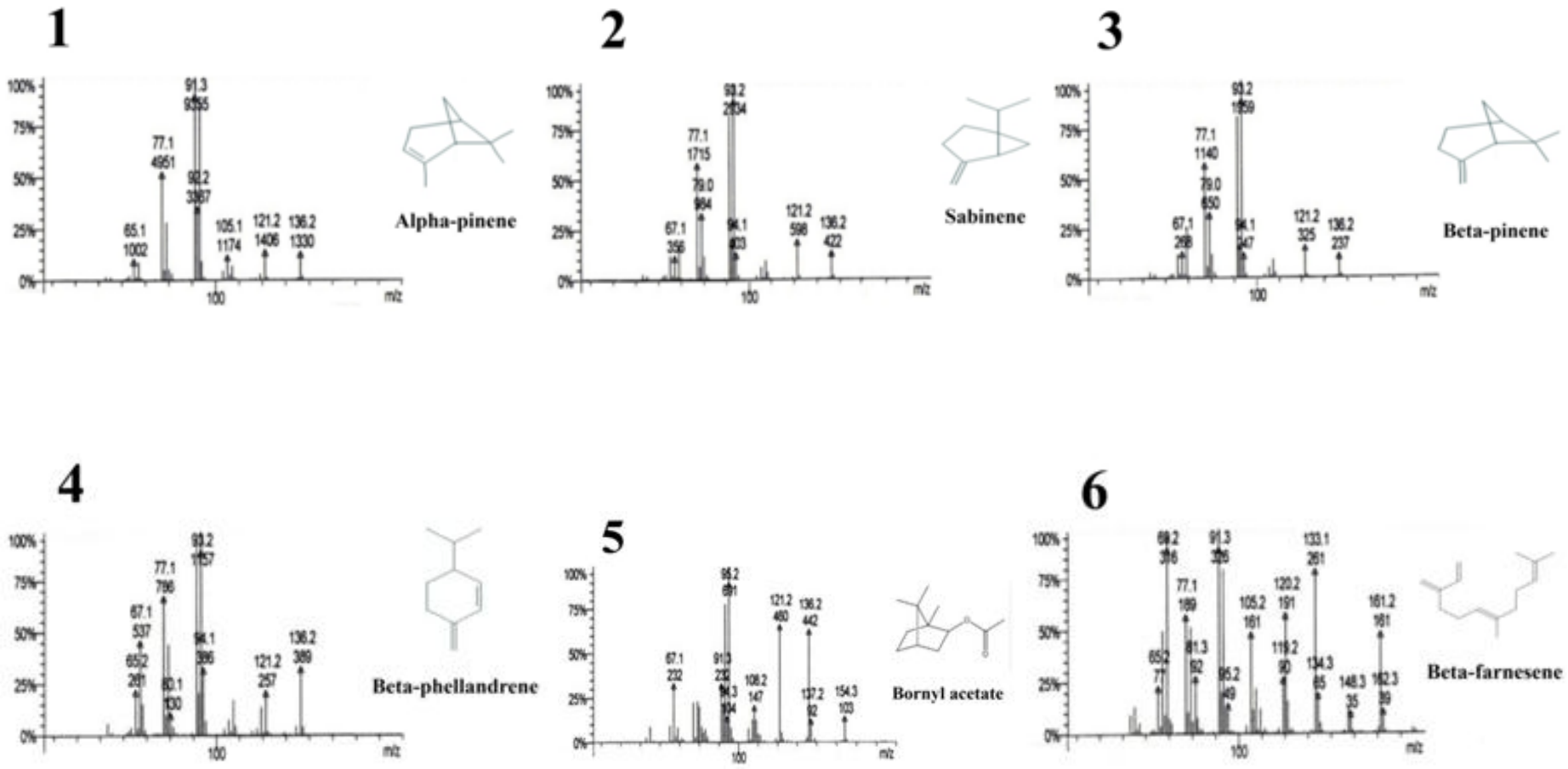

Figure 11. Mass spectra and chemical structures of the volatile components isolated from the essential oil of *C. atlantica* cones.

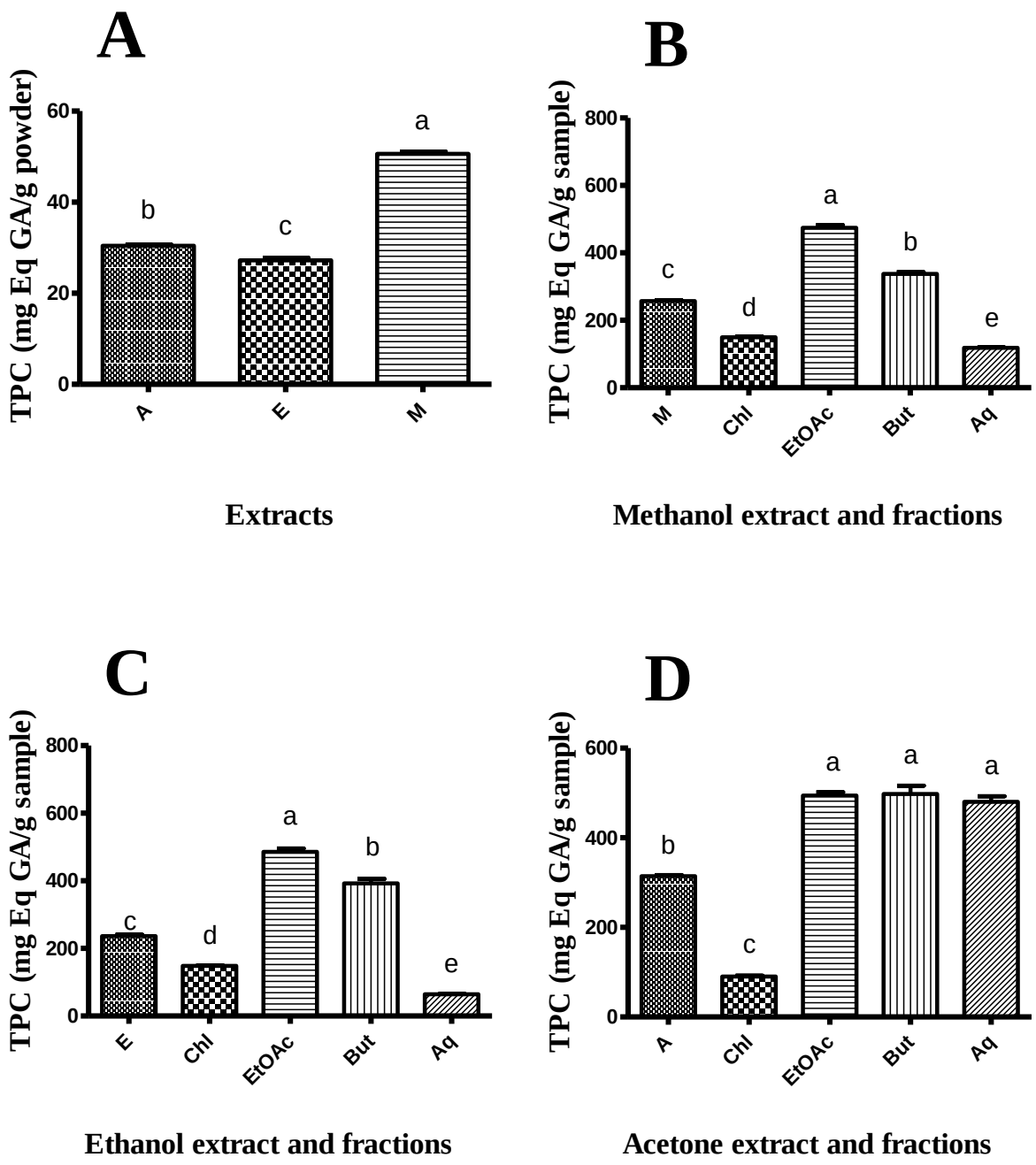

Figure 12. Total polyphenol content in *C. atlantica* branch extracts and fractions.

A: Acetone, E: Ethanol, M: Methanol, Chl: Chloroform, EtOAc: Ethyl acetate, But: n-Butanol, Aq: Aqueous. For each graph, values followed by different letter were significantly different ($P<0.05$). Values were expressed as mean ± SD (n=3).

The TPC values in the different fractions of the methanolic extract obtained from the powder of *C. atlantica* branches were as follows: 149.75±4.2, 474.39±13.34, 337.57±8.86 and 118.42±1.94 mg Eq GA/g of each fraction: Chl, EtOAc, But and Aq ($P < 0.05$); respectively (**Fig. 12B**). Similar tendencies were observed with the fractions of the ethanolic extract with TPC values of 148±2.72, 485.45±17.32, 392.12±23.41 and 64.12±1.77 mg Eq GA/g of each fraction: Chl, EtOAc, But and Aq ($P < 0.05$); respectively (**Fig. 12C**). Nevertheless, But, EtOAc, and Aq had the highest TPC values for the acetonic extract, at 497.87±31.96 mg Eq GA/g fraction, 494.24±12.73 mg Eq GA/g fraction and 480.30±21.55 mg Eq GA/g fraction ($P > 0.05$), respectively; followed by Chl at 90.42±2.97 mg Eq GA/g fraction ($P < 0.05$) (**Fig. 12D**).

TPC levels were higher in the Aq fraction of the acetonic extract than in the methanolic and ethanolic. As a result, the most of phenolic compounds are found in intermediate and polar fractions. The aforementioned results indicate that the extracted polyphenols have a better affinity mainly to intermediate and polar solvents. There has been no data published on the phytochemical investigation of *C. atlantica* branch extracts. However, Hofmann et al. (2020) reported significantly lower TPC for hydro-organic extracts of *C. atlantica* green cones obtained using an ultrasonic bath with acetone:water 80:20 v/v, ethanol:water 80:20 v/v, and methanol:water 80:20 v/v. Similarly, lower TPC levels have been reported for Atlas cedar tar traditionally produced by pyrolysis (Skanderi and Chouitah 2020).

III.1.4. Flavonoid content

The FC in *C. atlantica* extracts were determined using the aluminium chloride method, with Quercetin as a standard (**Fig. 13**). FC values were obtained from the calibration curve: Abs=0.029 [Q]-0.025, R^2=0.997. The highest FCs were achieved by ethanol and acetone ($P > 0.05$) with 5.89±0.04 mg Eq Q/g extract and 5.38±0.83 mg Eq Q/g extract, respectively. Methanol produced the lowest FC value of 2.47±0.27 mg Eq Q/g extract (**Fig. 13A**). Nonetheless, these levels were too low in comparison to the recorded TPC values, indicating that organic extracts from *C. atlantica* branches enclose small quantities of flavonoids. Furthermore, as shown in **Table XXI**, there was a weak correlation between the FC and TPC levels with a coefficient of determination (R^2) of 0.248.

For the methanolic extract (**Fig. 13B**), EtOAc had the highest FC level at 18.78±0.28 mg Eq Q/g fraction ($P < 0.05$), followed by Chl and But at 3.7±0.41 mg Eq Q/g fraction and 3.73±0.41 mg Eq Q/g fraction ($P > 0.05$), respectively. The Aq fraction had the lowest value at 2.98±0.07 mg Eq Q/g fraction ($P < 0.05$). A similar pattern was observed with ethanolic extract fractions, with EtOAc having the highest FC value at 18.20±0.52 mg Eq Q/g fraction ($P < 0.05$); followed by Chl, But, and Aq at 5.03±0.53 mg Eq Q/g fraction, 4.21±1.21 mg Eq Q/g fraction and 1.80±0.90 mg Eq Q/g fraction ($P > 0.05$), respectively (**Fig. 13C**). Likewie, the EtOAc fraction obtained from the acetonic extract had the highest FC level of 12.50±0.21 mg Eq Q/g fraction ($P < 0.05$); followed by Chl, But, and Aq at 6.18±0.94 mg Eq Q/g fraction, 4.09±0.59 mg Eq Q/g fraction and 0.85±0.14 mg Eq Q/g fraction ($P < 0.05$), respectively (**Fig. 13D**). However, Fadel et al. (2016) reported significantly higher FC from a crude hydro-ethanolic extract obtained by maceration of *C. atlantica* aerial parts. Indeed, flavonoids were found in higher concentrations in leaves and needles than in other plant parts (Jaakola and Hohtola 2010; Julkunen-Tiitto et al. 2015).

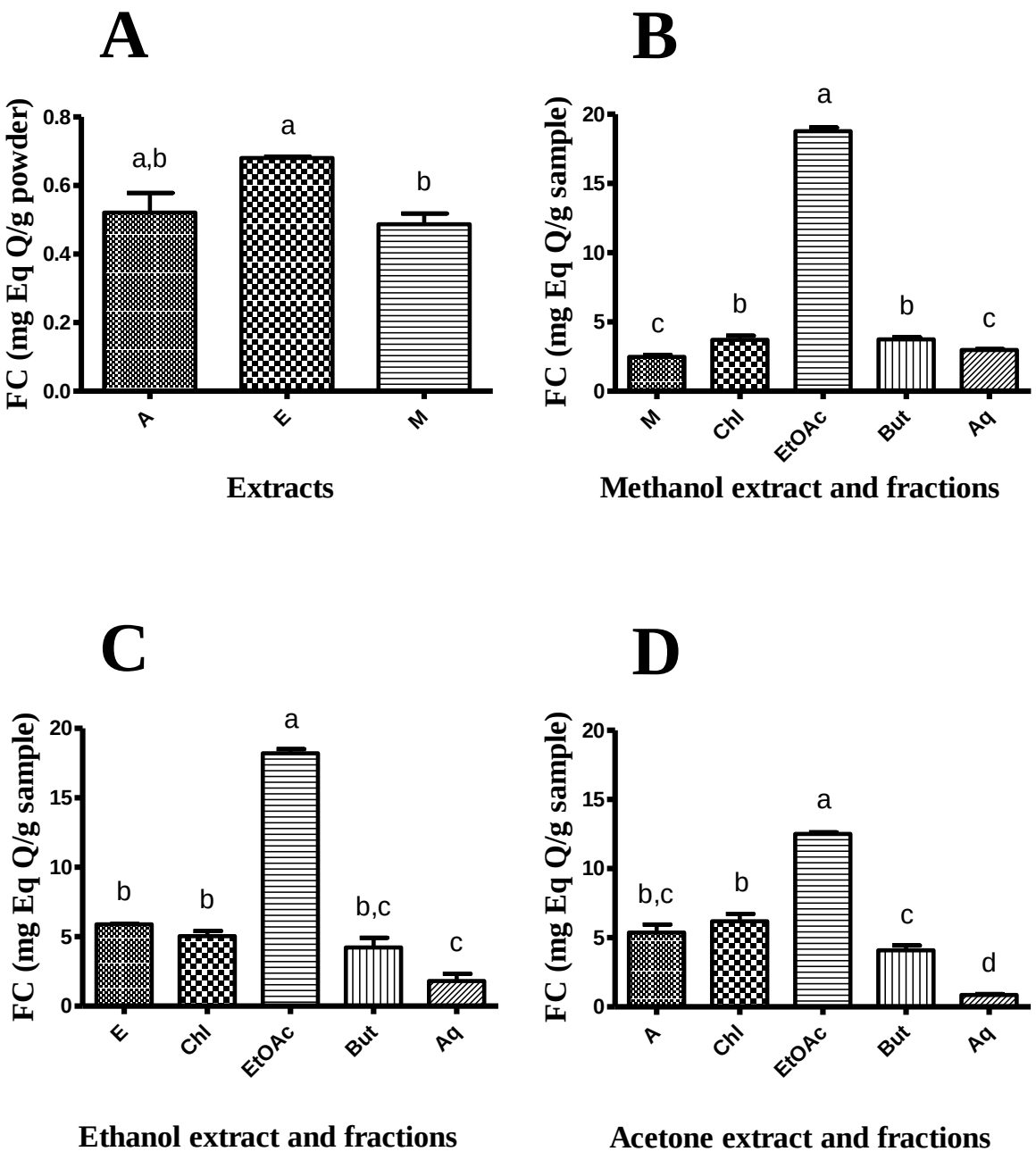

Figure 13. Flavonoid content in *C. atlantica* branch extracts and fractions.

A: Acetone, E: Ethanol, M: Methanol, Chl: Chloroform, EtOAc: Ethyl acetate, But: n-Butanol, Aq: Aqueous. For each graph, values followed by different letter were significantly different ($P<0.05$). Values were expressed as mean ± SD (n=3).

III.1.5. Condensed tannin content

The CTC in *C. atlantica* extracts were determined by the vanillin method, with Catechin as a standard (**Fig. 14**). CTC values were obtained from the calibration curve using simple regression analysis: Abs=0.002 [C] +0.038; R^2=0.992. The results showed that the solvent nature had a significant effect on CTC ($P < 0.05$), with 143.5±12.72 mg Eq C/g extract, 110.83±4.25 mg Eq C/g extract and 126.33±12.51 mg Eq C/g extract recorded for methanol, ethanol and acetone extracts, respectively (**Fig. 14A**).

The highest CTC value for the methanolic fractions (**Fig. 14B**) has been recoded for EtOAc at 504.83±18.9 mg Eq C/ g fraction ($P < 0.05$); followed by But, Aq, and Chl at 366±18.75 mg Eq C/ g fraction, 88.66±4.25 mg Eq C/ g fraction and 44±4.76 mg Eq C/ g fraction ($P < 0.05$), respectively. However, the EtOAc and But fractions of the ethanolic extract had the highest CTC levels, at 443.00±45.26 mg Eq C/ g fraction and 446.00±24.29 mg Eq C/ g fraction ($P < 0.05$), respectively; followed by Chl at 71.50±1.01 mg Eq C/ g fraction ($P< 0.05$), and Aq at 40.16±4.07 mg Eq C/ g fraction ($P < 0.05$) (**Fig. 14C**). On the other hand, for the acetonic extract, But fraction had the highest CTC value at 566.50±24.28 mg Eq C/ g fraction ($P < 0.05$), followed by EtOAc, Aq and Chl at 475.83±30.92 mg Eq C/ g fraction, 428.84±10.27 mg Eq C/ g fraction and 63.83±8.51 mg Eq C/ g fraction ($P < 0.05$), respectively (**Fig. 14D**). To the best of our knowledge, no data on CTC of extracts from *C. atlantica* branches have been published in the literature. However, Skanderi and Chouitah (2020) found that tar produced by dry distillation of wood had much lower CTC of 4.41±0.05 mg Eq C /g.

With a coefficient of determination R^2=0.948, CTC was found to be strongly correlated with TFC (**Table XXI**). This would be due to the fact that polyphenols extracted from *C. atlantica* branches were mostly condensed tannins.

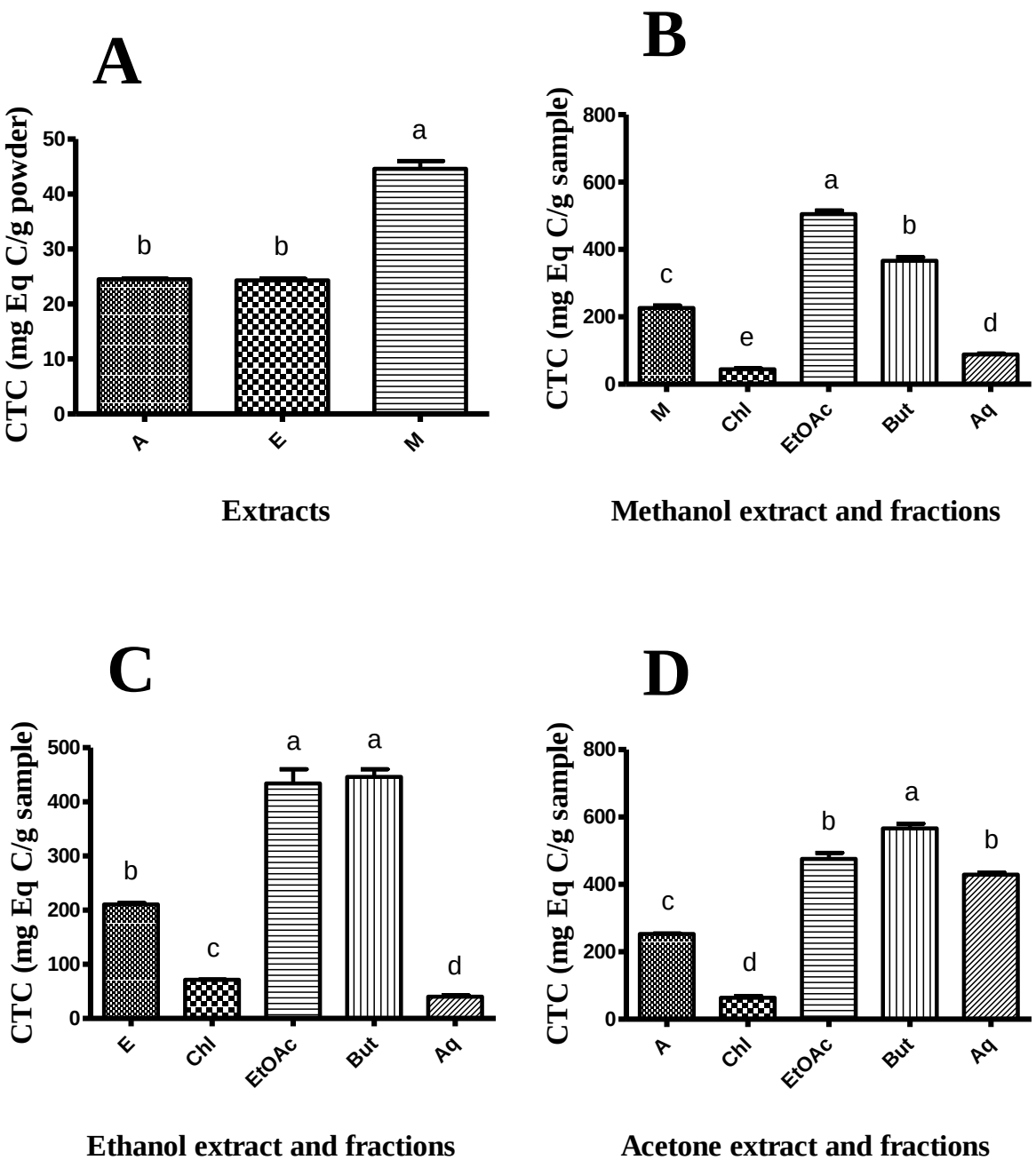

Figure 14. Condensed tannins content in *C. atlantica* branch extracts and fractions.

