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THESIS

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THEME

Applications of plants extracts in human health

Presented by: Miss BENSACI Narimen

Supervisor : Mr. AOUADI Saoudi — **Prof., University of Badji Mokhtar Annaba**

Co-Supervisor : Ms. ABDI Akila — **Prof., University of Badji Mokhtar Annaba**

Member of the Jury:

Chairman: Mr. SAKA Saad — **Prof., University of Badji Mokhtar, Annaba**

Examiner : Mr. HOUHAMDI Moussa — **Prof., University of 8 Mai 1945, Guelma**

Examiner : Mm. BIREM-KADI Assia — **MCA, University of Badji Mokhtar,Annaba**

Examiner: Mr. BOUKACHABIA Mourad — **MCA, University of Badji Mokhtar,Annaba**

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In the name of Allah, the Beneficent, the Merciful

DEDICATION

To my loving Parents, to Mom and Dad

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LIST OF CONTENTS page

Abbreviation, Chemical formulas & Symbols

Abbreviation	Significations
µm	Micrometer
AA	Ascorbic Acid
Ac	Acetyl group
ADH	Arginine DiHydrolase
AMY	amygdalin
API	Analytical Profile Index
Aq-E	Aqueous extract
ATCC	American Type Culture Collection.
AZWSP	Azarole Water Soluble Polysaccharide
BHT	Butylated Hydroxytoluene
CE	Catechin Equivalent
CFS	Cell-Free Supernatant
CIT	Citrate
D	Dalton
DAD	Diode-Array Detection
DHB	2,5-DiHydroxyBenzoic acid
DMSO	DiMethyl SulfOxide
DNS	DiNitroSalicylate
DPPH	2,2- DiPhenyl-1-PicrylHydrazyl
EC_{50}	Effective Concentration 50
EO	Essential Oil
Fe^{+3}	Ferric ion
$FeSO_4$ $7H_2O$	Ferrous Sulphate Heptahydrate
FRAP	Ferric-Reducing Ability Power
FT-IR	Fourier Transform InfraRed
GA eq	Gallic Acid Equivalent
GaM	GalactoMannans
GEL	Gelatinase
GGM	GalactoGlucoMannans
GI	Gastrointestinal
Glc	Glucose
GlcA	Glucose acid
GM	GlucoMannans

Abbreviation, Chemical formulas & Symbols

H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulfide production
HAz	Hydrolysat Azarole Polysaccharide
Hex	Hexose
HHDP	HexaHydroxyDiPhenoyl
HO	Hydroxyl group
HPAEC-PAD	High Performance Anion Exchange Chromatography Columns with Pulsed Amperometric Detection
HPLC	High Performance Liquid Chromatography
IC_{50}	Inhibitory Concentration 50
IND	Indole
KGM	Konjac GlucoMannans
LAB	Lactic Acid Bacteria
LDC	Lysine DeCarboxylase
LSTR	LysoStaphin Restisance
M/G	Mannose/Galactose
MAL	Mannitol
MALDI	Matrix-Assisted Laser Desorption, Ionisation
MBC	Minimum Bactericidal Concentration
MDG	Methyl-αD Glucopyranoside
Me	Methyl group
MEL	Melibiose
MeOH-E	Methanolic extract
MHB	Mueller Hinton Broth
MHz	Mega Hertz
MIC	Minimum Inhibitory Concentration
m-MRS	Modified Man Rogosa Sharpe
MRS	De Man, Rogosa and Sharpe
NAG	N-Acetyl glucosamine
NIT	Nitrate
NO	Nitric oxide
NO_2	Nitrite
NOE	Nuclear Overhauser Effect

Abbreviation, Chemical formulas & Symbols

O_2^-	Superoxide anions (O_2^-),
OD	Optic density
ODC	Ornithine DeCarboxylase
ONPG	Ortho NitroPhenyl-βD Galactopyranosidase
OX	Oxidase
PAL	Phosphatase ALcaline
PDA	Potato Dextrose Agar
Pent	Pentose
pH	Potential Hydrogen
ppm	Parts Per Million
QE	Quercetin Equivalent
RAF	Raffinose
ROO	Peroxide
RP-HPLC	Reversed-Phase high-performance liquid chromatography
rpm	Revolutions per minute
SCFA	Short-chain fatty acids
T	T lymphocyte
TAC	Total Antioxidant Capacity
TDA	Tryptophan Deaminase
Th1	Type 1 T helper
TLC	Thin Layer Chromatography
TOF	Time-Of-Flight
TRE	Trehalose
URE	Urease
UV-Vis	Ultra-Violet, visible
v/v	Volume/Volume
VP	Voges-Proskauer
w/v	Weight/Volume
XLT	Xylitol

Figures

Tables

INTRODUCTION

Introduction

The use of plant material in human health has a long tradition in many countries and is now recognised by international organisations (World Health Organisation 2002). There are two types of active substances in plants, those of the first type, produced by primary metabolite (mainly saccharides), are substances present in all green plants through photosynthesis and are essential for their life. Those of the second type are products of secondary metabolite, which result from processes that originate mainly in the assimilation of nitrogen. These products sometimes seem to be useless to the plant.

The aromatic substances, terpenes and alkaloids, are the main representatives, as well as the heterosides (anthocyanin, leuco-anthocyanin) of the second type of substances. The pharmaceutical industry makes frequent use of these substances.

On one side, the increasing appearance of resistant germs and, on the other, the use of antibiotics in certain regions of the world without precise microbiological analysis has led doctors to take an interest in natural remedies and medicinal plants. Thus, a wide variety of essential oils (EO) and plant extracts have been tested in recent years for their chemical and biological activities. EO's from aromatic plants, e.g. fennel (*Foeniculum vulgare*), peppermint (*mentha piperéta*), and Hyme (*Thymis vulgaris*) are been shown to be very effective against Gram positive and Gram-negative bacteria as well as yeasts, fungi and viruses (Wink 1999).

Other substances such as volatile phenolic compounds, flavonoids are available in most plants. Among the most interesting flavonoids are anthocyanins, flavonols, true tannins, flavones, rotenone and rotenoids (Guignard and Maurice Jay 1975). These compounds can be selectively extracted with organic or aqueous solvents. Herbal remedies (e.g. antioxidant activity, antimicrobial activity) trigger immense advantages over chemical treatments.

Much work has been done on *Tuberaria lignosa*, particularly in the Iberian Peninsula. The reports indicate that this plant is endowed with excellent antioxidant, anti-inflammatory, antimicrobial and antiproliferation antitumor properties: thus, we retained the antimicrobial and antioxidant properties to test *T. lignosa* harvested in the region of Chétaibi (Annaba, Algeria).

Hemicells, a product of primary metabolite, can account for up to 25% of the dry matter of many plant materials. These macromolecules are remarkable for the variety of polysaccharide structures present in plants. According to Joseleau, the different groups of hemicelluloses present in plant cell walls are xylans, mannans, galactans, arabinogalactans and glucans (Joseleau 1980).

The mannan group consists of mannan itself, the best known are extracted from the American *Phytelephas*. It is formed through the condensation of D-mannose units by β (1→4) glycosidic bonds. As well as galactomannans, it is composed of a β -D- mannopyranosyl main chain, (1 →4) linked with (1→ 6) branches formed of a single α-D galactopyranosyl unit. Galactomannans are hydrocolloids used in the food industry, along with glucomannans. From a structural point of view, typical glucomannans have the same structure, with D-glucose and D-mannose residues linked together by β (1→4) bonds. The most missing structural differences are in the occasional substitution of glucomannans from some plants on the C-6 of glucose and mannose by galactose units.

Glucomannans are found in angiosperm and also in softwoods (Shi et al. 2020). They have been extracted from leaves (acemannan), tubes or roots (Konjac flour and salep), bulbs and pseudobulbs (*Lilium* species and *cyrtopodium cardiochilium*), stems (*Dendrobium*), and seeds (Libyan dates) (Singh et al. 2018).

Many plants are capable to produce glucomannan, but only a few have an iterative process at the individual level. However, some have now reached the stage of commercialisation as the GMs of Konjac. We thought it would be interesting to compare some of their biological properties (prebiotic and antioxidant activities) under identical manipulation conditions. The knowledge of the primary structure of polysaccharides is the basis for their classification and correlations: structures and properties. Chemical and spectroscopic techniques were used to determine the primary structure of a glucomannan extracted from the fruits of *Crataegus azarolus* harvested in the eastern Algerian region.

The fact that antibiotic resistance has concerning increases, became a serious threat for public health. So, our investigation tends to discover and evaluate new safe and naturel substances that can replace antibiotics. There are two potential solutions to regulate the gut microbiota after getting multi-drug resistance infection; the pathogens growth is inhibited through a direct strategy applying antimicrobial compounds (polyphenols, bacteriocins) or an indirect strategy using lactic acid bacteria with probiotic property, this latter have the ability to eliminate competitors, and they can use polysaccharide as carbon source contrarily to pathogens which can ferment only simple sugar units. Both *Tuberaria lignosa* leaves and *Crataegus azarolus* fruits were tested in order to promote the colon health and the manage the gastrointestinal disorders.