A: Acetone, E: Ethanol, M: Methanol, Chl: Chloroform, EtOAc: Ethyl acetate, But: n-Butanol, Aq: Aqueous. For each graph, values followed by different letter were significantly different ($P<0.05$). Values were expressed as mean ± SD (n=3).

III.2. Biological activities evaluation

III.2.1. Antioxidant activity evaluation

There is no standardized method for evaluating plant extract antioxidant capacity. Therefore, using more than one method to provide more comprehensive information is recommended (Todorovic et al. 2015). In the current study, cones' essential oil, methanolic, ethanolic and acetonic extracts and fractions of *C. atlantica* branches were investigated for their *in vitro* antioxidant activity using three methods: antiradical scavenging activity (DPPH and ABTS assays) and ferric reducing antioxidant power (FRAP).

III.2.1.1. DPPH• radical scavenging capacity

DPPH• radical scavenging ability was measured in terms of the percentage of free radical DPPH• inhibited by antioxidants present in the various samples. The results are shown in **Figure 15**. All extracts, fractions, and essential oil scavenged the free radical DPPH• in a concentration dependent manner (**Fig. 15A**). The essential oil had a low antioxidant activity. Only the hydrodistilled essential oils from the cones and branches have been studied for their ability to scavenge DPPH• free radicals (Paun et al. 2013, Inaam et al. 2015). In fact, the essential oil of the cones had a 45% inhibition percentage (Paun et al. 2013).

Among the extracts, at the concentration of 12.5 µg/ml, the, methanolic, ethanolic, and acetonic extracts inhibited the free radical DPPH• at 43.84±1.18%, 38.55±6.94% and 39.95±7.28% ($P > 0.05$), respectively. On the other hand, standards, Vit C and BHA had the highest levels at 92.22±3.09% and 66.08±1.45% ($P < 0.05$), respectively. At the concentration of 25 µg/mL, Vit C showed the highest levels at 95.72±0.11% ($P < 0.05$); followed by the acetonic, ethanolic and methanolic extracts at 83.53±0.09%, 82.63±0.40% and 80.31±0.79% ($P > 0.05$), respectively; and BHA at 79.28±1.24% ($P > 0.05$).

Among the fractions obtained from the methanolic extract (**Fig. 15B**), at the concentration of 12.5 µg/ml, EtOAc exhibited the highest percentage of inhibition at 71.08±0.48% ($P < 0.05$); followed by But at 57.67±0.71% ($P < 0.05$); then Chl and Aq at 18.12±0.97% and 14.96±0.87% ($P < 0.05$), respectively. Similar trends were observed in the ethanolic extract fractions, with EtOAc and But having the highest percentages, followed by Chl and Aq (**Fig. 15C**). The Aq fraction partitioned from the acetonic extract, on the other hand, showed similar percentages of inhibition as EtOAc and But ($P > 0.05$) (**Fig. 15D**).

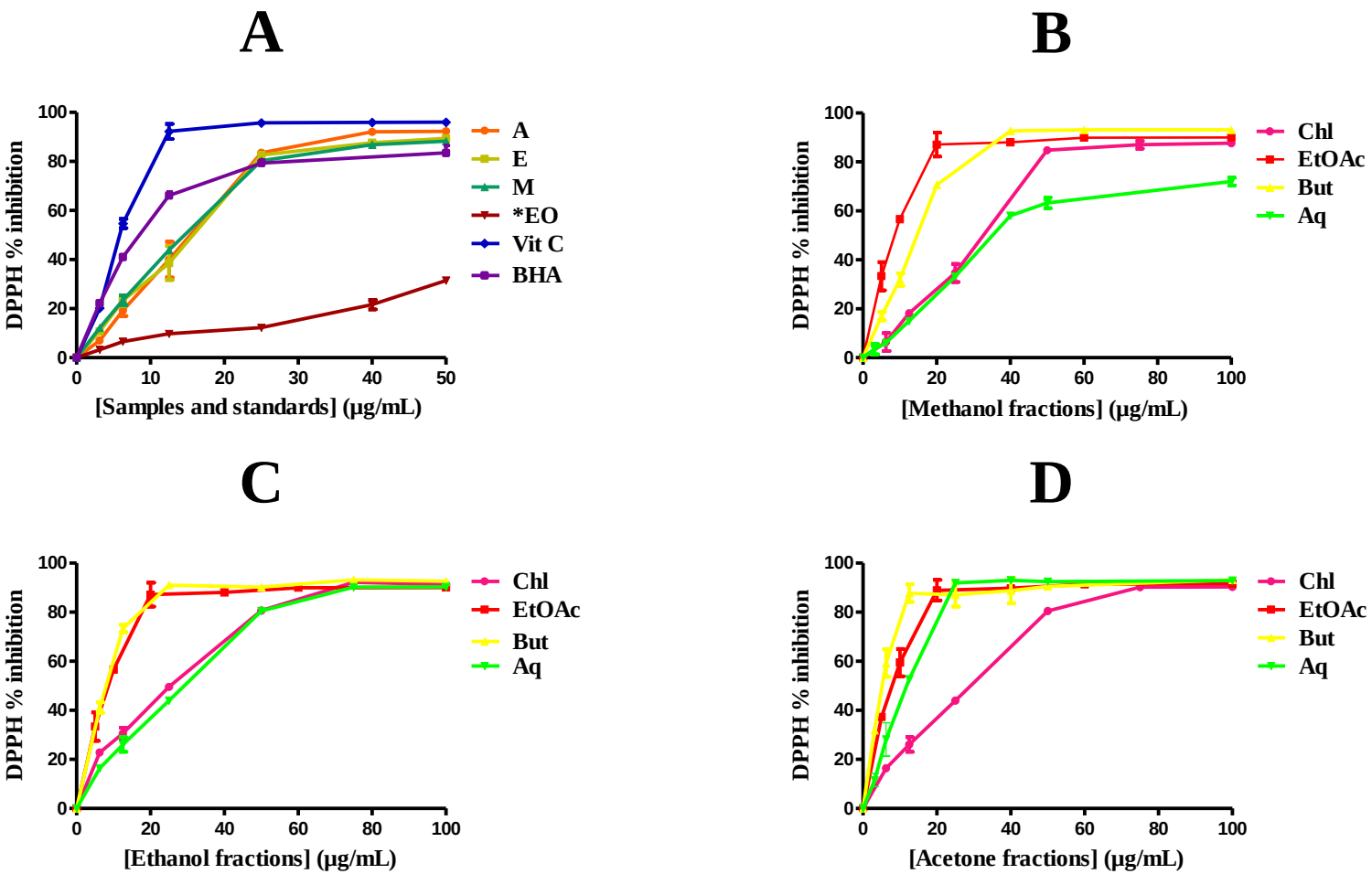

Figure 15. DPPH$^{\bullet}$ percentage inhibition of the standards and *C. atlantica* essential oil, extracts and fractions.

A: Acetone, E: Ethanol, M: Methanol, EO: Essential oil, Chl: Chloroform, EtOAc: Ethyl acetate, But: n-Butanol, Aq: Aqueous.* [EO] in mg/mL. Values were expressed as mean ± SD (n=3).

The respective IC_{50} values recorded for the standards, extracts and fractions were exhibited in **Figure 16**. The lowest IC_{50} value reflects the highest antioxidant activity. The essential oil's IC_{50} value was 133.67±5.12 mg/ml. However, it was lower than the essential oil of the branches, which was 315.85±0.97 mg/mL (Inaam et al. 2015).

All extracts had similar IC_{50} values of 13.05±0.31 µg/ml, 14.04±1.51 µg/ml and 14.05±1.39 µg/ml ($P > 0.05$), for the methanolic, ethanolic and acetonic, respectively (**Fig. 16A**). Among the fractions obtained from the methanolic extract (**Fig. 16B**), EtOAc demonstrated the best antioxidant capacity to scavenge the free radical $DPPH^{\bullet}$ with an IC_{50} value of 6.83±0.66 µg/ml ($P < 0.05$); followed by But with an IC_{50} value of 8.24±0.36 µg/ml ($P < 0.05$); and then Chl and Aq with IC_{50} values of 28.52±1.50 µg/ml and 26.70±1.19 µg/ml ($P > 0.05$), respectively. However, EtOAc and But of the ethanolic extract had the lowest IC_{50} values of 6.91±0.67 µg/ml and 6.87±0.19 µg/ml ($P > 0.05$), respectively; followed by Aq and Chl with IC_{50} values of 28.91±0.42 µg/ml and 30.43±0.24 µg/ml ($P > 0.05$), respectively (**Fig. 16C**). Finally, the EtOAc fraction obtained from the acetonic extract demonstrated the best antioxidant scavenging capacity of the free radical $DPPH^{\bullet}$ with an IC_{50} value of 6.16±0.01 µg/ml ($P < 0.05$), comparable to the standards BHA and Vit C with IC_{50} values of 6.03±0.13 µg/ml and 5.47±0.13 µg/ml ($P < 0.05$), respectively; followed by But with an IC_{50} value of 6.84±0.44 µg/ml ($P < 0.05$) (**Fig. 16D**). The Aq fraction exhibited the highest antioxidant activity compared to those fractionated from methanolic and ethanolic extracts with an IC_{50} value of 10.79±0.53 µg/ml ($P < 0.05$). Chl, on the other hand, had the lowest IC_{50} value of 31.83±4.09 µg/ml ($P < 0.05$). Therefore, the aforementioned results demonstrated that the extracts and fractions obtained from *C. atlantica* branches had strong antioxidant ability by scavenging the free radical $DPPH^{\bullet}$. Comparable results have been reported in the literature. In fact, with an IC_{50} value of 8.9 µg/ml, the hydro-ethanolic extract macerated from the aerial parts demonstrated a strong antioxidant power to scavenge the free radical $DPPH^{\bullet}$ (Fadel et al. 2016). The hydro-methanolic extract of cones exhibited a similar pattern, with an IC_{50} value of 14.91±2.00 µg/ml. The $DPPH^{\bullet}$ free radical scavenging activity of the ethanolic extract from seeds, on the other hand, was lower, with an IC_{50} value of 0.4 mg/ml (Naimi et al. 2015).

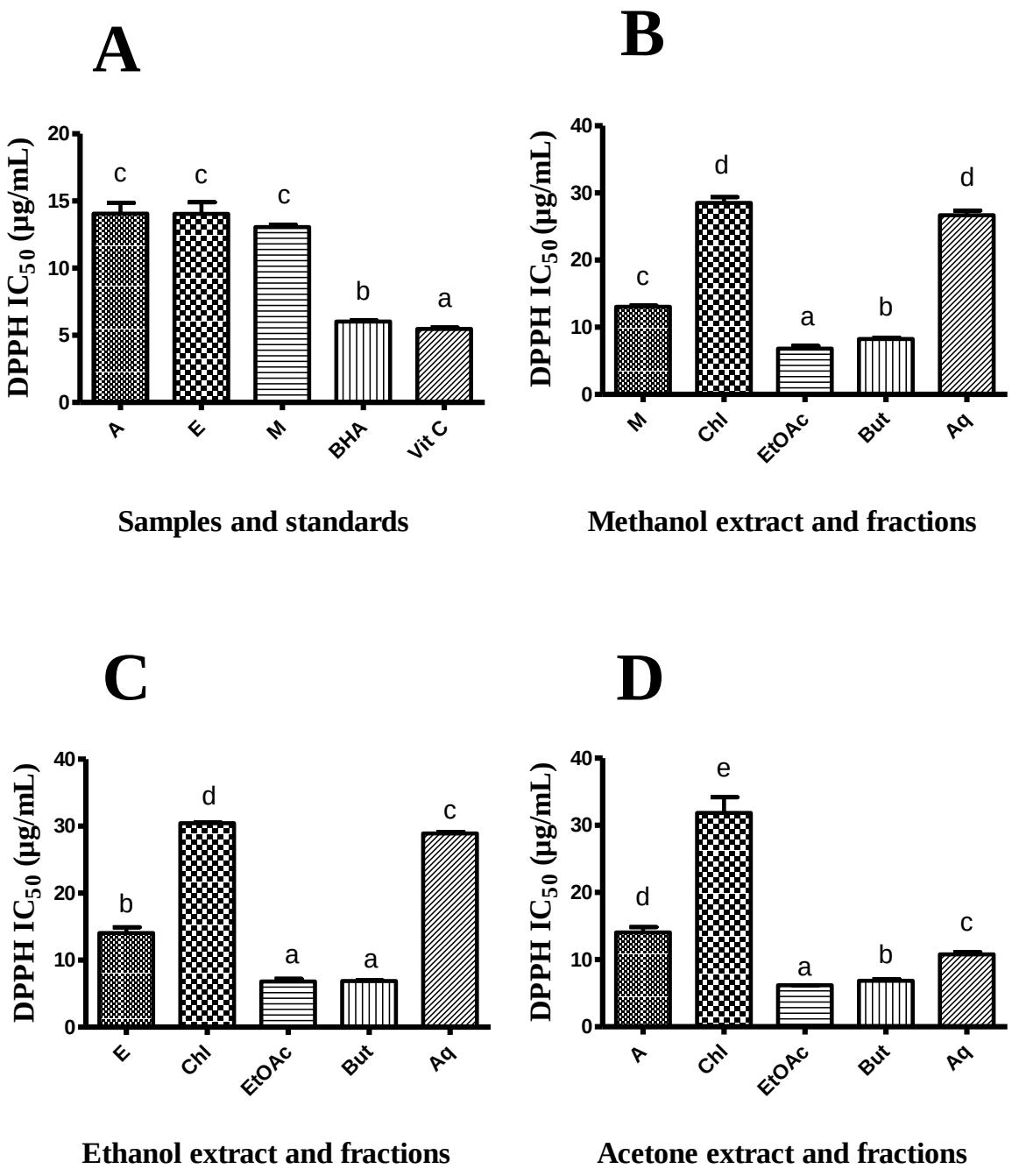

Figure 16. DPPH$^{\bullet}$ IC_{50} values of the standards and *C. atlantica* extracts and fractions.

A: Acetone, E: Ethanol, M: Methanol, Chl: Chloroform, EtOAc: Ethyl acetate, But: n-Butanol, Aq: Aqueous.. Values were expressed as mean ± SD (n=3). For each graph, values followed by different letter were significantly different ($P < 0.05$).

III.2.1.2. ABTS$^{\bullet+}$ radical scavenging capacity

The ABTS assay was carried out on the various extracts and fractions obtained from *C. atlantica*. **Figure 17** represents the results in terms of inhibition percentages.

The essential oil, extracts and fractions inhibited the free radical ABTS$^{\bullet+}$ in concentration dependent manner. The essential oil had a weak antioxidant capacity. Among the extracts (**Fig. 17A**), at the concentration of 100 µg/ml, the acetonic extract scavenged the highest percentage at 63.68±0.33% ($P < 0.05$); followed by the methanolic and ethanolic extracts at 48.43±0.35% and 47.38±0.85% ($P > 0.05$), respectively. However, the standards, Vit C and BHA, had the highest levels at 99.32±0.26% and 98.51±0.48% ($P > 0.05$), respectively.

The fractions partitioned from the three organic extracts with the highest antioxidant activity were EtOAc and But. In fact, at the concentration of 100 µg/ml, 97.71±0.43% and 67.56±0.59% ($P < 0.05$) have been inhibited respectively by EtOAc and But obtained from the methanolic extract (**Fig. 17B**); followed by Chl and Aq with 34.32±1.35% and 28.23±0.79% ($P < 0.05$), respectively. A similar trend was observed with the ethanolic extract fractions (**Fig. 17C**), where EtOAc and But had the highest percentages at 94.70±1.66% and 84.08±2.17% ($P < 0.05$), respectively; followed by Chl and Aq ($P < 0.05$). However, the EtOAc, But, and Aq fractions of the acetonic extract (**Fig. 17D**), had the highest percentages. The Chl fraction, on the other hand, had the lowest scavenging activity against the free radical ABTS$^{\bullet+}$.

The IC_{50} values recorded for the essential oil, extracts and their fractions were presented in **Figure 18**. The essential oil exhibited an IC_{50} value of 305.93±35.08 mg/ml ($P < 0.05$). According to solvent nature, the capacity of the extracts to scavenge the free radical ABTS$^{\bullet+}$ didn't show significant difference, with IC_{50} values of 158.4±8.57 µg/ml, 160.90±8.02 µg/ml and 147.46±3.91 µg/ml ($P > 0.05$) for the methanolic, ethanolic and acetonic, respectively (**Fig. 18A**).

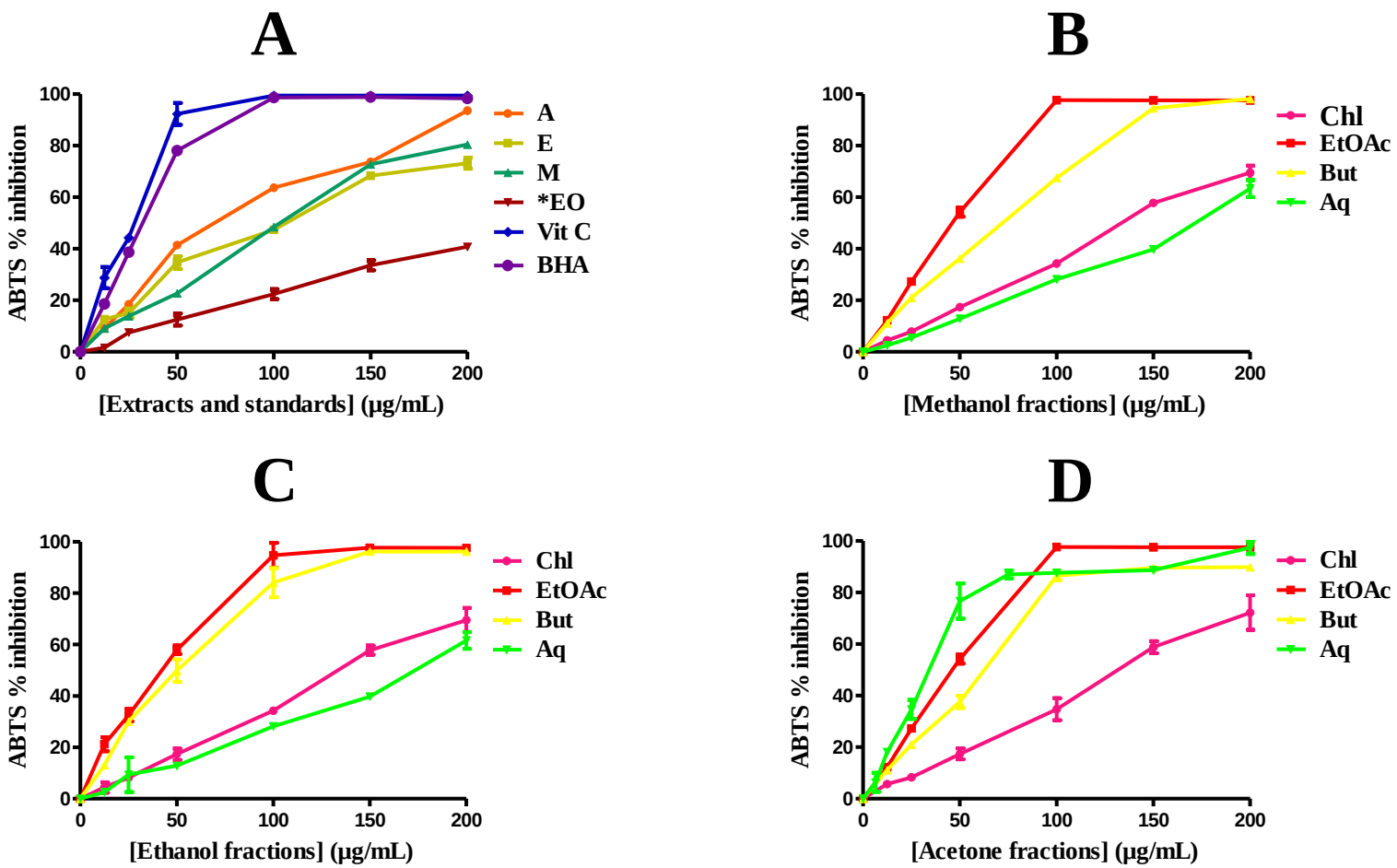

Figure 17. ABTS$^{\bullet+}$ percentage inhibition of the standards and *C. atlantica* essential oil, extracts and fractions.