LITERATURE REVIEW

I. Literature Review

I.1. Chapter I: Polyphenols

I.1.1. Botanical characteristics

I.1.1.1. Cistaceae family

I.1.1.1.1. Generality

Cistaceae is a small family of halophilous broadleaf plants, largely represented in Mediterranean shrublands. Their name comes from the Cists (Cistus family) and was given by Joseph Pitton de Tournefort from the Greek word "kisthos" meaning capsule. It includes about 170 to 200 species grouped into 8 genera (Christenhusz and Byng 2016): *Cistus, Crocanthemum, Fumana, Halimium, Helianthemum, Hudsonia, Lechea* and *Tuberaria* [=*Xolantha*], two of them considered main ones; *Helianthemum* and *Cistus*. Some of these plants are autochthonous and widespread in the southeast of the Iberian Peninsula, North-West Africa, Greece and Portugal. All species of the genera *Cistus, Fumana, Halimium* and *Tuberaria* are distributed almost exclusively in the Mediterranean basin, as well as half of the species of the genus *Helianthemum* (Thanos et al. 1992; Benoudina and Harieche 2018). The genus *Cistus* is particularly widespread in the Mediterranean area (Talavera, Gibbs, and Herrera 1993; Benoudina and Harieche 2018). In Algeria, it is distributed everywhere on the tell and the littoral, in forests, scrub, dry slopes, siliceous, rocky and calcareous soils (Beniston 1984).

According to Yahi et al. (2008) investigation of floristic and biogeographic diversity of Algerian cedar forests, it has been found that the Cistaceae family includes 3 genera, 8 Species and 3 endemics. However, based on our knowledge the genus of Cistaceae that were found and studied in Algeria are *Cistus, Helianthemum, Fumana* (Zitouni-Haouar et al. 2014) and *Tuberaria* (Dafri and Beddiar 2018). No more information was found about the number of species available in Algeria.

I.1.1.1.2. Botanical description

The species of this Rockrose family are shrubby or herbaceous, perennial or annual, with a low cone-shaped receptacle, bearing from bottom to top the perianth, androecium and gynoecium. They bear leaves, which are often opposite or ordinarily stipulated; flowers axillary or terminal, solitary, in spikes or arranged in cymes (simple inflorescence starting from the same point), racemose or paniculiform. They are generally hermaphroditic = bisexual (Guzmán and Vargas 2005).

I.1.1.1.3. Genus *Tuberaria*

The genus *Tuberaria* (Dunal) Spach includes short-cycle perennials and annuals, distributed in the Mediterranean area. This genus inhabits dry, stony sites, often close to the sea (Bedoya et al. 2001). The genus *Tuberaria* forms a group of about 12 species, which are characterised by rhizomatous and pubescent perennial (height: 0.6 m). The leaves are basal spatulate to oblanceolate, apex obtuse to acute (length: 36-65 mm, width: 11-18 mm), the distinct whorls of flower parts are terminal uniparous cymes, outer sepals linear lanceolate, inner ones oval lanceolate, and five obovate yellow petals (length: 10-15 mm). Moreover, the fruits have elliptic capsules shape (length: 6-8 mm). The plant *Tuberaria lignosa (Sweet) Samp* was listed on Herbarium de Belair (http://gdebelair.com) with voucher number ph015-50.

Synonyms: *Xolantha tuberaria, Cistus tuberaria, Helianthemum tuberaria, Helianthemum lignosum, Tuberaria vulgaris.*

I.1.1.1.3.1. Systematic classification

According to the APGIII classification (Reveal and Chase 2011) and the National Inventory of Natural Heritage (INPN), based on two chloroplast genes and one nuclear ribosome gene, the taxon *Tuberaria* (Dunal) Spach is classified as follows:

Domain	Biota
Kingdom	Plantae
Sub-kingdom	Viridaeplantae
Infra-kingdom	Streptophyta
Class	Equisetopsida
Cladus	Tracheophyta
Cladus	Spermatophyta
Sub-Class	Magnoliidae
Super-Order	Rosanae
Order	Malvales
Family	Cistaceae
Genus	*Tuberaria*

I.1.1.1.3.2. Folk medicine

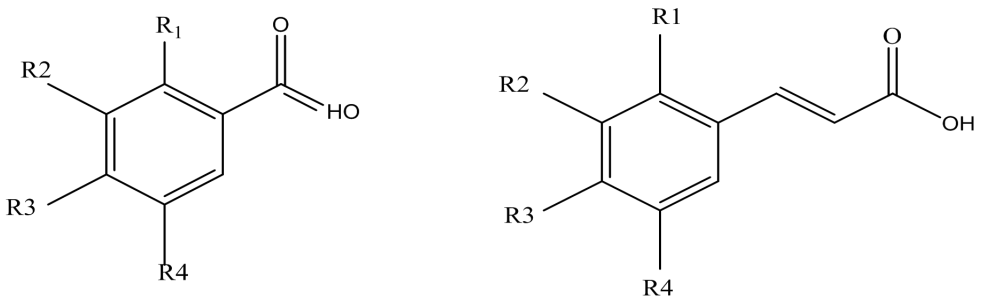
Tuberaria lignosa (Sweet) Samp, known as “Khayattet Eljirah” in Algeria, is one of the most popular medicinal plants in Mediterranean regions*, T. lignosa* herbal infusions or decoctions were used for treating various diseases, such as gastrointestinal disorders, influenza, heartburn, wounds,

skin infections and warts due to its vulnerary, anti-infectious and anti-inflammatory properties. The flowers, the leaves or the whole plant are prepared fresh or shade-dried in medicinal infusions and decoctions. Usually, infusion of leaves or inflorescences is used for hepato- depurative disorders (internal use) and decoction of the whole plant for skin inflammations (topical and external use) (Pinela et al. 2012). Moreover, crushed leaves can be directly used, mixed with honey, to heal skin burns and eschar.