A: Acetone, E: Ethanol, M: Methanol, EO: Essential oil, Chl: Chloroform, EtOAc: Ethyl acetate, But: n-Butanol, Aq: Aqueous. *[EO] in mg/mL. Values were expressed as mean ± SD (n=3).

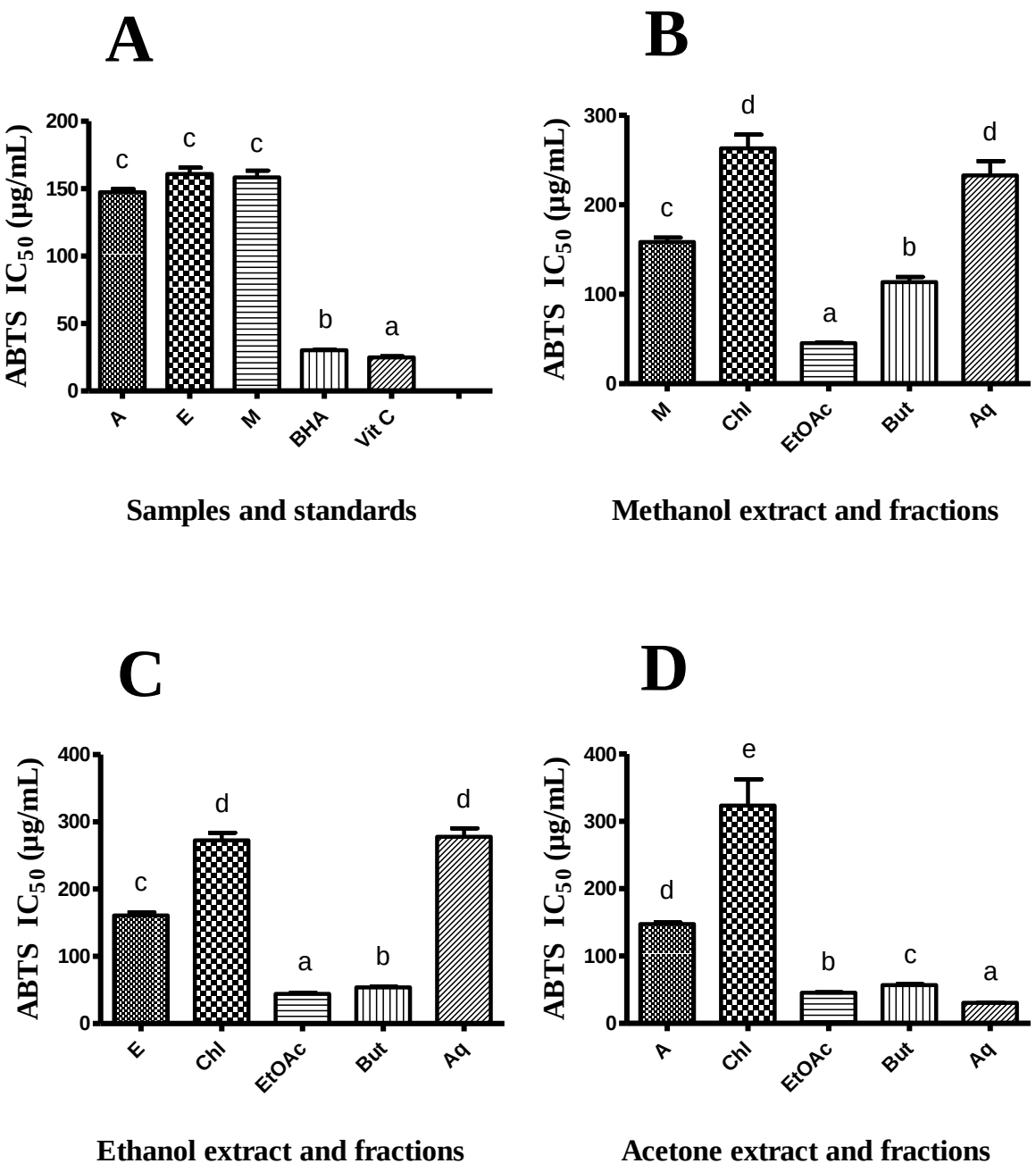

Figure 18. ABTS$^{\bullet+}$ IC_{50} values of the standards and *C. atlantica* extracts and fractions.

A: Acetone, E: Ethanol, M: Methanol, Chl: Chloroform, EtOAc: Ethyl acetate, But: n-Butanol, Aq: Aqueous..Values were expressed as mean ± SD (n=3). For each graph, values followed by different letter were significantly different ($P < 0.05$).

Among the methanolic extract fractions (**Fig. 18B**), EtOAc had the highest activity with an IC_{50} value of 45.34±1.53 µg/ml ($P < 0.05$); followed by But with an IC_{50} value of 113.53±10.17 µg/ml ($P < 0.05$); then Chl and Aq with IC_{50} values of 263.03±26.12 µg/ml and 232.03±27.97 µg/ml ($P > 0.05$), respectively. A similar trend was observed with the ethanolic extract fractions (**Fig. 18C**), where, EtOAc exhibited the highest antioxidant ability with an IC_{50} value of 44.18±3.03 µg/ml ($P < 0.05$); followed by But with an IC_{50} value of 53.00±1.68 µg/ml ($P < 0.05$); then Chl and Aq with IC_{50} values of 272.36±20.02 and 277.8±22.17 µg/ml ($P > 0.05$), respectively. On the other hand, for the acetonic extract fractions (**Fig. 18D**), the Aq fraction had the highest antioxidant capacity, with an IC_{50} value of 30.53±0.37 µg/ml ($P < 0.05$), which was close to values recorded for the standards BHA and Vit C, which were 30.23±0.54 µg/ml and 24.92±1.32 µg/ml ($P < 0.05$), respectively. The IC_{50} values for EtOAc, But, and Chl were 45.34±1.53 µg/ml, 56.93±2.21 µg/ml and 323.36±67.91 µg/ml ($P < 0.05$), respectively.

III.2.1.3. Ferric reducing antioxidant power

The FRAP test is an electron transfer based-assay. The pH was kept at 3.6 in order to increase iron solubility and allow for important drive electron transfer (Berdahl and McKeague 2015). The essential oil had a ferric reducing power value of 41.32±0.21 mg Eq Vit C/g sample ($P < 0.05$). There were significant differences ($P < 0.05$) based on the nature of the solvent (**Fig. 19A**). The acetonic and ethanolic extracts had the highest reducing capacity with 388.80±9.09 mg Eq Vit C/g extract, and 368.80±11.11 mg Eq Vit C/g extract, followed by the methanolic extract with 325.95±5.05 mg Eq Vit C/g extract ($P < 0.05$). The FRAP value of the cones' hydro-methanolic extract was 24.19±0.45 mg Eq AA/g dw (Hofmann et al. 2020).

Among the fractions, EtOAc fractionated from the methanolic extract (**Fig. 19B**), showed the best ferric reducing antioxidant power with 692.38±37.96 mg Eq Vit C/g fraction ($P < 0.05$); followed by But, Chl and Aq with 459.52±17.04 mg Eq Vit C/g fraction, 190.71±10.54 mg Eq Vit C/g fraction and 142.14±1.51 mg Eq Vit C/g fraction ($P < 0.05$), respectively. Similarly, EtOAc fraction obtained from the ethanolic extract had the highest FRAP value of 632.85±16.74 mg Eq Vit C/g fraction ($P < 0.05$); followed by But, Chl and Aq, which had 518.09±38.67 mg Eq Vit C/g fraction, 198.45±13.47 mg Eq Vit C/g fraction and 77.26±6.48 mg Eq Vit C/g fraction ($P < 0.05$), respectively (**Fig. 19C**).

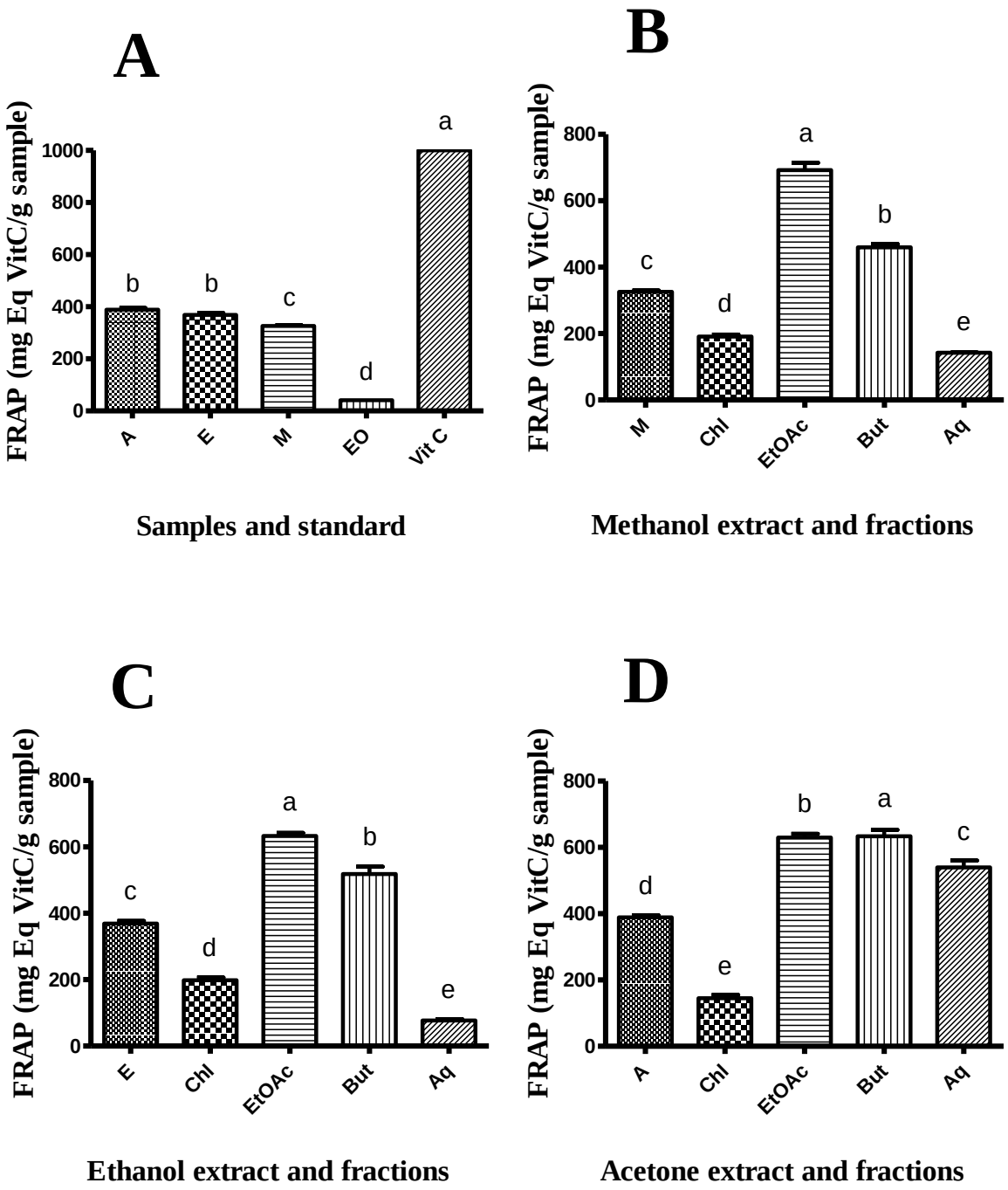

Figure 19. FRAP values of *C. atlantica* essential oil, extracts and fractions.

A: Acetone, E: Ethanol, M: Methanol, EO: Essential oil, Chl: Chloroform, EtOAc: Ethyl acetate, But: n-Butanol, Aq: Aqueous. For each graph, values followed by the same letter were not significantly different ($P<0.05$). Values were expressed as mean ± SD (n=3).

However, But and EtOAc fractions partitioned from the acetonic extract (**Fig. 19D**), showed the highest ferric reducing antioxidant capacity with 633.80±5.19 mg Eq Vit C/g fraction and 629.52±3.17 mg Eq Vit C/g fraction ($P > 0.05$), respectively; followed by Aq with 539.52±6.78 mg Eq Vit C/g fraction ($P < 0.05$); and Chl with 144.95±17.44 mg Eq Vit C/g fraction ($P < 0.05$).

III.2.1.4. Correlation between phenolic compounds contents and antioxidant activity

The antioxidant activity results obtained using various methods demonstrated a strong positive correlation with coefficient of determination values (R^2) of 0.915, 0.927 and 0.875 between ABTS-FRAP, DPPH-ABTS and DPPH-FRAP, respectively **(Table XXI)**. These findings demonstrated that *C. atlantica* is a good source of potent antioxidant components. TPC and TC levels were found to have a strong correlation with antioxidant activity (DPPH, ABTS and FRAP). FC, on the other hand, had a weak correlation with the three antioxidant assays. As a result, it could be suggested that the antioxidant capacity was mainly attributed to polyphenols, especially to condensed tannins and to a lesser extent to flavonoids.

Table XXI: Correlation matrix between phenolic compounds contents (TPC, FC, and CTC) and antioxidant activity (DPPH, ABTS, and FRAP).

	TPC	**FC**	**CTC**	**DPPH**	**ABTS**	**FRAP**
TPC	1	-	-	-	-	-
FC	0.248	1	-	-	-	-
CTC	0.948	0.206	1	-	-	-
DPPH	0.855	0.173	0.883	1	-	-
ABTS	0.942	0.180	0.922	0.927	1	-
FRAP	0.969	0.346	0.950	0.875	0.915	1

Coefficients of determination (R^2 values)

III.2.2. Antibacterial activity evaluation

The preliminary *in vitro* antibacterial activity results of the methanolic extract and fractions against six bacterial strains using the agar disc diffusion method are shown in **Table XXII**. The antibacterial susceptibilities of the tested bacterial strains to the various fractions and the methanolic extract differed. The crude extract had little activity against the Gram-positive bacterial strains *Staphylococcus aureus* ATCC 25923 and *Listeria innocua*. Gram negative strains, on the other hand, were resistant. However, *S. aureus* was found to be the most sensitive strain, with a zone of inhibition diameter of 25 mm measured for the EtOAc fraction at 60 mg/mL (**Fig. 20**). Likewise, at 60 mg/mL, EtOAc fraction inhibited the growth of *Escherichia coli* ATCC 25921, *Listeria innocua*, and *Bacillus cereus* ATCC 11778 with inhibition zone diameters of 19 mm, 12 mm, and 10 mm, respectively. The But fraction, however, demonstrated antibacterial activity against *S. aureus* ATCC 25923, *B. cereus* ATCC 11778, and *E. coli* ATCC 25921, with inhibition zone diameters of 19 mm, 13.5 mm, and 14 mm, respectively, at 60 mg/ml. The antibacterial activity of the aq fraction was the lowest against *S. aureus* ATCC 25923 and *E. coli* ATCC 25921. The Chl fraction, on the other hand, had no effect on the six bacterial strains.

Table XXII: Antibacterial effect of the *C. atlantica* methanolic extract and its fractions against the tested bacteria.

Samples	Concentration (mg/mL)	Inhibition zone diameter (mm)					
		Gram positive			Gram negative		
		S. aureus	*B. cereus*	*L. innocua*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
M	60	13	7	11,5	6	7	6
	30	9,5	6	10	6	6	6
	15	7,5	6	9	6	6	6
	7.5	6	6	6	6	6	6
	3.75	6	6	6	6	6	6
Chl	60	6	6	6	6	6	6
	30	6	6	6	6	6	6
	15	6	6	6	6	6	6

	7.5	6	6	6	6	6	6
	3.75	6	6	6	6	6	6
EtOAc	60	25	10	12	19	9	9
	30	16	9	8	16	6,5	7,5
	15	12	7	6	13,5	6	6
	7.5	10	6	6	8	6	6
	3.75	8	6	6	6	6	6
But	60	19	13,5	6	14	9	6
	30	12	12	6	11	8	6
	15	10	9,5	6	9,5	7	6
	7.5	8	7	6	7,5	6	6
	3.75	6	6	6	6	6	6
Aq	60	11,5	8	8	10	6	6
	30	8	6	6	8	6	6
	15	6	6	6	8	6	6
	7.5	6	6	6	6	6	6
	3.75	6	6	6	6	6	6

M: Methanol; Chl: Chloroform; EtOAc: Ethyl acetate ; But: n-Butanol; Aq: Aqueous.

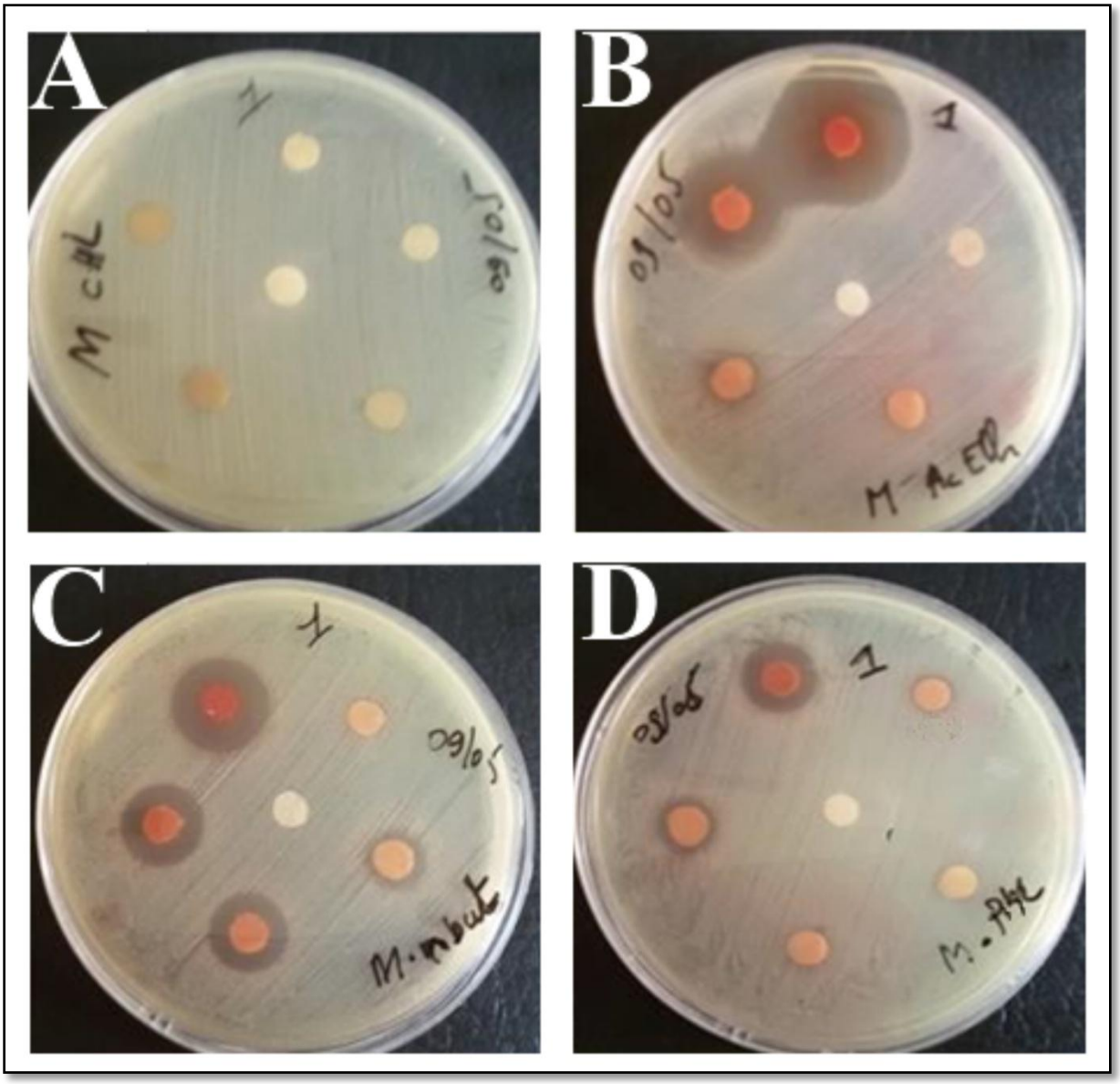

Figure 20. Effect of the methanolic fractions at different concentrations on *S. aureus*: A) Chl, B) EtOAc, C) But, and D) Aq.