I.1.2. Plant Polyphenols

Plant polyphenols found in higher plants are secondary metabolites. Known for their typical characteristics of water solubility, molecular weights that range from 500 to 4000 D and their intermolecular complexation. They have an excellent ability to form non-covalent and intermolecular complexes with each other's and with other molecules of variable size.

The phenolic acids are the most important dietary phenolics including hydroxybenzoic and hydroxycinnamic acids shown in Fig.1, polyphenols (hydrolysable and condensed tannins) and the most studied group is flavonoids (Fig. 2).



Benzoic acids derivative Hydroxycinnamic acids

Fig. 1. Basic monomeric phenolic acids structure

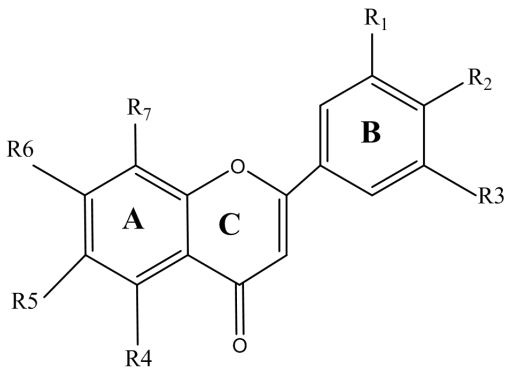


Fig. 2. Basic monomeric flavonoid structure

Polyphenols, as most secondary metabolites, are synthesised to perform certain functions:

- Protective function, defending the plant against pathogens such as moulds, fungi and bacteria, and protecting against ultraviolet radiation and any oxidative damages. That's the reason why they have been studied as antioxidant protectants for humans.
- Colouring function, the attractive colours of flowers and fruits are made to drag pollinating insects that transport seeds in the fruits.

I.1.3. Analytic methods

Researchers are more interested in simple and reliable analytic methods, attributed to determinate qualitatively and quantitatively the biologically active constituents. The amounts of these latter can be controlled by the used extraction technique. The solubility of phenolic compounds is influenced by the solvent polarity, phenolics' degree of polymerisation and the interaction of phenolics with other constituents; like the proteins forming insoluble complexes. So, the natural extracts are a mixture of different classes of phenolics that are soluble in the solvent used for extraction plus others substances. Therefore, an additional step is required to remove non-phenolic substances such as sugars, organic acids and chlorophylls. The most widely used techniques for purification are column chromatography, acidity based-fractionation and liquid-liquid fractionation (organic solvents).

I.1.3.1. Spectrophotometric Methods

Spectrophotometric methods particularly colourimetric methods are extensively used for the quantification of different classes of polyphenols: total phenolic content, tannins content, flavonoids and anthocyanins contents. These methods are rapid and simple. However, they lack the specificity for individual compounds. Interferences from non-polyphenolic components of the sample can also cause false readings and lead to erroneous results.

I.1.3.2. Separation and Identification Analysis

According to Ignat et al. (2013), it is almost impossible to develop a protocol for all polyphenols due to their structural diversity and chemical complexity. Still, some advanced analytical techniques are quite laborious and time-consuming for the identification of phenolic compounds such as the chromatographic technique HPLC.

I.1.3.2.1. High-Performance Liquid Chromatography

Liquid chromatography coupled with mass spectroscopy can be considered the method of choice for the structural analysis of phenolic compounds. It has allowed, for example, the characterisation

of anthocyanins from myrtle (*Myrtus Communis*) (Montoro et al. 2006) and also anthocyanins from waxberry fruit *(Myrica rubra Sieb. et Zucc*.) (Qin et al. 2011). It is possible to identify and quantify phenolic compounds by the HPLC method. However, to facilitate identification, it is necessary to perform acid hydrolysis to release the aglycone. Wannes and Marzouk were able to identify and qualify 13 phenolic compounds in myrtle (*Myrtus communis var. Italica*), using the HPLC-RP technique, coupled with a UV-Vis detector (Aidi Wannes and Marzouk 2013).

I.1.4. Chemical composition

A previous investigation of Pinela et al. (2012) evaluated the phytochemical composition of *Tuberaria lignosa* aerial parts, comparing the effects of drying and extraction methods on the extracts' composition and the antioxidant activity. Fig. 3 shows the chromatographic profile of the phenolic compounds detected in the infusion of a shade-dried sample, which includes mainly ellagitannins (Fig. 4), flavonoids (flavonols and flavones) and phenolic acid derivatives. Table 1 presents the identified compound obtained from HPLC-DAD MS analysis.

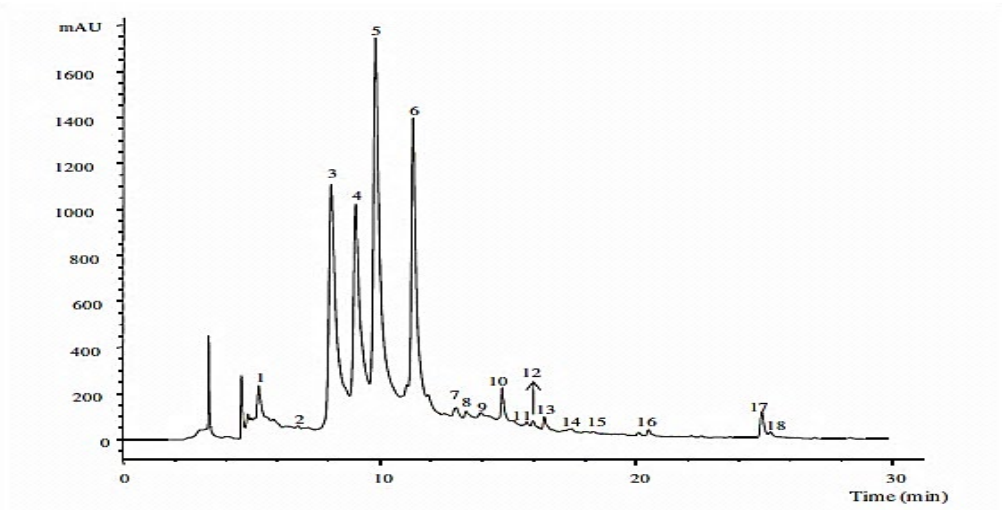


Fig. 3. HPLC profile recorded at 280 nm of *Tuberaria lignosa* phenolic compounds (Pinela et al. 2012).

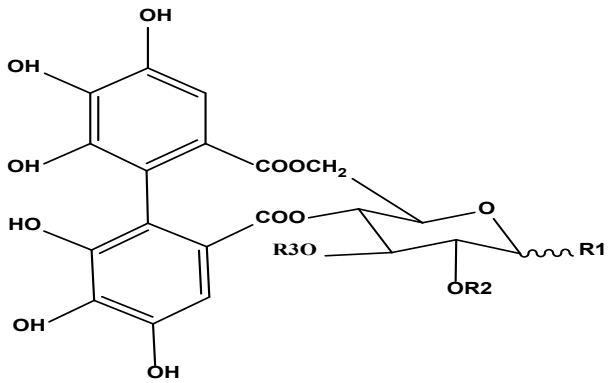


Fig. 4. General structure of ellagitannins (Yoshida et al. 2010)

Table 1. Tentative identification of the phenolic compounds in *Tuberaria lignosa* infusions.

Peak	*Tentative identification*	*Chemical Structure*
1	Punicalin	('Punicalin | C34H22O22 - PubChem' 2005)
2	Monogalloylglucose	('6-O-Galloylglucose | C13H16O10 - PubChem' 2005)
3	Punicalagin isomer 1	(L.-G. Chen et al. 2009)
4	Punicalagin gallate isomer 1	
5	Punicalagin isomer 2	
6	Punicalagin gallate isomer 2	
7	Luteolin-6-C-glucose-8-C-glucose	('FL157201 | 29428-58-8 | Luteolin 6,8-Di-C-Glucoside' 2023)
8	Apigenin-6-C-glucose-8-C-glucose	('Apigenin 6,8-Di-Glucopyranoside; | $C_{27}H_{30}O_{15}$ | TRC' 2018)
9	5-O-p-Coumaroylquinic acid	('5-p-Coumaroylquinic Acid,PubChem' 2005)
10	Luteolin-8-C-glucoside	('Luteolin-8-C-Glucoside - McCord Research' 2005)
11	Apigenin-8-C-glucoside	('Apigenin-8-C-Glucoside, PubChem-' 2005)
12	Quercetin-3-O-rutinoside	('Quercetin 3-Rutinoside (FDB002536) - FooDB' 2020)
13	Apigenin-6-C-glucoside	('6-Glucosylapigenin-Molbase' n.d.)
14	Kaempferol-3-O-rutinoside	('Kaempferol-3-Rutinoside PubChem' 2016)
15	Luteolin-6-C-glucoside	('Luteolin-6-C-Glucoside | CAS:4261-42-1 | Manufacturer ChemFaces' n.d.)
16	Kaempferol-O-rhamnoside-O-rutinoside	('Kaempferol 3-Rutinoside 7-Rhamnoside, PubChem' 2007)
17	Kaempferol-p-coumaroylglucoside-glutarate	('Kaempferol-3-Glucoside-2''-p-Coumaroyl | C30H26O13 | ChemSpider' 2023)
18	Kaempferol-p-coumaroylglucoside	('Kaempferol 3-(4''-p-Coumaroylglucoside) PubChem' 2009)

I.2. Chapter II: Polysaccharides

I.2.1. Botanical characteristics

I.2.1.1. Rosaceae family

I.2.1.1.1. Generality

Rosaceae is a moderately large angiosperm family in the order Rosales, it is mainly spread in temperate countries worldwide, providing habitats and foods for animals, producing many economically important fruits, and also it includes many ornamental flowers. The Latin name Rosaceae, the name of the rose family, was first published by Adanson in 1763. According to size, it is the 19th biggest plant family (Hummer and Janick 2009) and includes about 3000 species, 3 subfamilies, 16 tribes, and 88–100 genera. This richness in Rosaceae species could be partly related to polyploidisation and species radiation in the family history (Fougère-Danezan et al. 2015).