DMSO was used as negative control (Central disc).
Chl: Chloroform; EtOAc: Ethyl acetate; But: n-Butanol; Aq: Aqueous.

As a result, the broth microdilution assay for MIC and MBC determination of the methanolic fractions (EtOAc, But, and Aq) was performed on three bacterial strains: *S. aureus* ATCC 25923, *B. cereus* ATCC 11778, and *E. coli* ATCC 25921. The results of the *in vitro* antibacterial activity of the essential oil and the methanolic extract fractions (EtOAc, But, and Aq) against the three bacterial strains, as determined by the broth microdilution method, were shown in **Table XXIII**.

Table XXIII: Minimum inhibitory and bactericidal concentrations of the various plant samples and gentamicin against the tested bacteria.

Samples	*S. aureus*		*B. cereus*		*E. coli*	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
EtOAc	62.5[a]	125[a]	125[a]	250[a]	250[a]	1000[a]
But	62.5[a]	125[a]	250[a]	125[a]	250[a]	1000[a]
Aq	500[a]	1000[a]	1000[a]	2000[a]	1000[a]	2000[a]
EO	0.25[b]	0.5[b]	0.25[b]	0.5[b]	0.5[b]	1[b]
G	6.25[a]	6.25[a]	6.25[a]	12.5[a]	12.5[a]	25[a]

EtOAc: Ethyl acetate, But: n-Butanol, Aq: Aqueous, EO: Essential oil, G: Gentamicin
[a] Values in µg/ml
[b] Values in % (v/v)
n=3

The antibiotic gentamicin was effective against all strains. The essential oil had an antibacterial effect against all Gram positive and Gram negative bacteria tested, with MIC values of 0.25 and 0.5 percent (v/v) and MBC values of 0.5 and 1 percent (v/v), respectively. It seems reasonable to assume that this activity is attributable to monoterpene hydrocarbons, mainly α-pinene, found as major components in the analyzed oil. Minor components, on the other hand, may have some type of synergism with the active compounds (Marino et al. 2001; Rahman et al. 2011). There have been no reports in the literature on the antibacterial activity of cones-derived essential oil from *Cedrus atlantica*. However, some antimicrobial studies on *C. atlantica* essential oil derived from wood (Aberchane et al. 2003; Hammer et al. 1999; Zrira and Ghanmi 2016) and leaves (Derwich et al. 2010) have been conducted. To some extent, these findings were in agreement with the previous study performed by Derwich et al. (2010) on essential oil extracted from *C. atlantica* leaves grown in Morocco, finding that the main component was α-pinene. Similarly, several studies of essential oils rich in α-pinene revealed potential antibacterial activities (Magiatis et al. 1999, Dorman and Deans 2000, Derwich et al. 2010). Terpenes have been shown to attack cell membranes and cause toxicity

via chemiosmosis control dysfunction (Cox et al. 2001; Inoue et al. 2004; Rao et al. 2010). Furthermore, according to Bouyahya et al. (2017), the essential oil may modulate operon expression by inhibiting the mediators of self-inducers, as well as acting on the cell membrane, disrupting the cell's energy status, metabolic regulation, membrane-coupled energy transduction process and solute transport.

The MIC and MBC values of the tested fractions (EtOAc, But, and Aq) obtained from the methanolic extract, on the other hand, ranged from 31.25 µg/ml to 1000 µg/ml and 62.5 µg/ml to 2000 µg/ml, respectively. The broth microdilution assay yielded much lower active concentrations than the agar disc-diffusion testing. This could be because the substances should diffuse in the agar medium based on their physicochemical properties, in order to create a concentration gradient around the disc (Jorgensen and Turnidge 2015). According to our findings, the most active fractions were EtOAc and But, which had nearly identical activities. Our preliminary research revealed that the most apolar fraction isolated with chloroform had no antibacterial effect (**Table XXII**). This indicates that the most potent antibacterial agents were found in fractions obtained using intermediate polarity solvents. These fractions contained a high concentration of polyphenols (mainly tannins). This suggests that polyphenols, especially tannins, may be responsible for this activity. These phytochemical compounds from various plant sources were well known for their effective antimicrobial activities (Mendez et al. 2012). Scalbert (1991) reported several antimicrobial mechanisms of tannins, including: [1] enzyme inhibition and substrate deprivation due to their astringent character and chemical structure; [2] action on membranes via oxidative phosphorylation and electron transport system inhibition; and [3] metal ion deprivation via tannins-metal precipitates. To the best of our knowledge, no research on the antibacterial activity of *C. atlantica* branch extracts has been published. However, many studies on the antimicrobial potential of extracts from other *Cedrus* species, especially *C. libani* (Dığrak et al. 1999; Kizil et al. 2002; Loizzo et al. 2008) and *C. deodara* (Wu et al. 2016; Y. Wu et al. 2018c; Zeng et al. 2012a) have been conducted.

III.2.3. Cytotoxicity and MTT assays

In this study, the MTT assay of *C. atlantica* cones essential oil and branch methanolic extract fractions was performed on breast cancer cell lines MCF-7. **Figure 21 and 22** show the MTT assay results of doxorubicin on MCF-7, used as positive control.

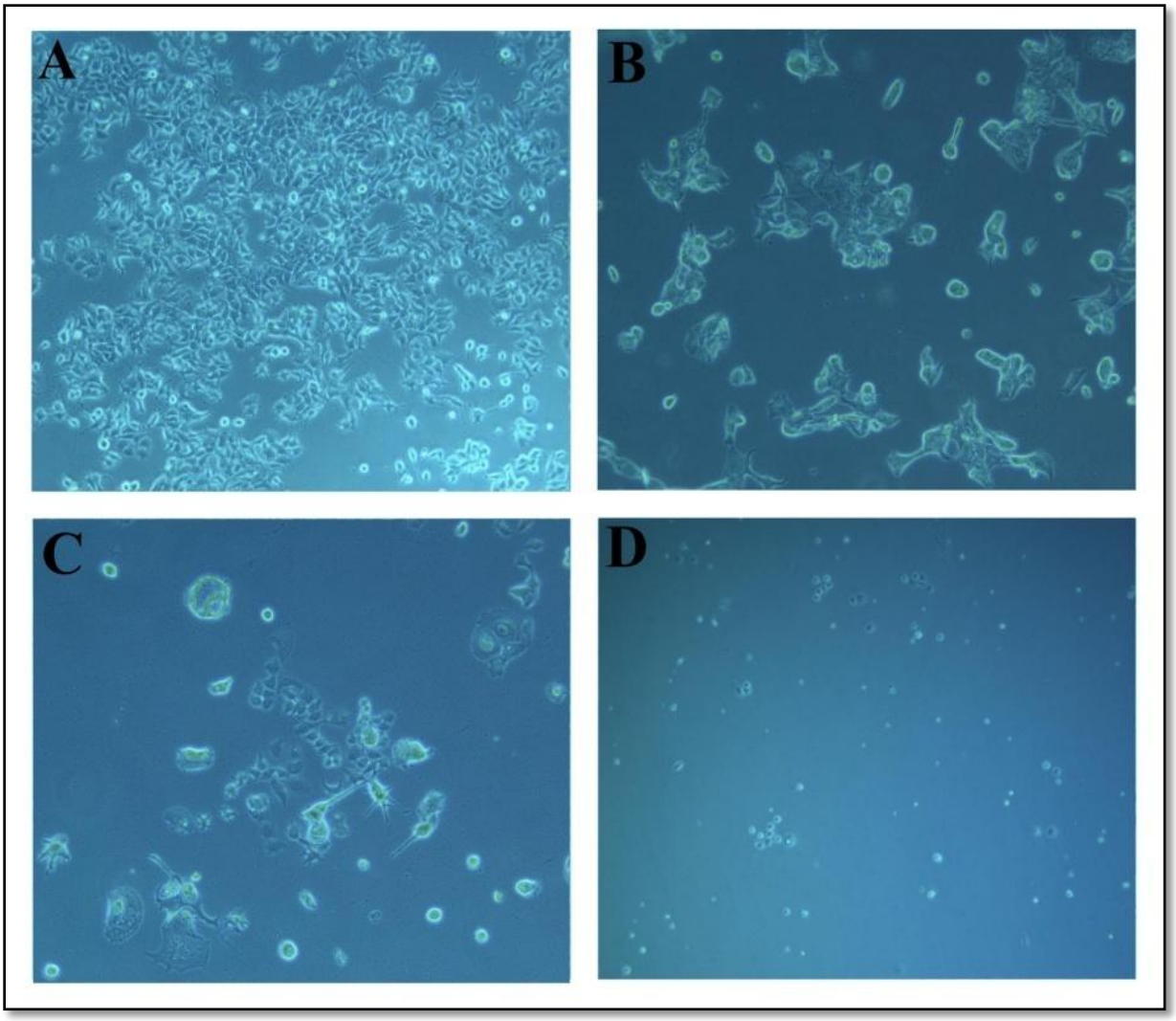

Figure 21. Images of MCF-7 cells (x10) treated with (A) Blank; (B) Doxorubicin 6 µM; (C) Doxorubicin 25 µM; and (D) Doxorubicin 100 µM.

The cytotoxic activity results (Cell viability percentage) of the essential oil and methanolic extract fractions against MCF-7 breast cancer cell lines were prensented in **Figure 22**. According to the MTT assay results, cancer cell lines were sensitive to doxorubicin with an IC_{50} value of 0.59±0.05 µg/ml. Fang et al. (2014) reported similar results with an IC_{50} value of 0.68±0.04 µg/ml.

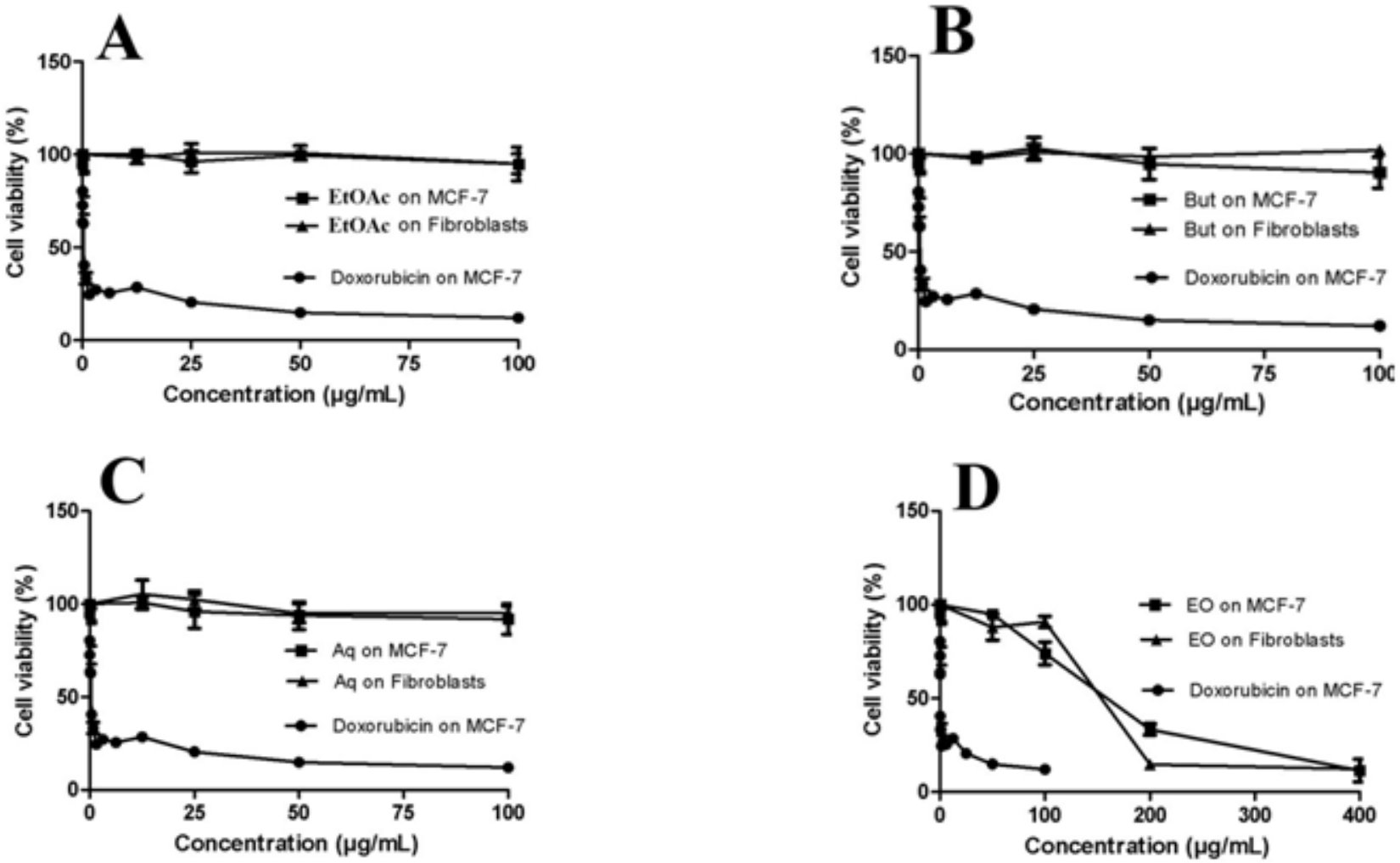

Figure 22. Cytotoxic effect on MCF-7 and fibroblasts treated with (A) EtOAc; (B) But; (C) Aq; and (D) EO (Cell viability percentage).

Doxorubicin was tested on MCF-7 as positive control. EtOAc: Ethyl acetate, But: n-Butanol; Aq: Aqueous, EO: Essential oil. The data represent experiments conducted in triplicate

According to our findings, there was no significant cytotoxic activity on MCF-7 cell lines treated with methanolic extract fractions EtOAc, But, and Aq within the tested concentration range (**Figure 22A**, **B**, and **C** and **Figure 23**). Comparably, similar results were obtained using fibroblast cell lines as normal cells. These findings revealed that *C. atlantica* fractions isolated from the methanolic extract of branches were inefficient against MCF-7 breast cancer cell lines and were not toxic to normal cells.

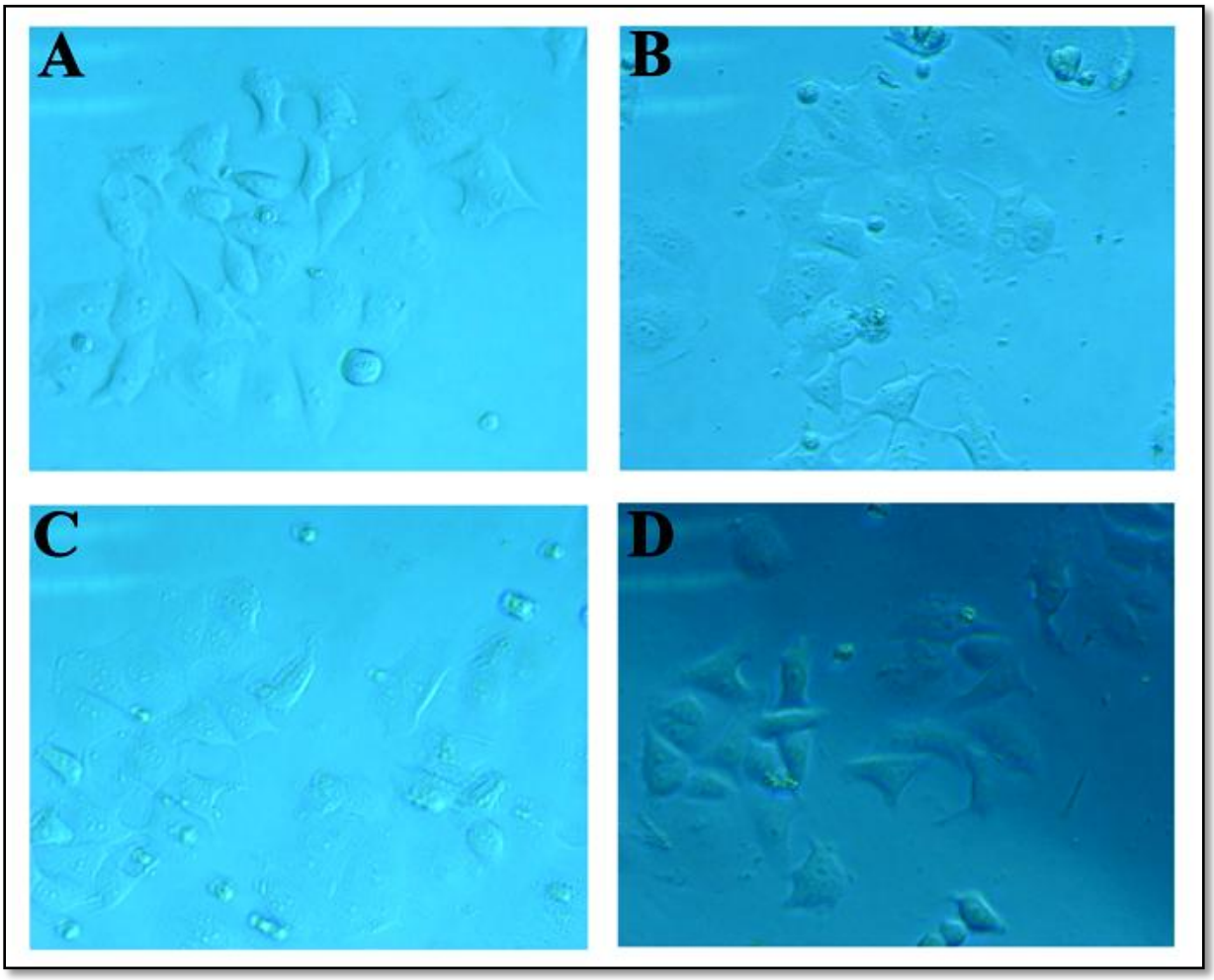

Figure 23. Images of MCF-7 cells (x40) treated with (A) Blank; (B) EtOAc (100 µg/mL); (C) But (100 µg/mL); and (D) Aq (100 µg/mL).

According to our thorough search, no studies on the cytotoxic effects of *C. atlantica* extracts against MCF-7 cells have been published. However, Barrero et al. (2005) demonstrated that the hexane extracts from *C. atlantica* cones had IC_{50} values greater than 5 µg/mL against a panel of cancer cell lines; A-549 (human lung carcinoma), H- 116 (human

colon carcinoma), PSN1 (human pancreatic adenocarcinoma), T98G (human caucasian gioblastoma), and SKBR3 (human breast carcinoma).

On the other hand, an IC_{50} value of 143.13±14.6 µg/ml was obtained with tested EO (**Figure 22D** and **Figure 24**); meanwhile, demonstrated cytotoxicity against fibroblasts normal cells with an IC_{50} value of 138.3±7.52 µg/ml.

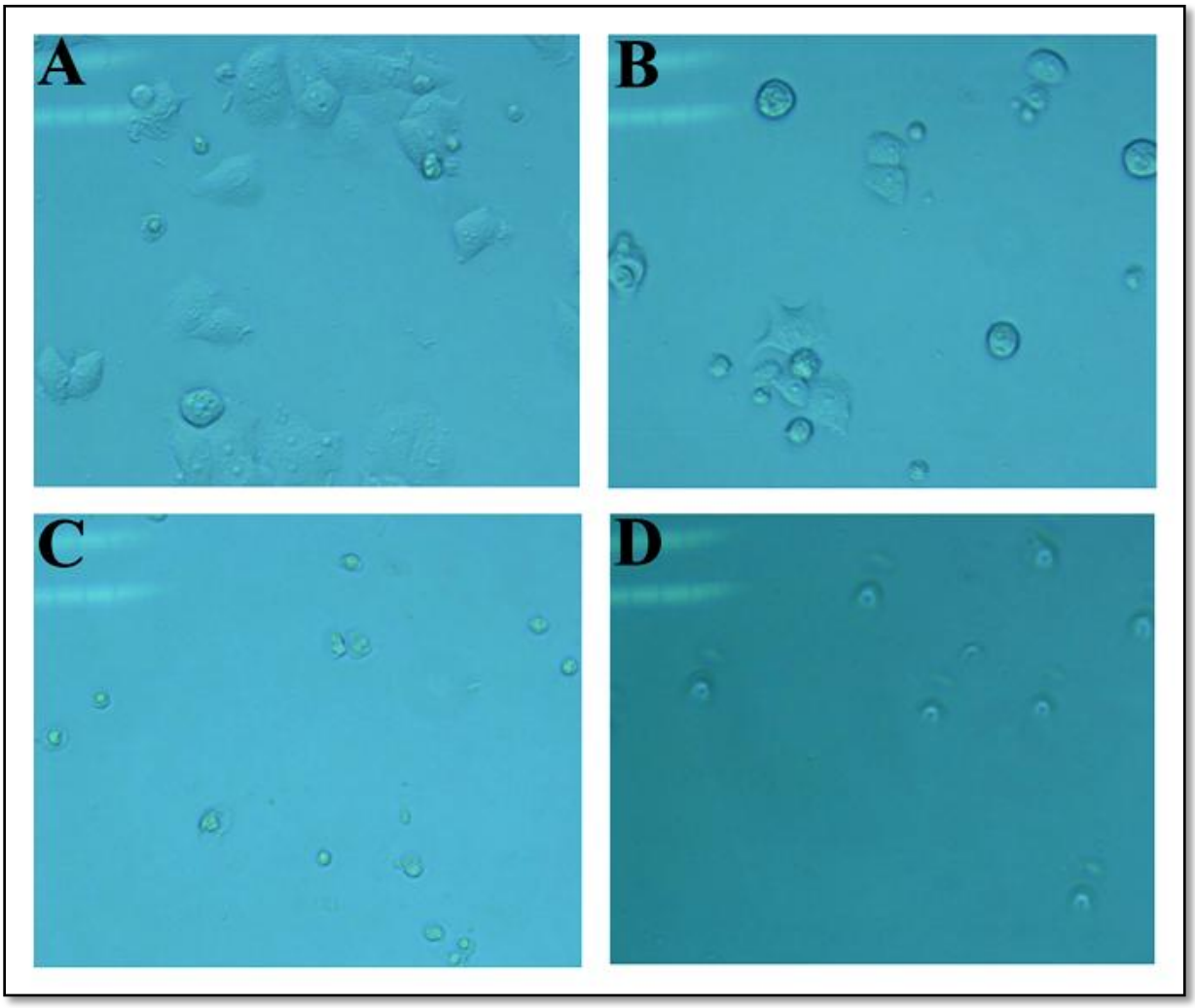

Figure 24. Images of MCF-7 cells (x40) treated with *C. atlantica* essential oil at (A) 50 µg/ml; (B) 100 µg/ml; (C) 200 µg/ml; and (D) 400 µg/ml.