I.2.1.1.2. Botanical description

This family include plants with widely varied leaf habits and patterns, from ephemeral plants to long-lived woody shrubs and trees. Herbaceous annuals, perennials, shrubs and small trees with alternate leaves that could bear stipules. The leaves are often simple, highly dissected, compound, entire or toothed. Generally, flowers found red, pink, yellow or white, with five separate sepals, five separate petals and numerous stamens attached to a shallow, cup- or bowl-shaped hypanthium, arising from an enlargement of the floral receptacle ('Rosaceae: The Rose Family. Course Hero' 2021).

I.2.1.1.2. Genus *Crataegus*

In the anglophone countries, the *Crataegus* fruits are known by the famous name "Azerole Hawthorne". The generic name Crataegus for hawthorns, meaning in Latin "crataegon" or "crataegos". The *Crataegus azarolus* may be from the Greek (kratos) in reference to the hardness of the wood (Couplan 2000). Its common name azerolus comes from the Spanish 'acerola' which refers to the fruit, this word has an Arabic origin 'az-zou'rour or 'az-zucrur' (Meier 1965). Longley (1924) published that 750 species of *Crataegus* have been listed worldwide. According to the herbarium of Gérard de Belair ('Herbier GdB' 2019) and Algeria native plants (Marouf 2021), many *Crataegus* species were found in Algeria, especially in the northeast area (Skika, Guelma and Souk Ahras). The Arabic name "Zaaroura" is given to both *Crataegus azarolus L*. and *Crataegus azarolus*

X oxyacantha, "Bou mekherri or aïne el-begra" are given to *Crataegus laciniata Ucria* and *Crataegus oxyacantha L.* in addition to the *Crataegus monogyna Jacq* specie.

The hawthorn tree is a shrub of 5 to 10m in height, resistant to drought, cold, strong winds and urban pollution. It prefers sunny areas and dry, heavy and clayey soils (Koyuncu et al. 2007). The Hawthorn, also known as the "West Indian cherry", is a small apple-shaped fruit, 1 to 3 cm in diameter. When ripe, its skin turns from creamy white to yellow. Its flesh is delicately fruity, but it has a very acid taste and two seeds (Espiard 2002). The plant was listed on Herbarium de Belair (http://gdebelair.com) with voucher number 012-17.

I.2.1.1.2.1. Systematic classification

The classification was according to Centre for invasive species and ecosystem health. So, the taxon *Crataegus* is classified as follows:

Domain	**Biota**
Kingdom	Plantae
Sub-kingdom	Tracheobionta
Phylum	Magnoliophyta
Class	magnoliopsida
Sub-class	Rosideae
Order	Rosales
Family	Rosaceae
Sub-family	Maloideae
Genus	*Crataegus*

I.2.1.2.2. Folk medicine

In Arab traditional medicine, a decoction of leaves and unripe fruits from *Crataegus azarolus*, is used to treat diabetes, cardiovascular diseases and sexual weakness. In herbal folk medicine, the flowers and fruits of many hawthorns are well-known as a heart tonic. Also, they have a hypotensive effect and act as a direct mild heart tonic. They are especially indicated in the treatment of weak hearts combined with high blood pressure. It is normally used either as a tea or a tincture, but prolonged use is necessary to be efficacious (Foster and Duke 1990). The Hawthorn fruits are edible raw or cooked. Also, it can be used fresh or dried for later use.

I.2.2. Plant polysaccharides

Carbohydrates are ubiquitous and have been found to play fundamental roles in biological systems encompassing cell proliferation, immune response, cell adhesion and cell-cell recognition. Carbohydrates are a major source of energy and they are prime biological substances, of which tons and tons are produced every year through the process of photosynthesis by plants and microorganisms. There exists a great relationship between carbohydrate structure and many biological functions due to their diversity and complexity.

The study of plant polysaccharides begins with an awareness of their wide structural variability, which is partly related to their many biological functions. Most often separated into three categories (Warrand 2004), a distinction is made between:

- Reserve polysaccharides (starch, galactomannan)
- Structural polysaccharides (celluloses, hemicelluloses, pectins)
- Exudates, gums or mucilages

The structure of the plant wall varies according to the plant species, age and type of tissue involved. In general, it is considered to be a superposition of several layers of lignin, cellulose, hemicellulose and pectin. It is the combination of these polymers that provides the mechanical and physicochemical properties of the wall. And that provides the main characteristics to be used as thickeners, stabilisers, gelling and binding agents in the food, pharmaceutical and cosmetic industries (Funami 2017).

Overall, the skeletal part of the wall is composed of two types of polysaccharide fibres: cellulose microfibrils, responsible for the rigidity of the wall, and chains of other polysaccharides collectively called hemicelluloses. These different polysaccharide chains can be assembled to form the two main types of plant walls.

I.2.2.1. Hemicelluloses

The scientific community and industry consider the Hemicelluloses the centre of interest, in the context of biorefining plant materials (Ruff 2008). There are Heteropolymeric in nature (glucomannans, glucuronoxylans, glucuronoarabinoxylans, arabinoxylans, xyloglucans), also some homopolymeric hemicelluloses such as mannans, xylans and β-glucans (Habibi et al. 2004). Therefore, Hemicelluloses can be divided into four major classes, namely Xylans, Mannans, Xyloglucans and Mixed-bond β-glucans (Ruff 2008). Consisting of neutral monosaccharides (Xyl,

Ara, Man, Glc, Gal) and uronic acids (GlcA, 4-Me-GlcA), hemicelluloses are extractable and soluble at alkaline pH (Benaoun 2017). Also, they are highly soluble and easily extractable in water.

I.2.2.1.1. Mannans

Mannans, are polysaccharides described as hemicelluloses and storage polysaccharides in the cell wall of plant seeds. They can be separated into two groups comprising galactomannans (GaM) and both glucomannans (GM) and galactoglucomannans (GGM). The galactomannans, present in many plant seeds, are considered storage polysaccharides and not directly associated with hemicelluloses. GM and GGM have a carbon skeleton composed of both β-(1,4)-linked D-Man*p* and β-(1,4)-linked D-Glc*p* residues. The side chains can be composed of α-D Gal*p* residues linked in the O-6 position on the main mannan chain, in different proportions depending on the polymer type. If we consider the threshold value of 15% of Gal*p* unit, it is thus possible to distinguish GM from GGM (Stephen 1983). It should also be noted that acetyl groups substitute the O-2 and O-3 positions of Man*p*. These macromolecules have a conformation close to cellulose with the same polymorphism. GM and GGM, which were extensively studied in the 1960s, are common components within the plant cell wall. Extractable under alkaline conditions, GMs and GGMs of the secondary cell wall often show a low degree of substitution by Gal*p* in contrast to those extracted under aqueous conditions at neutral pH where the proportions of Glc*p* and Gal*p* are much higher (Benaoun 2017).

The KGM (from *Amorphophallus konjac*) consists of a sequence of Man*p* units separated by Glc*p* units (Fig.5) with acetyl groups every 6-19 Glc*p* residues (Petera 2016). GaMs also consist of a Man*p* sequence with branches of α-Gal*p* units at position O-6.

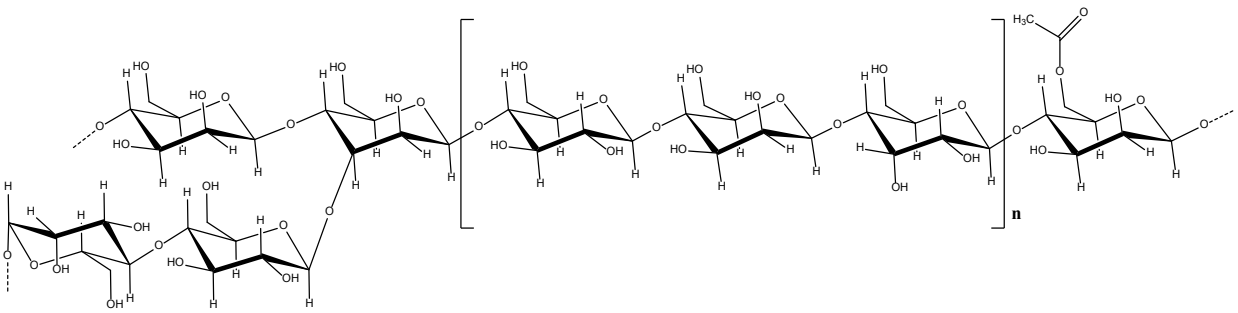


Fig. 5. General structure of a section appertaining to KGM repeating unit (Li et al. 2013).

These are separated into several categories according to the M/G ratio (which corresponds to the D-Man*p*/D-Gal*p* ratio). This ratio as well as the size of the macromolecular chains varies according to the plant species, climatic conditions, variety, age, cultivation conditions and also extraction conditions (Dakia et al. 2008).

I.2.3. Methods of analysis

The structural analysis of polysaccharides is an elemental progress in glycoscience; however, it is still an underdeveloped field because of some limitations of the approaches. In order to study the structure, the first step should be to isolate and extract the polymers. So, in the aqueous solution, the polysaccharides are recovered by ethanol, isopropanol or acetone precipitation. Then comes the step of purification, using fractional precipitation, for example, the complexes formed between polysaccharides and metal ions. Fehling's liquor has often led to fractionation and good purifications.