There was no scientific proof of anticancer activity of *C. atlantica* essential oils against MCF-7 breast cancer cell lines in the literature. However, the *in vitro* evaluation of the anti-proliferative activity of wood essential oils from *C. atlantica* against K562 human chronic myelogenous leukaemia cells revealed an IC_{50} value of 59.37 ± 2.6 µg/ml (Saab al. 2012b). Several studies on the evaluation of the cytotoxic effect of extracts from other *Cedrus*

species have been published (Shashi et al. 2006, Singh et al. 2007, Saxena et al. 2010, Saab et al. 2012a).

III.3. Acute toxicity study

The acute toxicity evaluation was carried out in accordance with OECD guidelines, with the number of animals kept to a minimum. Nine female wistar mice were used in the experiments. Indeed, according to the OECD Guidance document on acute oral toxicity, traditional tests reported in the literature on the determination of the LD_{50} revealed few differences between sexes, and when they did occur, females were generally more sensitive (OECD 2001).

III.3.1. Signs of toxicity observation

The toxicity signs displayed by the mice were listed in **Table XXIV**. Hair straightening and drowsiness were observed in all animals within the first 4 hours of administration of the crude extract. Furthermore, all except one of the animals displayed hypo-activity. However, only one or two mice were anorexic and isolated. In addition to the aforementioned symptoms, one animal displayed tachycardia, loss of appetite, weakness, and laboured breathing, and died after 24 hours. During the two weeks following the day of dosing, all remaining mice displayed normal behaviour, with no signs of toxicity. However, all survived animals gained body weight, with gains of (0.9 g to 1.4 g) and (1.2 g to 2.1 g) after week one and week two, respectively.

III.3.2. Determination of the median lethal dose (LD_{50})

The experiment started with three mice being given a dose of 2000 mg/kg every 48 hours. The three mice all survived and showed no signs of toxicity. Therefore, the experiment was repeated on three additional animals using the same dose. As a result, only one of the three treated mice died after 24 hours, while the other two showed no signs of death. After that, one mouse was given a dose of 5000 mg/Kg and survived. Then, two more mice were given the same dose and survived as well. As a result, the LD_{50} was evaluated to be greater than 5000 mg/kg (OECD 2001). According to Viau and Tardif (2003), the crude extract of *C. atlantica* obtained from branches is either little toxic or non-toxic.

Table XXIV: Toxicity symptoms observed in the animals following administration of the crude extract.

Animal	Dose (mg/Kg)	Toxicity signs		
		4 h	One week	Two weeks
1	2000	Hair straightening, drowsiness and hypo-activity.	Normal	Normal
2	2000	Hair straightening, drowsiness and hypo-activity.	Normal	Normal
3	2000	Hair straightening and drowsiness.	Normal	Normal
4	2000	Hair straightening, drowsiness and hypo-activity.	Normal	Normal
5	2000	Hair straightening, drowsiness and hypo-activity.	Normal	Normal
6	2000	Hair straightening, drowsiness, hypo-activity, tachycardia, loss of appetite, weakness and laboured breathing.	Died after 24 h	-
7	5000	Hair straightening, drowsiness, hypo-activity, anorexia and isolation.	Normal	Normal
8	5000	Hair straightening, drowsiness, hypo-activity and isolation.	Normal	Normal
9	5000	Hair straightening, drowsiness, hypo-activity and isolation.	Normal	Normal

III.4. Effect of ultrasonic power

Several studies have been conducted to improve the extraction yield by using ultrasound waves (Al-Juhaimi et al. 2016; Liao et al. 2015; Yin et al. 2016). In this work, the physicochemical properties of the methanolic extract were evaluated after application of the ultrasonic power. The effect of acoustic waves on the viscosity of the extract is exhibited in **Figure 25A**. The viscosity parameter was significantly influenced by ultrasonic power. In fact, after US exposure at 42 KHz frequency, for 10 min and 20 min, the viscotity was decreased from 97.66 ± 2.51 cP to 67.70 ± 3.56 cP and 57.43 ± 3.09 cP ($P < 0.05$), respectively. However, increasing sonication time to 30 min resulted in no further significant difference ($P > 0.05$). Venegas-Sanchez et al. (2013) demonstrated that US exposure at 43 KHz for 5 min significantly reduced the viscosity of aqueous polymer solutions and came to the conclusion that exposure to the US influenced hydrogen-bond interactions between the polymer's OH groups and water molecules in the aqueous medium.

The effect of US exposure at different sonication durations on solubility was shown in **Figure 25B**. The solubility was significantly influenced by ultrasonic waves. In fact, after US application at 42 KHz for 10 min and 20 min, the solubility level was significantly increased from 0.126 ± 0.008 g/mL to 0.164 ± 0.009 g/mL and 0.189 ± 0.001 g/mL ($P < 0.05$), respectively. However, increasing US exposure to 30 min produced no further significant effect. This increase could be attributed in part to a decrease in particle size diameter, which results in an increase in specific surface area (Belkacem et al. 2015); as well as the ability of US to control the polymorphism (Hatakka et al. 2010).

The effect of ultrasonic waves on the $DPPH^{\bullet}$ scavenging activity was exhibited in **Figure 25C**. In terms of $DPPH^{\bullet}$ percentage inhibition, the application of power ultrasound on the *C. atlantica* extract at different sonication durations had no effect on its constituents' ability to scavenge the free radical $DPPH^{\bullet}$. Similarly, after 10 min and 20 min of sonication, the recorded IC_{50} values showed no significant differences (**Figure. 25D**). However, US exposure of 30 min resulted in a statistically significant but still negligible decrease. These findings suggested that the chemical structure of *C. atlantica* methanolic extract components would be unaffected by US power.

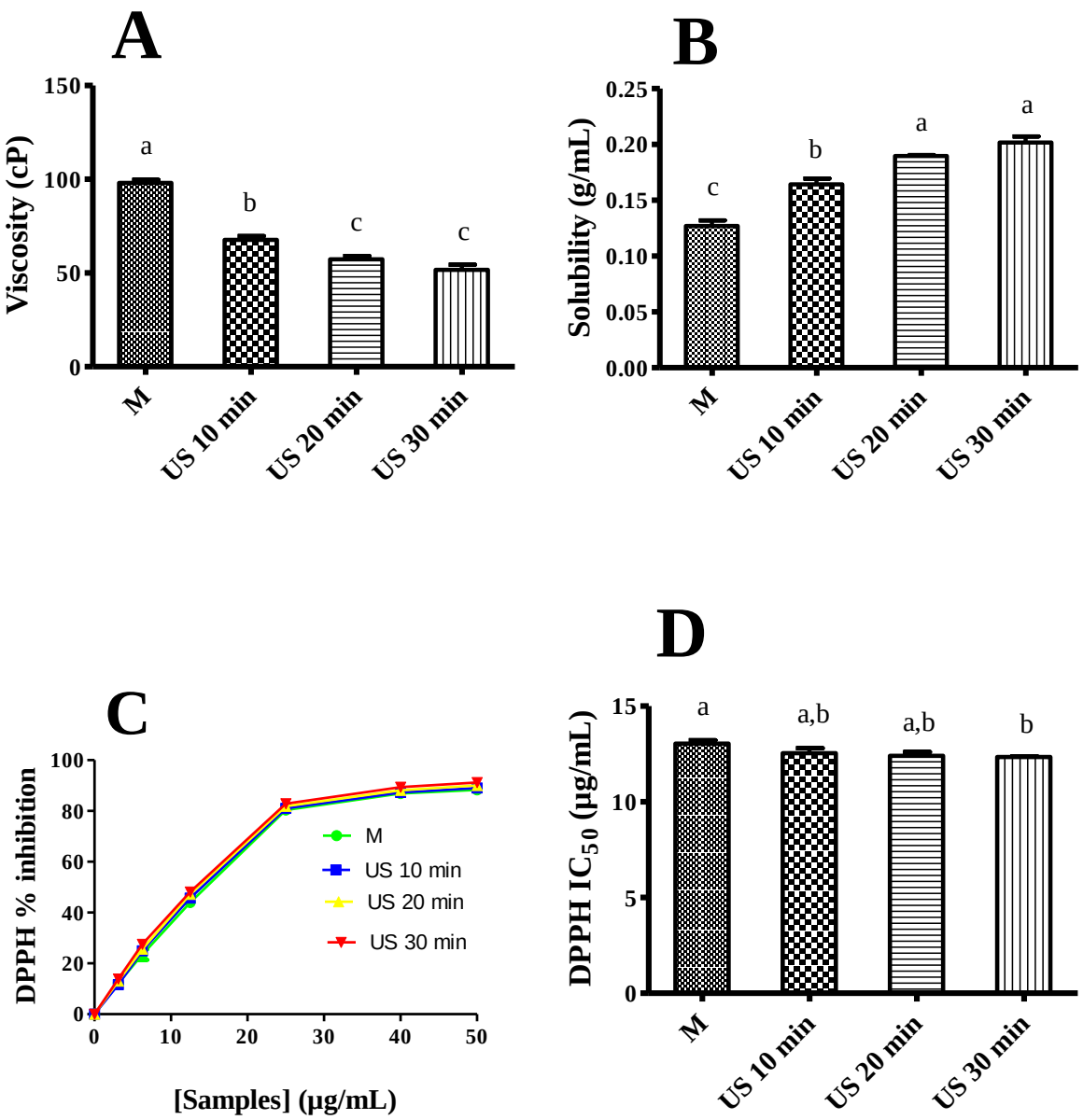

Figure 25. Effect of power ultrasound at various sonication times on: A) Viscosity; B) solubility; C) DPPH$^{\bullet}$ scavenging percentage; and D) DPPH$^{\bullet}$ IC_{50}.

M: Methanol, US: Ultrasound. For each graph, values followed by different letter were significantly different ($P<0.05$).

It can be concluded that the use of ultrasonic power provides a non-destructive technique that could be used to improve the extraction yield and enhance the physicochemical properties of the extracts, in particular the solubility, which is an important parameter in several experimental tests especially those that require the administration of extracts orally, such as *in vivo* studies.

.

Conclusion

Conclusion

The chemical composition, antioxidant, antibacterial, and cytotoxic activities of essential oils extracted from *Cedrus atlantica* cones and various branch extracts were evaluated. In addition, the crude methanolic extract was investigated for its acute toxicity and the effect of ultrasound on its physico-chemical properties.

Six compounds were identified in the cones' essential oil, with alpha-pinene being the most abundant monoterpene hydrocarbon component, followed by sabinene, β-phellandrene, β-pinene, and β-farnesene. The essential oil also contained a trace of bornyl acetate with a miscellaneous structure. The extracts and fractions from branches, on the other hand, were found to be rich in polyphenols, particularly tannins.

The antioxidant activity results obtained using different methods (DPPH, ABTS and FRAP) showed strong positive correlation with TPC and TC values. These findings demonstrated that *C. atlantica* is a good source of potent antioxidant components. It could be suggested that the antioxidant capacity was mainly attributed to polyphenols, precisely to tannins and to a lesser extent to flavonoids.

The essential oil and fractions (EtOAc, But, and Aq) of *C. atlantica* had antibacterial activity against *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, and *Escherichia coli* ATCC 25921, with MIC values ranging from 0.25 percent (v/v) to 0.5 percent (v/v) and from 31.25 µg/ml to 1000 µg/ml, respectively. Bactericidal activity was also recorded for the essential oil and fractions with MBC values ranging from 0.5 percent (v/v) to 1 percent (v/v) and from 62.5 µg/ml to 2000 µg/ml, respectively.

C. atlantica essential oil demonstrated cytotoxic activity against MCF-7 breast cancer cell lines with an IC_{50} value of 143.13±14.6 µg/ml, as well as cytotoxic activity against normal fibroblasts with an IC_{50} value of 138.3±7.52 µg/ml. On the other hand, the fractions partitioned from the crude methanolic extract had no significant cytotoxic effect on either cancer or normal cells.

The acute toxicity study conducted in accordance with the OECD recommendations with a starting dose of 2000 mg/Kg revealed that the *C. atlantica* methanolic extract obtained from branches is either little toxic or non-toxic.

The use of ultrasonic power on the *C. atlantica* methanolic extracts, using an ultrasonicator water bath, in the objective to improve their physicochemical properties, demonstrated that the viscosity was reduced, the solubility increased and the DPPH$^{\bullet}$ scavenging capacity conserved. However, it is worth investigating this non-destructive method on other natural extracts as well as on purified phytochemical compounds intended for oral administration as food supplements or phytomedicines, such as, resveratrol and quercetine.

Therefore, it was revealed that *C. atlantica* can be a good source of potent antioxidant and antimicrobial agents or serve as leading compounds. Furthermore, *Cedrus* by-products, particularly in cedarwood-producing countries, could be valorized, especially in the extraction and use of essential oils in traditional applications. However, the isolation and elucidation of the chemical structures of the pure components are essential to evaluate their *in vitro* and *in vivo* pharmacological effects. This might be a first step in a long process to develop new chemical entities with therapeutic potential.

References

References

Abdel-Maksoud, G., A. Elamin and F. Afifi (2019). "Evaluation of cedar wood oil (cedrus libani a. rich) for the control of common egyptian mummies‟ insect pest (dermestes maculatus)." Sci Cult. **5**(2): 31-36.

Aberchane, M., M. Fechtal and A. Chaouch (2004). "Analysis of Moroccan atlas cedarwood oil (*Cedrus atlantica* Manetti)." J. Essent. Oil Res., **16**(6): 542-547.

Aberchane, M., B. Satrani, M. Fechtal and A. Chaouch (2003). "Effet de l'infection du bois de Cèdre de l'Atlas par *Trametes pini* et *Ungulina officinalis* sur la composition chimique et l'activité antibactérienne et antifongique des huiles essentielles." Acta Bot Gall **150**(2): 223-229.

Abu-Lafi, S., M. S. Al-Natsheh, R. Yaghmoor and F. Al-Rimawi (2017). "Enrichment of phenolic compounds from olive mill wastewater and in vitro evaluation of their antimicrobial activities." Evid Based Complementary Altern Med. **2017**.

Adams, R. P. (2007). Identification of essential oil components by gas chromatography/mass spectrometry, Allured Publishing Corporation.

Ainane, A., F. Khammour, S. Charaf, M. Elabboubi, M. Elkouali, M. Talbi, R. Benhima, S. Cherroud and T. Ainane (2019). "Chemical composition and insecticidal activity of five essential oils: *Cedrus atlantica, Citrus limonum, Rosmarinus officinalis, Syzygium aromaticum* and *Eucalyptus globules*." Mater Today Proc. **13**: 474-485.

Al-Dabbas, M. M., T. Suganuma, K. Kitahara, D.-X. Hou and M. Fujii (2006). "Cytotoxic, antioxidant and antibacterial activities of *Varthemia iphionoides* Boiss. extracts." J. Ethnopharmacol **108**(2): 287-293.

Al-Juhaimi, F., O. Q. Adiamo, K. Ghafoor and E. E. Babiker (2016). "Optimization of ultrasonic-assisted extraction of phenolic compounds from fenugreek (*Trigonella foenum-graecum* L.) seed." CYTA J Food. **14**(3): 369-374.

Amin, A., H. Gali-Muhtasib, M. Ocker and R. Schneider-Stock (2009). "Overview of major classes of plant-derived anticancer drugs." Int J Biomed Sci. **5**(1): 1.

Arshan, M. M. K. and H. Gul (2020). "In vitro antimicrobial activity of some medicinal plants against selected pathogenic microbes." Asian J. Adv Res: 1-5.

Arts, J. W., K. Kramer, S. S. Arndt and F. Ohl (2014). "Sex differences in physiological acclimatization after transfer in Wistar rats." Animals **4**(4): 693-711.

Ba, K., E. Tine, J. Destain, N. Cissé and P. Thonart (2010). "Étude comparative des composés phénoliques, du pouvoir antioxydant de différentes variétés de sorgho sénégalais et des enzymes amylolytiques de leur malt." Biotechnol agron soc environ **14**(1): 131-139.

Bai, J., Y. Wu, X. Liu, K. Zhong, Y. Huang and H. Gao (2015). "Antibacterial activity of shikimic acid from pine needles of *Cedrus deodara* against *Staphylococcus aureus* through damage to cell membrane." Int. J. Mol. Sci. **16**(11): 27145-27155.

Barrero, A. F., J. F. Q. del Moral, M. M. Herrador, J. F. Arteaga, M. Akssira, A. Benharref and M. Dakir (2005). "Abietane diterpenes from the cones of *Cedrus atlantica*." Phytochemistry **66**(1): 105-111.

Basli, A., N. Belkacem and I. Amrani (2017). Health Benefits of Phenolic Compounds Against Cancers. Phenolic Compounds-Biological Activity. M. Soto-Hernández, InTech**:** 193-210.

Baylac, S. and P. Racine (2003). "Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts." Int J Aromather **13**(2-3): 138-142.

Belkacem, N., B. Khettal, M. Hudaib, Y. Bustanji, B. Abu-Irmaileh and C. S. M. Amrine (2021). "Antioxidant, antibacterial, and cytotoxic activities of *Cedrus atlantica* organic extracts and essential oil." Eur. J. Integr. Med. **42**: 101292.

Belkacem, N., M. A. S. Salem and H. S. AlKhatib (2015). "Effect of ultrasound on the physico-chemical properties of poorly soluble drugs: antisolvent sonocrystallization of ketoprofen." Powder Technol. **285**: 16-24.

Bennouna, F., M. Lachkar, S. El Abed and S. I. Koraichi (2019). "*Cedrus atlantica* essential oil: Antimicrobial activity and effect on the physicochemical properties of cedar wood surface." Moroccan J. Biol. **2019**(16): 35-45.

Benouaklil, F., F. Hamaidi-Chergui, M. S. Hamaidi and F. Saidi (2017). "Chemical composition and antimicrobial properties of Algerian *Cedrus atlantica* m. Essential oils." AgroBiologia **7**(1): 355-362.

Benzie, I. F. and J. J. Strain (1996). "The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay." Anal biochem **239**(1): 70-76.

Berdahl, D. R. and J. McKeague (2015). Handbook of antioxidants for food preservation. Handbook of antioxidants for food preservation. S. F. (Eds.). Cambridge, England, Elsevier**:** pp. 177-217.

Bhagat, M., A. Kumar and R. Suravajhala (2020). "*Cedrus deodara* (bark) essential oil induces apoptosis in human colon cancer cells by inhibiting nuclear factor kappa b." Curr. Top. Med. Chem. **20**(22): 1981-1992.

Blowman, K., M. Magalhães, M. Lemos, C. Cabral and I. Pires (2018). "Anticancer properties of essential oils and other natural products." Evid Based Complement Alternat Med. **2018**.

Bouchra, C., A. Mohamed, I. H. Mina and M. Hmamouchi (2003). "Antifungal activity of essential oils from several medicinal plants against four postharvest *Citrus pathogens*." Phytopathol. Mediterr. **42**(3): 251-256.

Boudarene, L., A. Baaliouamer, B. Y. Meklati and C. Scharff (2004a). "Composition of the seed oils from Algerian *Cedrus atlantica* G. Manetti." J Essent Oil Res **16**(1): 61-63.

Boudarene, L., L. Rahim, A. Baaliouamer and B. Y. Meklati (2004b). "Analysis of Algerian essential oils from twigs, needles and wood of *Cedrus atlantica* G. Manetti by GC/MS." J. Essent. Oil Res. **16**(6): 531-534.

Boutos, S., E.-M. Tomou, A. Rancic, M. Socović, D. Hadjipavlou-Litina, K. Nikolaou and H. Skaltsa (2020). "Composition of the essential oil of *Cedrus brevifolia* needles Evaluation of its antimicrobial and antioxidant activities." Am. J. Essent. Oil. **8**(2): 01-05.

Bouyahya, A., Y. Bakri, A. Et-Touys, A. Talbaoui, A. Khouchlaa, S. Charfi, J. Abrini and N. Dakka (2017). "Résistance aux antibiotiques et mécanismes d'action des huiles essentielles contre les bactéries." Phytothérapie: 1-11.

Brunetti, M., E. L. De Capua, N. Macchioni and S. Monachello (2001). "Natural durability, physical and mechanical properties of Atlas cedar (*Cedrus atlantica* Manetti) wood from Southern Italy." Ann. For. Sci. **58**(6): 607-613.

Buneri, I. D., M. Yousuf, M. Attaullah, S. Afridi, S. I. Anjum, H. Rana, N. Ahmad, M. Amin, M. Tahir and M. J. Ansari (2019). "A comparative toxic effect of *Cedrus deodara* oil on larval protein contents and its behavioral effect on larvae of mealworm beetle (*Tenebrio molitor*)(Coleoptera: Tenebrionidae)." Saudi J. Biol. Sci. **26**(2): 281-285.

Cetin, H., Y. Kurt, K. Isik and A. Yanikoglu (2009). "Larvicidal effect of *Cedrus libani* seed oils on mosquito *Culex pipiens*." Pharm. Biol. **47**(8): 665-668.

Chakraborty, S., N. Kar, L. Kumari, A. De and T. Bera (2017). "Inhibitory effect of a new orally active cedrol-loaded nanostructured lipid carrier on compound 48/80-induced mast cell degranulation and anaphylactic shock in mice." Int J Nanomedicine. **12**: 4849.

Chalchat, J.-C., R.-P. Garry, A. Miehet and B. Benjilali (1994). "Essential oil components in sawdust of *Cedrus atlantica* from Morocco." J. Essent. Oil Res. **6**(3): 323-325.

Chao, S., G. Young, C. Oberg and K. Nakaoka (2008). "Inhibition of methicillin-resistant *Staphylococcus aureus* (MRSA) by essential oils." Flavour Fragr. J. **23**(6): 444-449.

Chao, S. C., D. G. Young and C. J. Oberg (2000). "Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses." J. Essent. Oil Res. **12**(5): 639-649.

Chaudhari, L., B. A. Jawale, S. Sharma, H. Sharma, C. Kumar and P. A. Kulkarni (2012). "Antimicrobial activity of commercially available essential oils against *Streptococcus mutans*." J Contemp Dent Pract **13**(1): 71-74.

Chaudhary, A., S. Sood, P. Kaur, N. Kumar, A. Thakur, A. Gulati and B. Singh (2012a). "Antifungal sesquiterpenes from *Cedrus deodara*." Planta Med. **78**(02): 186-188.

Chaudhary, A. K., S. Ahmad and A. Mazumder (2012b). "Study of antibacterial and antifungal activity of traditional *Cedrus deodara* and *Pinus roxburghii* Sarg." CELLMED **2**(4): 37.31-37.34.

Chaudhary, A. K., S. Ahmad and A. Mazumder (2014). "Cognitive enhancement in aged mice after chronic administration of *Cedrus deodara* Loud. and *Pinus roxburghii* Sarg. with demonstrated antioxidant properties." J. Nat. Med. **68**(2): 274-283.

Chaudhary, A., P. Sharma, G. Nadda, T. Dhananjay Kumar and S. Bikram (2011). "Chemical composition and larvicidal activities of the Himalayan cedar, Cedrus deodara essential oil and its fractions against the diamondback moth, Plutella xylostella." J. Insect Sci. **11**(1).

Chaudhary, A. K., S. Ahmad and A. Mazumder (2015). "Isolation, structural elucidation and in vitro antioxidant activity of compounds from chloroform extract of *Cedrus deodara* (Roxb.) Loud." Nat. Prod. Res. **29**(3): 268-273.

Chauhan, R. and A. Joshi (2018). "Anti-Cancer properties of herbs in cell culture system." J. Immunol Immunopathol. **20**(si): 107-120.

Chebli, B., M. Hmamouchi, M. Achouri and L. I. Hassani (2004). "Composition and in vitro fungitoxic activity of 19 essential oils against two post-harvest pathogens." J. Essent. Oil Res. **16**(5): 507-511.

Chen, Y., L. Chi, X. Liang, Y. Shi, T. Wu, M. Ye, P. Han, L. Lin, L. Zhang and P. Xu (2020). "Essential Oils of *Cedrus deodara* Leaves Exerting Anti-inflammation on TPA-induced Ear Edema by Inhibiting COX-2/TNF-α/NF-κB Activation." J. Essent. Oil-Bear. Plants. **23**(3): 422-431.

Chinou, I. (2008). Primary and secondary metabolites and their biological activity. Chromatographic science series. Thin layer chromatography in phytochemistry, CRC Press. **99:** 59-76.

Choi, W.-I., E.-H. Lee, B.-R. Choi, H.-M. Park and Y.-J. Ahn (2003). "Toxicity of plant essential oils to Trialeurodes vaporariorum (Homoptera: Aleyrodidae)." J. Econ. Entomol. **96**(5): 1479-1484.

Cox, S., C. Mann and J. Markham (2001). "Interactions between components of the essential oil of Melaleuca alternifolia." J. Appl. Microbiol. **91**(3): 492-497.

Cretu, E., J.-P. Salminen, M. Karonen, A. Miron, C. Charalambous, A. I. Constantinou and A. C. Aprotosoaie (2014). "In vitro antioxidant activity and phenolic content of *Cedrus brevifolia* bark." Nat. Prod. Commun. **9**(4).

Cretu, E., A. Trifan, A. C. Aprotosoaie and A. Miron (2013). "15-Lipoxygenase inhibition, superoxide and hydroxyl radicals scavenging activities of *Cedrus brevifolia* bark extracts." Rev. Med. Chir. Soc. Med. Nat. Iasi **117**: 250-256.

Dagher-Kharrat, M. B., S. Mariette, F. Lefèvre, B. Fady, G. Grenier-de March, C. Plomion and A. Savouré (2007). "Geographical diversity and genetic relationships among *Cedrus* species estimated by AFLP." Tree Genet. Genomes. **3**(3): 275-285.

Daher, C., R. Iskandar, N. D. El Jalbout, V. Dwairi, M. Zgheib, P. Ibrahim, J. Saad, P. Bakhos, N. Chelala and C. Daou (2016). "The antitumor promoting activity of 2-himachalen-7-ol in two-stage mouse skin carcinogenesis test." Planta Med. **82**(S 01): P994.

Dakir, M., F. El Hanbali, F. Mellouki, M. Akssira, A. Benharref, J. Quilez Del Moral and A. Barrero (2005). "Antibacterial diterpenoids from *Cedrus atlantica*." Nat. Prod. Res. **19**(7): 719-722.

Dakir, M., F. El Hanbali, F. Mellouki, M. M. Herrador, A. Barrero, A. Benharref and M. Akssira (2014). "Chemical and antibacterial studies of essential oils of scales and seeds of *Cedrus atlantica* Endl." J. Nat. Prod. Plant Resour **4**(6): 15-18.

Debazac, E.-F. (1964). "Manual of conifers." Manual of conifers.

Demirci, A. N., N. Çömlekçioğlu and A. Aygan (2020). "Determination of the chemical composition, antimicrobial activity and flavonoid content of the essential oils of *Cedrus libani* and *Pinus nigra* subsp. Pallasiana." Turk J. Food Agric. Sci. Tech. **8**(8): 1747-1754.

Derwich, E., Z. Benziane and A. Boukir (2010). "Chemical composition and in vitro antibacterial activity of the essential oil of *Cedrus atlantica*." Int. J. Agric. Biol **12**(3): 381-385.

Devmurari, V., P. Shivanand, S. Vaghani, K. Jagganath, M. Goyani and N. Jivani (2010). "Antihyperglycemic activity of ethanolic extract of *Cedrus deodara* wood in alloxan induced hyperglycemic rat." Int. J. Chem. Sci. **8**(1): 483-488

Dhayabaran, D., E. J. Florance, K. Nandakumar and A. Puratchikody (2010). "Anxiolytic and anticonvulsant activity of alcoholic extract of heart wood of *Cedrus deodara* Roxb. in rodents." J. Med. Plant Res. **4**(14): 1374-1381.

Dhayabaran, D., E. J. Florance, K. Nandakumar, A. Shanmugarathinam and A. Puratchikody (2014). "Anticonvulsant activity of fraction isolated from ethanolic extract of heartwood of *Cedrus deodara*." J. Nat. Med. **68**(2): 310-315.

Dhayabaran, D., J. Florance, K. Nandakumar and A. Puratchikody (2012). "Isolation and anxiolytic activity of 3, 4-bis (3, 4-dimethoxyphenyl) furan-2, 5-dione from the ethanolic extract of heart wood of *Cedrus deodara*." Med Chem Res. **21**(11): 3460-3464.

Dığrak, M., A. İlçim and M. Hakkı Alma (1999). "Antimicrobial activities of several parts of *Pinus brutia*, *Juniperus oxycedrus*, *Abies cilicia*, *Cedrus libani* and *Pinus nigra*." Phytother Res. **13**(7): 584-587.

Do, Q. D., A. E. Angkawijaya, P. L. Tran-Nguyen, L. H. Huynh, F. E. Soetaredjo, S. Ismadji and Y.-H. Ju (2014). "Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica." J Food Drug Anal **22**(3): 296-302.

Dorman, H. D. and S. G. Deans (2000). "Antimicrobial agents from plants: antibacterial activity of plant volatile oils." J appl microbiol **88**(2): 308-316.

Douros, A., D. Hadjipavlou-Litina, K. Nikolaou and H. Skaltsa (2018). "The occurrence of flavonoids and related compounds in *Cedrus brevifolia* A. Henry ex Elwes & A. Henry needles. Inhibitory potencies on lipoxygenase, linoleic acid lipid peroxidation and antioxidant activity." Plants **7**(1): 1.

Elias, A., W. N. Shebaby, B. Nehme, W. Faour, B. S. Bassil, J. El Hakim, R. Iskandar, N. Dib-Jalbout, M. Mroueh and C. Daher (2019). "In Vitro and In Vivo Evaluation of the Anticancer and Anti-inflammatory Activities of 2-Himachelen-7-ol isolated from *Cedrus libani*." Sci. Rep. **9**(1): 1-9.

Emer, A. A., N. N. Donatello, A. P. Batisti, L. A. O. Belmonte, A. R. Santos and D. F. Martins (2018). "The role of the endocannabinoid system in the antihyperalgesic effect of *Cedrus atlantica* essential oil inhalation in a mouse model of postoperative pain." J Ethnopharmacol **210**: 477-484.

Fadel, H., F. Benayache and S. Benayache (2016). "Antioxidant properties of four Algerian medicinal and aromatic plants *Juniperus oxycedrus* L., *Juniperus phoenicea* L., *Marrubium vulgare* L. and *Cedrus atlantica* (Manetti ex Endl)." Der Pharm Lett **8**(3): 72-79.

Fady, B., M. Bariteau, D. Fallour, E. Giroud and F. Lefevre (2000). Isozyme gene markers and taxonomy of Mediterranean Cedrus species. Final Conference of the European Union, Mytilene, Greece.

Fahed, L., M. Khoury, D. Stien, N. Ouaini, V. Eparvier and M. El Beyrouthy (2017). "Essential oils composition and antimicrobial activity of six conifers harvested in Lebanon." Chem. Biodivers. **14**(2.

Fang, X. J., H. Jiang, Y. Q. Zhu, L. Y. Zhang, Q. H. Fan and Y. Tian (2014). "Doxorubicin induces drug resistance and expression of the novel CD44st via NF-κB in human breast cancer MCF-7 cells." Oncol. Rep. **31**(6): 2735-2742.

Fantini, M., M. Benvenuto, L. Masuelli, G. Frajese, I. Tresoldi, A. Modesti and R. Bei (2015). "In vitro and in vivo antitumoral effects of combinations of polyphenols, or polyphenols and anticancer drugs: perspectives on cancer treatment." Int. J. Mol. Sci. **16**(5): 9236-9282.

Farjon, A. (1990). Pinaceae. Drawings and descriptions of the genera Abies, Cedrus, Pseudolarix, Keteleeria, Nothotsuga, Tsuga, Cathaya, Pseudotsuga, Larix and Picea. D-6240 Königstein, Federal Republic of Germany., Koeltz scientific books.

Ferraro, M. J. (2009). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, NCCLS.

Fidah, A., M. Rahouti, B. Kabouchi and A. Famiri (2019). Durable woods and antifungal activity of their essential oils: Case of *Tetraclinis articulata* (Vahl) masters and *Cedrus atlantica* manetti. Essential Oils-Oils of Nature, IntechOpen.

Fidah, A., N. Salhi, M. Rahouti, B. Kabouchi, M. Ziani, M. Aberchane and A. Famiri (2016). "Natural durability of *Cedrus atlantica* wood related to the bioactivity of its essential oil against wood decaying fungi." Maderas-Cienc Tecnol. **18**(4): 567-576.

Gaidhani, S., A. Singh, S. Kumari, G. Lavekar, A. Juvekar, S. Sen and M. Padhi (2013). "Evaluation of some plant extracts for standardization and anticancer activity." Indian j. tradit. knowl. **12**(4): 682-687.

Gene Lim, E., H. Sik Roh, T. A. Coudron and C. Gyoo Park (2011). "Temperature-dependent fumigant activity of essential oils against twospotted spider mite (Acari: Tetranychidae)." J. Econ. Entomol. **104**(2): 414-419.

Hadzri, H. M., M. A. C. Yunus, S. Zhari and F. Rithwan (2014). "The effects of solvents and extraction methods on the antioxidant activity of *P. Niruri*." J Technol **68**(5): 47-52.

Hammer, K. A., C. F. Carson and T. V. Riley (1999). "Antimicrobial activity of essential oils and other plant extracts." J. Appl. Microbiol. **86**(6): 985-990.

Hatakka, H., H. Alatalo, M. Louhi-Kultanen, I. Lassila and E. Hæggström (2010). "Closed-loop control of reactive crystallization PART II: polymorphism control of L-glutamic acid by sonocrystallization and seeding." Chem Eng Technol. 33(5): 751-756.

Hayet, E., M. Maha, A. Samia, M. M. Ali, B. Souhir, K. Abderaouf, Z. Mighri and A. Mahjoub (2009). "Antibacterial, antioxidant and cytotoxic activities of extracts of *Conyza canadensis* (L.) Cronquist growing in Tunisia." Med Chem Res. **18**(6): 447-454.

Hofmann, T., E. Visi-Rajczi and L. Albert (2020). "Antioxidant properties assessment of the cones of conifers through the combined evaluation of multiple antioxidant assays." Ind Crops Prod. **145**: 111935.

Huang, X.-F., K.-F. Chang, S.-C. Lee, G.-T. Sheu, C.-Y. Li, J.-C. Weng, C.-Y. Hsiao and N.-M. Tsai (2020). "Extract derived from *Cedrus atlantica* acts as an antitumor agent on hepatocellular carcinoma growth in vitro and In vivo." Molecules. **25**(20): 4608.

Hung, P.-H., M.-C. Hsieh, S.-C. Lee, X.-F. Huang, K.-F. Chang, S.-Y. Chen, M.-S. Lee and N.-M. Tsai (2020). "Effects of *Cedrus atlantica* extract on acute myeloid leukemia cell cycle distribution and apoptosis." Mol. Biol. Rep. **47**(11): 8935-8947.

Inaam, E.-m., H. Sara, L. Saadia, E. Mohamed and L. Abdeslam (2015). "Study of antioxidant activity of essential oils extracted from Moroccan medicinal and aromatic plants." Eur J Med Plants. **10**(2):1-12.

Inan, M., M. Kirpik, D. A. Kaya and S. Kirici (2011). "Effect of harvest time on essential oil composition of *Thymbra spicata* L. growing in flora of Adıyaman." Adv Environ Biol. **5**(2): 356-358.

Inoue, Y., A. Shiraishi, T. Hada, K. Hirose, H. Hamashima and J. Shimada (2004). "The antibacterial effects of terpene alcohols on *Staphylococcus aureus* and their mode of action." FEMS microbiol lett. **237**(2): 325-331.

Jaakola, L. and A. Hohtola (2010). "Effect of latitude on flavonoid biosynthesis in plants." Plant Cell Environ. **33**(8): 1239-1247.

Jain, A., A. Joshi, J. Joshi, M. Tatawat, S. Saeed, S. Telang, Y. Choubey and P. Puri (2019). "Comparative study of phytochemical screening and antibacterial activity of four medicinal plants." J. Med. Plant Res. **7**(4): 81-89.

Jain, S., A. Jain, S. Jain, N. Malviya, V. Jain and D. Kumar (2015). "Estimation of total phenolic, tannins, and flavonoid contents and antioxidant activity of *Cedrus deodara* heart wood extracts." Egypt. Pharm. J. **14**(1): 10.

Jain, S., A. Jain, N. Malviya, D. Kumar, V. Jain and S. Jain (2014). "Antidiabetic activity of *Cedrus deodara* aqueous extract and its relationship with its antioxidant properties." J. Pharm. Sci. Pharmacol. **1**(3): 187-194.

Jain, S. (2020). "Evaluations of additive effect of two Indigenous medicinal plants *Cedrus Deodara* and Mucuna pruriens towards the treatment of Parkinson's Disease." Parkinsonism Relat Disord.**79**(2): e64.

Jorgensen, J. H. and J. D. Turnidge (2015). Susceptibility test methods: dilution and disk diffusion methods. Manual of clinical microbiology**:** 1253-1273.

Joshi, S., K. Parikshit, P. Prabha and S. Sati (2018). "A comparative evaluation of Kumaun Himalayan gymnosperms for their antifungal potential against plant pathogenic fungi." Int. J. Phytopharm. **7**(3): 230-241.

Julkunen-Tiitto, R., N. Nenadis, S. Neugart, M. Robson, G. Agati, J. Vepsäläinen, G. Zipoli, L. Nybakken, B. Winkler and M. A. Jansen (2015). "Assessing the response of plant flavonoids to UV radiation: an overview of appropriate techniques." Phytochem Rev. **14**(2): 273-297.

Kadam, A. A., S. Singh and K. K. Gaikwad (2021). "Chitosan based antioxidant films incorporated with pine needles (*Cedrus deodara*) extract for active food packaging applications." Food Control **124**: 107877.

Kala, S., N. Sogan, S. Naik, A. Agarwal and J. Kumar (2020). "Impregnation of pectin-cedarwood essential oil nanocapsules onto mini cotton bag improves larvicidal performances." Scie. Rep. **10**(1): 1-12.

Kamarudin, N. A., M. Markom and J. Latip (2016). "Effects of solvents and extraction methods on herbal plants *Phyllanthus niruri*, *Orthosiphon stamineus* and *Labisia pumila*." Indian J Sci Technol. **9**(21): 1-5.

Kaoutar, F., B. Najiba, Z. Rabea, B. El Hassan and G. Najib (2019). "Evaluation of the aphicide activity of two essential oils on the survival of the black citrus aphid *Toxoptera aurantii* (boyer de fonscolombe) (homoptera: Aphididae)." Int. J. Sci. Res. **8**(11): 421 - 426.

Kar, K., V. Puri, G. Patnaik, R. N. Sur, B. Dhawan, D. Kulshrestha and R. Rastogi (1975). "Spasmolytic constituents of *Cedrus deodara* (Roxb.) Loud: pharmacological evaluation of himachalol." J. Pharm. Sci **64**(2): 258-262.

Karam, M.-J., M. Aouad, A. Roig, A. Bile, M. B. Dagher-Kharrat, E. K. Klein, B. Fady and F. Lefèvre (2019). "Characterizing the genetic diversity of Atlas cedar and phylogeny of Mediterranean *Cedrus* species with a new multiplex of 16 SSR markers." Tree Genet. Genomes. **15**(4): 1-12.

Kazi, D. (2017). Role of Zinc Ascorbic Acid and *Cedrus deodara* Root Oil in Prevention Cyclophosphamide induced Nephrotoxicity in Rat Model, Thesis. Isra University, Hyderabad.

Khanavi, M., H. Vatandoost, N. K. Dehaghi, A. S. Dehkordi, M. M. Sedaghat, A. Hadjiakhoondi and F. Hadjiakhoondi (2013). "Larvicidal activities of some Iranian native plants against the main malaria vector, *Anopheles stephensi*." Acta Med Iran. **51**(3): 141-147.

Kizil, M., G. Kizil, M. Yavuz and Ç. Aytekin (2002). "Antimicrobial activity of resins obtained from the roots and stems of *Cedrus libani* and *Abies cilicia*." Appl Biochem Microbiol **38**(2): 144-146.

KT, S., S. Gupta and J. Lal (1998). "Pharmacodynamic effects of *Cedrus deodara* wood essential oil." Indian J. Pharm. Sci. **60**(1): 20.

Kuete, V. (2014). Toxicological Survey of African Medicinal Plants, Elsevier.

Kumar, A., R. Suravajhala and M. Bhagat (2020). "Bioactive potential of *Cedrus deodara* (Roxb.) Loud essential oil (bark) against *Curvularia lunata* and molecular docking studies." SN Appl. Sci. **2**: 1-9.

Kumar, A., V. Singh and A. K. Chaudhary (2011). "Gastric antisecretory and antiulcer activities of *Cedrus deodara* (Roxb.) Loud. in Wistar rats." J Ethnopharmacol. **134**(2): 294-297.

Kumar, M., P. Kumar, N. Kumar, A. N. Singh and M. Lata (2014a). "Antimicrobial Activity of *Cedrus deodara* Linn. and *Hemidesmus indicus* Linn. Plants Against Clinically Important Micro-organism." Am J Phytomed Clin Ther. **8**(2): 952-956.

Kumar, N., D. Dhayabaran, M. Nampoothiri, K. Nandakumar, A. Puratchikody, N. Lalani, K. Dawood and A. Ghosh (2014b). "Atypical antidepressant activity of 3, 4-Bis (3, 4-dimethoxyphenyl) furan-2, 5-dione isolated from heart wood of *Cedrus deodara*, in rodents." Korean J Physiol Pharmacol. **18**(5): 365.

Kumar, S., S. Pandey and A. K. Pandey (2014c). "In vitro antibacterial, antioxidant, and cytotoxic activities of *Parthenium hysterophorus* and characterization of extracts by LC-MS analysis." BioMed Res. Int. **2014**.

Lahlou, M. (2003). "Composition and molluscicidal properties of essential oils of five Moroccan Pinaceae." Pharm. Biol. **41**(3): 207-210.

Lamiri, A., S. Lhaloui, B. Benjilali and M. Berrada (2001). "Insecticidal effects of essential oils against Hessian fly, Mayetiola destructor (Say)." Field Crops Res. **71**(1): 9-15.

Le, K., F. Chiu and K. Ng (2007). "Identification and quantification of antioxidants in *Fructus lycii*." Food Chem. **105**(1): 353-363.

Lefèvre, F., F. Courbet, C. Ripert, N. Ricodeau, E. Collin and A. Pierangelo (2016). "Cèdre de l'ʿAtlas." hal-02795429.

Liang, X., Y. P. Wu, J. H. Qiu, K. Zhong and H. Gao (2014). "A Potent Antibrowning Agent from Pine Needles of *Cedrus deodara*: 2R, 3R-Dihydromyricetin." J. Food Sci. **79**(9): C1643-C1648.

Liao, J.-Q., N. Zheng and S.-G. Xiao (2015). "Optimization of ultrasonic frequency for the improvement of extraction yields of bufadienolides from the Chinese medicine ChanSu by using a novel ultrasonic system." RSC Adv. **5**(61): 49480-49486.

Liu, J.-L., L.-C. Yang, X.-J. Zhu, W.-J. Wang and G.-D. Zheng (2018). "Combinational effect of pine needle polysaccharide and kudzu flavonoids on cell differentiation and fat metabolism in 3T3-L1 cells." Food Sci. Technol. **24**(5): 903-910.

Loizzo, MR., A. Saab, G. Statti and F. Menichini (2007). "Composition and α-amylase inhibitory effect of essential oils from *Cedrus libani*." Fitoterapia **78**(4): 323-326.

Loizzo, M. R., A. Saab, R. Tundis, G. A. Statti, I. Lampronti, F. Menichini, R. Gambari, J. Cinatl and H. W. Doerr (2008). "Phytochemical analysis and in vitro evaluation of the biological activity against *Herpes simplex* virus type 1 (HSV-1) of *Cedrus libani* A. Rich." Phytomedicine **15**(1-2): 79-83.

M'hirit, O. and M. Benzyane (2006). Le Cèdre de l'Atlas: Mémoire du temps, Editions Mardaga.

Magiatis, P., E. Melliou, A.-L. Skaltsounis, I. B. Chinou and S. Mitaku (1999). "Chemical composition and antimicrobial activity of the essential oils of Pistacia lentiscus var. chia." Planta medica. **65**(08): 749-752.

Mahajan, D., Z. Bhat and S. Kumar (2016). "Pine needles (*Cedrus deodara* (Roxb.) Loud.) extract as a novel preservative in cheese." Food Packag. Shelf Life. **7**: 20-25.

Marino, M., C. Bersani and G. Comi (2001). "Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae." Int J Food Microbiol. **67**(3): 187-195.

Martins, D. F., A. A. Emer, A. P. Batisti, N. Donatello, M. G. Carlesso, L. Mazzardo-Martins, D. Venzke, G. A. Micke, M. G. Pizzolatti and A. Piovezan (2015). "Inhalation of *Cedrus atlantica* essential oil alleviates pain behavior through activation of descending pain modulation pathways in a mouse model of postoperative pain." J Ethnopharmacol **175**: 30-38.

Martynov, V., O. Titov, T. Kolombar and V. Brygadyrenko (2019). "Influence of essential oils of plants on the migration activity of Tribolium confusum (Coleoptera, Tenebrionidae)." Biosyst. Divers. **27**(2): 177-185.

Mashaal, K., R. Perveen, Z. Zia, S. M. Mahmood, K. Minhas, S. Ibrahim and H. Naeem (2020). "Histopathological effects on stomach, liver and kidney associated with *Cedrus deodara* root oil in ulcer induced rats (Wistar strain)." Pak. J. Pharm. Sci. **33**(6): 2483-2488.

Maya, B. M., A. Abedini, S. C. Gangloff, A. Kabouche, Z. Kabouche and L. Voutquenne-Nazabadioko (2017). "A new δ-tocotrienolic acid derivative and other constituents from the cones of *Cedrus atlantica* and their in vitro antimicrobial activity." Phytochem. Lett. **20**: 252-258.

Mendez, M., R. Rodríguez, J. Ruiz, D. Morales-Adame, F. Castillo, F. D. Hernández-Castillo and C. N. Aguilar (2012). "Antibacterial activity of plant extracts obtained with alternative organics solvents against food-borne pathogen bacteria." Ind Crops Prod. **37**(1): 445-450.

Metreveli, M., A. Meskhidze, G. Mepharishvili, L. Gorgilade and L. Koiava (2020). "Antimicrobial Activity of the Himalayan Cedar (*Cedrus Deodara* Loud.) in Seasonal Dynamics." Bull. Georg. Natl. Acad. Sci **14**(2): 88-94.

Mhirit, O. (1999). "Le cèdre de l'Atlas à travers le réseau Silva mediterranea" cèdre". Bilan et perspectives." Forêt méditerranéenne.

Mohd, A., S. Ramji, L. Mehi, A. Irshad, Z. Mohammad and K. Santosh (2015). "Evaluation of different plant based essential oils against *Rhizoctonia solani* causing sheath blight of rice." Agriways **3**(1): 10-13.

Naik, P. M. and J. M. Al-Khayri (2016). Abiotic and biotic elicitors-role in secondary metabolites production through in vitro culture of medicinal plants. Abiotic and biotic stress in plants-recent advances and future perspectives, InTech.

Naimi, F., D. Bousta, M. Balouiri and A. E. Meskaoui (2015). "Antioxidant and free radical-scavenging properties of seeds flavonoids extract of *Cedrus atlantica* Manetti, *Linum usitatissimum* L. and *Ocimum basilicum* L. species." J. Appl. Pharm. Sci. **5**(08): 095-099.

Nam, A.-M., M. Paoli, V. Castola, J. Casanova and A. Bighelli (2011). "Identification and quantitative determination of lignans in *Cedrus atlantica* resins using 13C NMR spectroscopy." Nat. Prod. Commun. **6**(3): 379 - 385

Nam, A. M., A. Bighelli, M. Ghanmi, B. Satrani, J. Casanova and F. Tomi (2015). "Deodarone isomers in *Cedrus atlantica* essential oils and tar oils." Nat. Prod. Commun. **10**(11): 1905 - 1906

Narayan, S., C. P. Thakur, S. Bahadur, M. Thakur, S. N. Pandey, A. K. Thakur, D. K. Mitra and P. K. Mukherjee (2017). "*Cedrus deodara*: In vitro antileishmanial efficacy & immumomodulatory activity." Indian J Med Res. **146**(6): 780.

Nisha, M., M. Kalyanasundaram, K. Paily, P. Vanamail and K. Balaraman (2007). "In vitro screening of medicinal plant extracts for macrofilaricidal activity." Parasitol Res. **100**(3): 575-579.

Norin, T. and B. Winell (1971). "Diterpenoids of cones from two Cedrus species." Phytochemistry 10(11): 2818-2821.

OCDE (2014). "Partie B: Méthodes de détermination de la toxicité etdes autres effets sur la santé." The Hershberger 601: 858.

OECD (2001). "Guidance Document on acute oral toxicity." Environmental health and safety monograph series on testing and assessment 24.

Orchard, A., S. F. van Vuuren and A. M. Viljoen (2019). "Commercial essential oil combinations against topical fungal pathogens." Nat. Prod. Commun. **14**(1).

Ormerod, A., I. Hay and M. Jamieson (2000). "Treatment of hair loss." PCT Int. Appl. .

Oudjehih, B. (1999). "Première étude de la croissance et de la productivité du Cèdre de l'Atlas (*Cedrus atlantica* Manetti) dans le massif de Bélezma (Aurés Algérie)." Forêt méditerranéenne.

Pandey, S. (2018). "In Vitro Drug Release and Study of Anti-inflammatory Effect of Rasna Saptak Kwath." Asian J Pharm **11**(04) S727.

Panetsos, K., A. Christou and A. Scaltsoyiannes (1992). "First analysis on allozyme variation in cedar species (*Cedrus* sp.)." Silvae Genet. **41**(6): 339-342.

Paoli, M., A. M. Nam, V. Castola, J. Casanova and A. Bighelli (2011). "Chemical variability of the wood essential oil of *Cedrus atlantica* Manetti from Corsica." Chem. Biodivers. **8**(2): 344-351.

Parveen, R., M. A. Azmi, R. Tariq, S. Mahmood, M. Hijazi, S. Mahmud and S. Naqvi (2010). "Determination of antifungal activity of *Cedrus deodara* root oil and its compounds against *Candida albicans* and *Aspergillus fumigatus*." Pak. J. Bot **42**(5): 3645-3649.

Patil, S., T. Prakash, D. Kotresha, N. R. Rao and N. Pandy (2011). "Antihyperlipidemic potential of *Cedrus deodara* extracts in monosodium glutamate induced obesity in neonatal rats." Indian J. Pharmacol. **43**(6): 644.

Paun, G., S. Zrira, A. Boutakiout, O. Ungureanu, D. Simion, C. Chelaru and G. L. Radu (2013). "Chemical composition, antioxidant and antibacterial activity of essential oils from moroccan aromatic herbs." Rev Roum Chim **58**(11-12): 891-897.

Perrot, É., R. Paris and R. Pâris (1971). Les plantes médicinales, Presses universitaires de France.

Phrompittayarat, W., T. Hongratanaworakit, S. Tadtong, V. Sareedenchai and K. Ingkaninan (2014). "Survey of acetylcholinesterase inhibitory activity in essential oil derived from aromatic plants." Int. J. Med. Aromat. Plants **4**: 1-5.

Pijut, P. M. (2000). "Cedrus-the true cedars." J. Arboric. **26**(4).

Pinelo, M., M. Rubilar, J. Sineiro and M. Nunez (2004). "Extraction of antioxidant phenolics from almond hulls (*Prunus amygdalus*) and pine sawdust (*Pinus pinaster*)." Food Chem **85**(2): 267-273.

Popović, T., Z. Milićević, V. Oro, I. Kostić, V. Radović, A. Jelušić and S. Krnjajić (2018). "A preliminary study of antibacterial activity of thirty essential oils against several important plant pathogenic bacteria." Pestic. fitomed. **33**(3-4): 185-195.

Pradhan, G., S. Podder and S. C. Mahapatra (2016). "Effect of petroleum ether extract of *Cedrus deodara* on body weight in diabetic rats." Int. j. clin. exp. physiol. **3**(3): 140-143.

Qian-Da, X., Z. Zhi-Qiang, H. Qiang, S. Qun and Z. Wei-Cai (2020). "Antioxidant activity and structure-activity relationship of ethanol extract from pine needle of *Cedrus deodara*." Food Ind. Tech. **41**(20): 295-302.

Qiao, C.-Y., J.-H. Ran, Y. Li and X.-Q. Wang (2007). "Phylogeny and biogeography of *Cedrus* (Pinaceae) inferred from sequences of seven paternal chloroplast and maternal mitochondrial DNA regions." Ann. Bot. **100**(3): 573-580.

Quettier-Deleu, C., B. Gressier, J. Vasseur, T. Dine, C. Brunet, M. Luyckx, M. Cazin, J.-C. Cazin, F. Bailleul and F. Trotin (2000). "Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour." J Ethnopharmacol. **72**(1-2): 35-42.

Quézel, P. and S. Santa (1962, 1963). Nouvelle flore de l'Algérie et des régions désertiques méridionales, 2 Tomes, Editions CNRS, Paris.

Raghavendhar, S., P. K. Tripati, P. Ray and A. K. Patel (2019). "Evaluation of medicinal herbs for Anti-CHIKV activity." Virology. **533**: 45-49.

Rahman, A., V. K. Bajpai, N. T. Dung and S. C. Kang (2011). "Antibacterial and antioxidant activities of the essential oil and methanol extracts of Bidens frondosa Linn." Int J Food Sci Tech. **46**(6): 1238-1244.

Rahman, M. A. and M. S. Islam (2013). "Antioxidant, antibacterial and cytotoxic effects of the phytochemicals of whole *Leucas aspera* extract." Asian Pac J Trop Biomed. **3**(4): 273.

Ramadass, M., S. A. Hakeem, A. Y. Chandran, G. Vadivelu and P. Thiagarajan (2019). "Formulation and Characterization of *Cedrus deodara* Oil Emulsion and studies on its activity against representative food and plant pathogens." Res J Pharm Technol. **12**(3): 1333-1337.

Ramesh, C., N. Krishnadas, R. Radhakrishnan, S. Rangappa, G. L. S. Viswanatha, D. Rajesh, M. Gopal and S. Talwar (2010). "Anti-urolithiatic activity of heart wood extract of *Cedrus deodara* in rats." J. Complement. Integr. Med. **7**(1).

Ramzan, M., R. M. Obodo, S. Mukhtar, S. Ilyas, F. Aziz and N. Thovhogi (2021). "Green synthesis of copper oxide nanoparticles using *Cedrus deodara* aqueous extract for antibacterial activity." Mater Today Proc. **36**: 576-581.

Rao, K., B. Ch, L. M. Narasu and A. Giri (2010). "Antibacterial activity of *Alpinia galanga* (L) Willd crude extracts." Appl Biochem Biotechnol. **162**(3): 871-884.

Rather, R. A. and M. Bhagat (2018). "Cancer chemoprevention and piperine: molecular mechanisms and therapeutic opportunities." Front. Cell Dev. Biol. **6**: 10.

Reddy, S. E. and S. K. Dolma (2018). "Acaricidal activities of essential oils against two-spotted spider mite, *Tetranychus urticae* Koch." Toxin Rev. **37**(1): 62-66.

Reddy, S. E., S. Kirti Dolma, R. Koundal and B. Singh (2016). "Chemical composition and insecticidal activities of essential oils against diamondback moth, *Plutella xylostella* (L.)(Lepidoptera: Yponomeutidae)." Nat. Prod. Res. **30**(16): 1834-1838.

Reichardt, C. and T. Welton (2011). Solvents and solvent effects in organic chemistry, John Wiley & Sons.

Rhafouri, R., B. Strani, T. Zair, M. Ghanmi, A. Aafi, M. El Omari and A. Bentayeb (2014). "Chemical composition, antibacterial and antifungal activities of the *Cedrus atlantica* (Endl.) Manetti ex Carrière seeds essential oil." Mediterr. J. Chem. **3**(5): 1034-1043.

Rostagno, M. A. and J. M. Prado (2013). Natural product extraction: principles and applications, R. Soc. Chem.

Saab, A., F. Harb and W. Koenig (2005). "Essential oils components in the leaves of *Cedrus libani* and *Cedrus deodara*." Minerva Biotecnol. **21**(4): 201.

Saab, A. M., R. Gambari, G. Sacchetti, A. Guerrini, I. Lampronti, M. Tacchini, A. El Samrani, S. Medawar, H. Makhlouf and M. Tannoury (2018). "Phytochemical and pharmacological properties of essential oils from Cedrus species." Nat. Prod. Res. **32**(12): 1415-1427.

Saab, A. M., A. Guerrini, G. Sacchetti, S. Maietti, M. Zeino, J. Arend, R. Gambari, F. Bernardi and T. Efferth (2012a). "Phytochemical analysis and cyto-toxicity towards multidrug-resistant leukemia cells of essential oils de-rived from lebanese medicinal plants." Planta Med. **78**(18): 1927-1931.

Saab, A. M., I. Lampronti, M. Borgatti, A. Finotti, F. Harb, S. Safi and R. Gambari (2012b). "*In vitro* evaluation of the anti-proliferative activities of the wood essential oils of three *Cedrus* species against K562 human chronic myelogenous leukaemia cells." Nat. Prod. Res. **26**(23): 2227-2231.

Saab, A. M., I. Lampronti, A. Grandini, M. Borgatti, A. Finotti, G. Sacchetti, R. Gambari and A. Guerrini (2011). "Antiproliferative and erythroid differentiation activities of *Cedrus libani* seed extracts against K562 human chronic myelogenus leukemia cells." Int J Pharm Biol Arch. **2**(6): 1744-1748.

Sabatier, S., P. Baradat and D. Barthelemy (2003). "Intra-and interspecific variations of polycyclism in young trees of *Cedrus atlantica* (Endl.) Manetti ex. Carrière and *Cedrus libani* A. Rich (Pinaceae)." Ann. For. Sci. **60**(1): 19-29.

Satrani, B., M. Aberchane, A. Farah, A. Chaouch and M. Talbi (2006). "Composition chimique et activité antimicrobienne des huiles essentielles extraites par hydrodistillation fractionnée du bois de *Cedrus atlantica* Manetti." Acta Bot. Gall. **153**(1): 97-104.

Savill, P. and S. Wilson (2015). "*Cedrus*, true cedars: silviculture and properties." Q J Forest. **109**(3): 168-173.

Saxena, A., A. Saxena, J. Singh and S. Bhushan (2010). "Natural antioxidants synergistically enhance the anticancer potential of AP9-cd, a novel lignan composition from *Cedrus deodara* in human leukemia HL-60 cells." Chem Biol Interact. **188**(3): 580-590.

Saxena, S., V. Uniyal and R. Bhatt (2012). "Inhibitory effect of essential oils against *Trichosporon ovoides* causing Piedra Hair Infection." Brazilian Journal of Microbiology **43**(4): 1347-1354.

Scalbert, A. (1991). "Antimicrobial properties of tannins." Phytochemistry **30**(12): 3875-3883.

Scaltsoyiannes, A. (1999). "Allozyme differentiation and phylogeny of cedar species." Silvae Genet. **48**(2): 61-68.

Semerci, A. B., D. İnceçayir, T. Konca, H. Tunca and K. Tunç (2020). "Phenolic constituents, antioxidant and antimicrobial activities of methanolic extracts of some female cones of gymnosperm plant." Indian J. Biochem. Biophys. **57**(3): 298-303.

Senol, F. S., I. E. Orhan and O. Ustun (2015). "In vitro cholinesterase inhibitory and antioxidant effect of selected coniferous tree species." Asian Pac. J. Trop. Med. **8**(4): 269-275.

Shashi, B., S. Jaswant, R. J. Madhusudana, S. A. Kumar and Q. G. Nabi (2006). "A novel lignan composition from *Cedrus deodara* induces apoptosis and early nitric oxide generation in human leukemia Molt-4 and HL-60 cells." Nitric oxide **14**(1): 72-88.

Shebaby, W., A. Elias, M. Mroueh, B. Nehme, N. D. El Jalbout, R. Iskandar, J. C. Daher, M. Zgheib, P. Ibrahim and V. Dwairi (2020). "Himachalol induces apoptosis in B16-F10 murine melanoma cells and protects against skin carcinogenesis." J Ethnopharmacol. **253**: 112545.

Shi, X., R. Du, J. Zhang, Y. Lei and H. Guo (2019). "Evaluation of the anti-cancer potential of *Cedrus deodara* total lignans by inducing apoptosis of A549 cells." BMC Compl Alternative Med. **19**(1): 1-7.

Shi, X., D. Liu, J. Zhang, P. Hu, W. Shen, B. Fan, Q. Ma and X. Wang (2016). "Extraction and purification of total flavonoids from pine needles of *Cedrus deodara* contribute to anti-tumor in vitro." BMC Compl Alternative Med. **16**(1): 1-9.

Shinde, U., K. Kulkarni, A. Phadke, A. Nair, A. Mungantiwar, V. Dikshit and M. Saraf (1999a). "Mast cell stabilizing and lipoxygenase inhibitory activity of *Cedrus deodara* (Roxb.) Loud. wood oil." Indian J Exp Biol. **37**(3): 258-261.

Shinde, U., A. Phadke, A. Nair, A. Mungantiwar, V. Dikshit and M. Saraf (1999b). "Preliminary studies on the immunomodulatory activity of *Cedrus deodara* wood oil." Fitoterapia. **70**(4): 333-339.

Shinde, U., A. Phadke, A. Nair, A. Mungantiwar, V. Dikshit and M. Saraf (1999c). "Studies on the anti-inflammatory and analgesic activity of *Cedrus deodara* (Roxb.) Loud. wood oil." J Ethnopharmacol. **65**(1): 21-27.

Shirwaikar, A., K. Rajendran and I. S. R. Punitha (2006). "In vitro antioxidant studies on the benzyl tetra isoquinoline alkaloid berberine." Biol. Pharm. Bull. **29**(9): 1906-1910.

Singh, S. K., M. Shanmugavel, H. Kampasi, R. Singh, D. Mondhe, J. M. Rao, M. Adwankar, A. Saxena and G. Qazi (2007). "Chemically standardized isolates from *Cedrus deodara* stem wood having anticancer activity." Planta Med. **73**(06): 519-526.

Singh, P., R. Khosa and G. Mishra (2013). "Evaluation of antidiabetic activity of ethanolic extract of *Cedrus deodara* (Pinaceae) stem bark in streptozotocin induced diabetes in mice." Niger J Exp Clin Biosci. **1**(1): 33.

Skanderi, I. and O. Chouitah (2020). "Chemical characterization and antioxidant activity of *Cedrus atlantica* manetti tar (Atlas cedar tar)." Fr. Ukr. J. Chem. **8**(2): 244-255.

Song, X., H. Li, C. Li, J. Xu and D. Hu (2016). "Effects of VOCs from leaves of *Acer truncatum* Bunge and *Cedrus deodara* on human physiology and psychology." Urban For Urban Green. **19**: 29-34.

Sporn, M. B. (1976). "Approaches to prevention of epithelial cancer during the preneoplastic period." Cancer Res. **36**(7 Part 2): 2699-2702.

Sung, H., J. Ferlay, R. L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal and F. Bray (2021). "Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries." CA Cancer J Clin **71**(3): 209-249.

Suryavanshi, S., A. Zanwar, M. Hegde and R. Kaul-Ghanekar (2014). "Standardization of a polyherbal formulation (HC9) and comparative analysis of its cytotoxic activity with the individual herbs present in the composition in breast cancer cell lines." Pharmacogn. J. **6**(2): 87-95.

Takci, H. A. M., F. U. Turkmen and M. Sari (2019). "In vitro mutagenic effect of cedar (*Cedrus libani* A. Rich) tar in the salmonella/microsome assay system." Banats J Biotechnol. **10**(20): 13-18.

Talluri, M., S. R. Yathapu and D. K. Bharatraj (2018). "Evaluation of Rasna panchaka (indigenous drug) as oxidative stress down-regulator using serum-free explant culture system." Indian J. Pharmacol. **50**(6): 326.

Tanwar, G., A. G. Mazumder, V. Bhardwaj, S. Kumari, R. Bharti, D. Singh, P. Das and R. Purohit (2019). "Target identification, screening and in vivo evaluation of pyrrolone-fused benzosuberene compounds against human epilepsy using Zebrafish model of pentylenetetrazol-induced seizures." Sci. Rep. **9**(1): 1-12.

Tarranum, A., U. Malhotra, A. Ghildiyal and P. Chandola (2014). "Antimicrobial activity of plants (*Cinnamomum zeylanicum, Cedrus deodara, Eucalyptus globulus, Rosmarinus officinalis*) essential oils against some bacterial and fungal strains." Octa. J. Biosci. **2**(1): 49-52.

Telci, I., I. Demirtas and A. Sahin (2009). "Variation in plant properties and essential oil composition of sweet fennel (*Foeniculum vulgare* Mill.) fruits during stages of maturity." Ind Crops Prod **30**(1): 126-130.

Thabrew, M. I., M. Dharmasiri and L. Senaratne (2003). "Anti-inflammatory and analgesic activity in the polyherbal formulation Maharasnadhi Quathar." J Ethnopharmacol. **85**(2-3): 261-267.

Thaipong, K., U. Boonprakob, K. Crosby, L. Cisneros-Zevallos and D. H. Byrne (2006). "Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts." J Food Compost Anal. **19**(6-7): 669-675.

Todorovic, V., I. R. Redovnikovic, Z. Todorovic, G. Jankovic, M. Dodevska and S. Sobajic (2015). "Polyphenols, methylxanthines, and antioxidant capacity of chocolates produced in Serbia." J Food Compost Anal. **41**: 137-143.

Toth, Jean and R. Moreau (2005). Le cèdre de France. Etude approfondie de l'espèce. France, L'Harmattan.

Truchan, M., H. Tkachenko, L. Buyun, N. Kurhaluk, A. Góralczyk, V. Tomin and Z. Osadowski (2019). "Antimicrobial activities of three commercial essential oils derived from plants belonging to family pinaceae." Agrobiodivers. Improv. Nutr., Health Life Qual.(3): 111–126.

Tumen, I., E. K. Akkol, I. Süntar and H. Keleş (2011). "Wound repair and anti-inflammatory potential of essential oils from cones of Pinaceae: Preclinical experimental research in animal models." J Ethnopharmacol. **137**(3): 1215-1220.

Uniyal, V., S. Saxena and R. Bhatt (2013). "Screening of some essential oils against Trichosporon species." J. Environ. Biol. **34**(1): 17.

Upton, R., A. Graff, G. Jolliffe, R. Laenger and E. Williamson (2011). American Herbal Pharmacopoeia: botanical pharmacognosy-microscopic characterization of botanical medicines, American Herbal Pharmacopoeia/CRC Press.

Uwineza, M., B. El Yousfi and A. Lamiri (2018a). "Activités antifongiques in vitro des huiles essentielles de Mentha pulegium, Eugenia aromatica et Cedrus atlantica sur Fusarium culmorum et Bipolaris sorokiniana." Rev Mar Pro Pla(12): 19-32.

Van de Loosdrecht, A., R. Beelen, g. Ossenkoppele, M. Broekhoven and M. Langenhuijsen (1994). "A tetrazolium-based colorimetric MTT assay to quantitate human monocyte mediated cytotoxicity against leukemic cells from cell lines and patients with acute myeloid leukemia." J Immunol Methods. **174**(1-2): 311-320.

Venditti, A., F. Maggi, A. Saab, M. Bramucci, L. Quassinti, D. Petrelli, L. Vitali, G. lupidi, A. El Samrani and M. Borgatti (2020). "Antiproliferative, antimicrobial and antioxidant properties of *Cedrus libani* and *Pinus pinea* wood oils and *Juniperus excelsa* berry oil." Plant Biosyst.: 1-20.

Venegas-Sanchez, J. A., M. Tagaya and T. Kobayashi (2013). "Effect of ultrasound on the aqueous viscosity of several water-soluble polymers." Polym. J **45**(12): 1224-1233.

Verma, R. K., L. Chaurasia and M. Kumar (2011). "Antifungal activity of essential oils against selected building fungi." Indian J Nat Prod Resour. **2**(4): 448-451

Viau, C. and R. Tardif (2003). Toxicologie. Environnement et santé publique: Fondements et pratiques. M. Gérin, P. Gosselin, S. Cordier et al. Acton Vale / Paris, Édisem/Tec & Doc**:** 119-143.

Wong, C.-C., H.-B. Li, K.-W. Cheng and F. Chen (2006). "A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay." Food chem. **97**(4): 705-711.

Wu, Y-P., J.-R. Bai, E. Grosu, K. Zhong, L.-J. Liu, M.-M. Tang, Y.-N. Huang and H. Gao (2018a). "Inhibitory effect of 2R, 3R-dihydromyricetin on biofilm formation by *Staphylococcus aureus*." Foodborne Pathog. Dis. **15**(8): 475-480.

Wu, Y-P., J.-R. Bai, K. Zhong, D.-D. Bai, Y.-N. Huang, K. Xiao, Y. Ran and H. Gao (2018b). "Antibacterial Effect of 2R, 3R-dihydromyricetin on the Cellular Functions of *Staphylococcus aureus*." Biosci. Biotechnol. Biochem. **82**(1): 135-138.

Wu, Y-P., X. Liang, X.-Y. Liu, K. Zhong, B. Gao, Y.-N. Huang and H. Gao (2015). "*Cedrus deodara* pine needle as a potential source of natural antioxidants: Bioactive constituents and antioxidant activities." J. Funct. Foods. **14**: 605-612.

Wu, Y-P., X.-Y. Liu, J.-R. Bai, H.-C. Xie, S.-L. Ye, K. Zhong, Y.-N. Huang and H. Gao (2019). "Inhibitory effect of a natural phenolic compound, 3-p-trans-coumaroyl-2-hydroxyquinic acid against the attachment phase of biofilm formation of *Staphylococcus aureus* through targeting sortase A." RSC Adv. **9**(56): 32453-32461.

Wu, Y., J. Bai, K. Zhong, Y. Huang, H. Qi, Y. Jiang and H. Gao (2016). "Antibacterial activity and membrane-disruptive mechanism of 3-p-trans-coumaroyl-2-hydroxyquinic acid, a novel phenolic compound from pine needles of *Cedrus deodara*, against *Staphylococcus aureus*." Molecules **21**(8): 1084.

Wu, Y., J. Bai, X. Liu, L. Liu, K. Zhong, Y. Huang and H. Gao (2018c). "Antibacterial effect of 3-p-trans-coumaroyl-2-hydroxyquinic acid, a phenolic compound from needles of *Cedrus deodara*, on cellular functions of *Staphylococcus aureus*." RSC advances. **8**(9): 4969-4975.

Xu, F., D. Gu, M. Wang, L. Zhu, T. Chu, Y. Cui, J. Tian, Y. Wang and Y. Yang (2017). "Screening of the potential α-amylase inhibitor in essential oil from *Cedrus deodara* cones." Ind Crops Prod. **103**: 251-256.

Yang, C. S., J. M. Landau, M.-T. Huang and H. L. Newmark (2001). "Inhibition of carcinogenesis by dietary polyphenolic compounds." Annu. Rev. Nutr. **21**(1): 381-406.

Yasmeen, F., T. Rauf, M. Babar, S. Ali and S. Andleeb (2015). "*Cedrus deodara* (Deodar) and *Zanthoxylum armatum* (Timur) Evaluated as Antimicrobial and Antioxidant Agents." J. Pharm. Sci. Pharmacol. **2**(2): 110-118.

Yassaa, N., B. Y. Meklati and A. Cecinato (2000). "Evaluation of monoterpenic biogenic volatile organic compounds in ambient air around *Eucalyptus globulus*, *Pinus halepensis* and *Cedrus atlantica* trees growing in Algiers city area by chiral and achiral capillary gas chromatography." Atmos. Environ. **34**(17): 2809-2816.

Yeşilada, E., İ. Gürbüz and H. Shibata (1999). "Screening of Turkish anti-ulcerogenic folk remedies for anti-*Helicobacter pylori* activity." J Ethnopharmacol. **66**(3): 289-293.

Yeşilada, E., E. Sezik, T. Fujita, S. Tanaka and M. Tabata (1993). "Screening of some Turkish medicinal plants for their antiulcerogenic activities." Phytother Res. **7**(3): 263-265.

Yin, X., Q. You, Z. Jiang and X. Zhou (2016). "Optimization for ultrasonic-microwave synergistic extraction of polysaccharides from *Cornus officinalis* and characterization of polysaccharides." Int. J. Biol. Macromol. **83**: 226-232.

Yousef, I., S. Oran, Y. Bustanji, D. Al-Eisawi and B. Abu-Irmaileh (2018). "Cytotoxic effect of selected wild medicinal plant species from Jordan on two different breast cancer cell lines, MCF7 and T47D." Biol Med. **10**(443): 433-433C.

Yu, Z., R. Dhital, W. Wang, L. Sun, W. Zeng, A. Mustapha and M. Lin (2019). "Development of multifunctional nanocomposites containing cellulose nanofibrils and soy proteins as food packaging materials." Food Packag. Shelf Life. **21**: 100366.

Yuan, H., Q. Ma, L. Ye and G. Piao (2016). "The traditional medicine and modern medicine from natural products." Molecules. **21**(5): 559.

Zaman, T., M. S. Syed, S. Isfaq and M. S. Khan (2018). "Biological activities of stem, leaves and essential oil of *Cedrus deodara* from district Poonch, Rawalakot Azad Kashmir, Pakistan." Turk J. Food Agric. Sci. Tech. **6**(9): 1114-1119.

Zeng, W-C., Q. He, Q. Sun, K. Zhong and H. Gao (2012a). "Antibacterial activity of water-soluble extract from pine needles of *Cedrus deodara*." Int J Food Microbiol **153**(1-2): 78-84.

Zeng, W-C., Z. Zhang and L.-R. Jia (2014). "Antioxidant activity and characterization of antioxidant polysaccharides from pine needle (*Cedrus deodara*)." Carbohydr. Polym. **108**: 58-64.

Zeng, W-C., Z. Zhang, H. Gao, L. R. Jia and Q. He (2012b). "Chemical composition, antioxidant, and antimicrobial activities of essential oil from pine needle (*Cedrus deodara*)." J. Food Sci. **77**(7): C824-C829.

Zhang, Z., X. Lyu, Q. Xu, C. Li, M. Lu, T. Gong, B. Tang, L. Wang, W. Zeng and Y. Li (2020). "Utilization of the extract of *Cedrus deodara* (Roxb. ex D. Don) G. Don against the biofilm formation and the expression of virulence genes of cariogenic bacterium *Streptococcus mutans*." J Ethnopharmacol. **257**: 112856.

Zhao, Y., W. Huang, J. Wang, Y. Chen, W. Huang and Y. Zhu (2018a). "Taxifolin attenuates diabetic nephropathy in streptozotocin-induced diabetic rats." Am. J. Transl. Res. **10**(4): 1205.

Zhao, Z., Z. Dong, J. Ming and Y. Liu (2018b). "Cedrin identified from *Cedrus deodara* (Roxb.) G. Don protects PC12 cells against neurotoxicity induced by Aβ1–42." Nat. Prod. Res. **32**(12): 1455-1458.

Zoubi, Y. E., E. Fouad, A. Farah, K. Taghzouti and A. E. O. Lalami (2017). "Chemical composition and larvicidal activity of Moroccan Atlas Cedar (*Cedrus atlantica* Manetti) against *Culex pipiens* (Diptera: Culicidae)." J Appl Pharm. **7**(07): 030-034.

Zrira, S. and M. Ghanmi (2016). "Chemical composition and antibacterial activity of the essential of *Cedrus atlantica* (Cedarwood oil)." J Essent Oil-Bear Plants. **19**(5): 1267-1272.

Abstract

In the present study, chemical composition, antioxidant, antibacterial and cytotoxic activities of samples from *Cedrus atlantica* cones and branches were evaluated. The essential oils derived from the cones were analysed by gas chromatography/mass spectrometry (GC/MS). Furthermore, ultrasound power has been applied in the objective to enhance the physicochemical properties of the extracts. Ferric reducing antioxidant power (FRAP) and free radical scavenging assays (DPPH and ABTS) were conducted to measure the antioxidant activity. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were recorded using three Gram-positive and negative bacteria. The cytotoxic activity of extracts and essential oil was assessed on MCF-7 breast cancer cell line using MTT assay. GC/MS revealed that the major compound of the essential oil was α-pinene. The extracts and fractions from branches were found rich in polyphenols mainly tannins. Ethyl acetate fraction exhibited the best antioxidant activity. *Staphylococcus aureus* was found the most susceptible strain. Essential oil and tested fractions have not demonstrated interesting effects against MCF-7 cell lines. The acute toxicity study showed that the crude extract is little toxic or not toxic. Finally, ultrasound power application reduced the extract viscosity, increased the solubily and conserved the antiaxidant capacity. In conclusion, *C. atlantica*'s samples showed antioxidant, antibacterial, and anticancer activities. Thereby, the ethnobotanical use of *C. atlantica* in traditional preparations is worth investigating as the plant appears to be a potential source of interesting metabolites.

Keywords: *Cedrus atlantica*, chemical composition, antioxidant, antibacterial, cytotoxic.

Résumé

Dans la présente étude, la composition chimique, les activités antioxydantes, antibactériennes et cytotoxiques d'échantillons de cônes et de branches de *Cedrus atlantica* ont été évaluées. Les huiles essentielles (HE) dérivées des cônes ont été analysées par chromatographie en phase gazeuse / spectrométrie de masse (GC / MS). En outre, l'ultrasonication a été appliquée dans l'objectif d'améliorer les propriétés physico-chimiques des extraits. Le pouvoir réducteur ferrique (FRAP) et les essais de piégeage des radicaux libres (DPPH et ABTS) ont été réalisés pour mesurer l'activité antioxydante. La concentration minimale inhibitrice (CMI) et la concentration bactéricide minimale (MBC) ont été enregistrées sur trois bactéries Gram-positives et négatives. L'activité cytotoxique des extraits et de l'HE a été évaluée sur la lignée cellulaire de cancer du sein MCF-7 en utilisant le test MTT. La GC / MS a révélé que le composé principal de l'HE était l'α-pinène. Les extraits et fractions de branches se sont révélés riches en polyphénols principalement en tanins. La fraction d'acétate d'éthyle a présenté la meilleure activité antioxydante. *Staphylococcus aureus* a été trouvé la souche la plus sensible. L'HE et les fractions testées n'ont pas démontré d'effets intéressants contre les lignées cellulaires MCF-7. L'étude de toxicité aiguë a montré que l'extrait brut est peu ou pas toxique. Enfin, l'application des ultrasons a fait diminuer la viscosité de l'extrait, augmenter la solubilité et préserver son pouvoir antioxydant. En conclusion, les échantillons de *C. atlantica* ont montré des activités antioxydantes, antibactériennes et anticancéreuses. Ainsi, l'utilisation ethnobotanique de *C. atlantica* dans les préparations traditionnelles est intéressante d'être étudiée car la plante semble être une source potentielle de métabolites intéressants.

Mots clés: *Cedrus atlantica*, composition chimique, antioxydant, antibactériien, cytotoxique.

الملخص

في هذه الدراسة ، تم تقييم التركيب الكيميائي ، ومضادات الأكسدة ، والأنشطة المضادة للبكتيريا والسمية للخلايا لعينات من مستخلصات الأرز الأطلسي ، وتم تحليل الزيوت الأساسية المشتقة من الأقماع بواسطة كروماتوجرافيا الغاز / مطياف الكتلة. علاوة على ذلك ، تم تطبيق قوة الموجات فوق الصوتية بهدف تعزيز الخصائص الفيزيائية والكيميائية للمستخلصات. تم إجراء اختبارات القدرة المضادة للأكسدة الحديديك واختبارات إزالة المؤكسدات الحرة لقياس نشاط مضادات الأكسدة. تم تسجيل أدنى تركيز مثبط وأدنى تركيز مبيد للجراثيم باستخدام ثلاثة أنواع من البكتيريا موجبة وسالبة الجرام. تم تقييم النشاط السام للخلايا للمستخلصات والزيوت الأساسية على خلايا سرطان الثدي . تم العثور على المستخلصات غنية بالبوليفينول . أظهر خلات الإيثيل أفضل نشاط مضاد للأكسدة. تم الكشف على أن البكتيريا الموجبة أكثر السلالات حساسية. لم يُظهر الزيت الأساسي والمستخلصات المختبرة تأثيرات مثيرة للاهتمام ضد خلايا سرطان الثدي. أظهرت دراسة السمية الحادة أن المستخلص الخام قليل السمية أو غير سام. أخيرًا، قلل استخدام قوة الموجات فوق الصوتية من لزوجة المستخلص، وزاد من الذوبان وحافظ على قدرة مضادات الأكسدة. في الختام ، أظهرت عينات الأرز الأطلسي نشاطًا مضادًا للأكسدة ومضادًا للبكتيريا ومضادًا للسرطان. وبالتالي، فإن استخدام الأرز الأطلسي في المستحضرات التقليدية يستحق التحقيق حيث يبدو أن النبات مصدر محتمل للمركبات المثيرة للاهتمام..

الكلمات المفتاحية : التركيب الكيميائي ، مضادات الأكسدة ، مضاد للجراثيم ، سام للخلايا