Elucidation of the primary structure of polysaccharides requires knowledge of the nature and ring size (pyranose or furanose) of each monosaccharide, including the presence of any substituents, the type and anomeric configuration of each inter glycosidic bond and the sequence of monosaccharide residues in the polymer. Many chemical, enzymatic and spectroscopic techniques can be used to determine the primary structure of polysaccharides. Recently methods have been developed for the structural determination of polysaccharides. They are based on the knowledge of the monosaccharide composition and data from proton (^{1}H) NMR, carbon (^{13} C) NMR, infrared spectroscopy (FT-IR) and mass spectroscopy.

The determination of the monomer conditions of a polysaccharide uses total hydrolysis methods. This hydrolysis can be carried out with different mineral or organic acids: sulphuric acid, hydrochloric acid, trifluoroacetic acid, formic acid or by methanolysis. The separation of the bones can be obtained by different methods, the main ones being paper chromatography, thin layer chromatography, and gas chromatography after the transformation of the sugars into volatile derivatives (in the form of trimethylsilyl ethers or trifluoroacetylated esters). In addition, high performance anion exchange chromatography columns with pulsed amperometric detection (HPAEC-PAD) have been developed for the separation of neutral and acid monosaccharides (Cui 2005). Meanwhile, the spectroscopic (infrared) and spectrometric (NMR, MS) methods are very useful tools for the structural determination of polysaccharides.

I.2.3.1. Infrared spectroscopy

Each bond in molecule, such as OH, and each group of three or more atoms, such as $^{1}CH_3$ or NH_2, absorb infrared radiation (electromagnetic radiation) at certain wave numbers to give quantified excited valence and strain states. Each type of inter-atomic bond (single or multiple) correspond to two types of fundamental vibrations:

- The valence or stretching vibrations, between two atoms considered, in which the distance between the two increases and decreases, while they remain in the axis of the bond.
- The vibrations of angular deformation (bending) in which the position of the atom's changes concerning the axis of the original bond.

Table 2, reports the characteristic absorption frequencies of some functional groups classically found in polysaccharides (Heinze et al. 2006). The FT-IR spectrum can be used to examine the presence of certain chemical functions within a polymer, e.g. O-H groups, the C=O of acetyl or the COOH of a uronic acid. Fig. 6 shows a typical IR spectrum of heteropolysaccharides isolated from the leaves of *Taxus chinensis var. mairei*. by Habibi et al. (2004). These spectroscopic methods can also be used to analyse the configuration of monosaccharides: the region between 1200 cm $^{-1}$ and 800 cm $^{-1}$. Indeed, in this region (1200 cm $^{-1}$ and 800 cm $^{-1}$), Černá et al. (2003) developed a mathematical programme for the characterisation of foodstuff polysaccharides from FT-IR spectra.

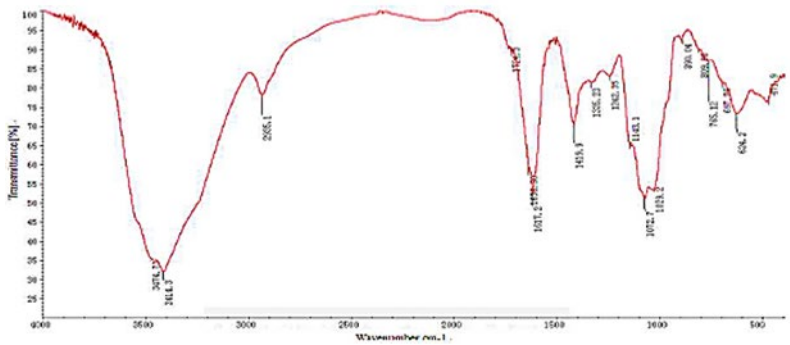


Fig. 6. IR spectrum of heteropolysaccharides isolated from the leaves of *Taxus chinensis var. mairei*. (Habibi et al. 2004).

Table 2. General assignment of FTIR spectra of polysaccharides (Heinze et al. 2006).

Wave number (cm^{-1})	Assignment
3450-3570	OH stretch, intramolecular H-bridge between the OH groups
3200-3400	OH stretch, intramolecular H-bridge between the OH groups (bonded)
2933-2981	CH_1 antisymmetric stretch
2850-2904	CH_2 antisymmetric stretch
1725-1730	C=O stretch from acetyl- or COOH groups
1635	Adsorption of water
1455-1470	CH_2 symmetric ring stretch at pyran ring: OH in plane deformation
1416-1430	CH_2 scissors vibration
1374-1375	CH deformation
1335-1336	OH in-plane deformation
1315-1317	CH_2 tip vibration
1277-1282	CH deformation
1225-1235	OH in-plane deformation, also in COOH groups
1200-1205	OH in-plane deformation
1125-1162	C-O-C antisymmetric stretch
1107-1110	Ring antisymmetric stretch
1015-1060	C-O stretch
985-996	C-O stretch
925-930	Pyran ring stretch
892-895	C-anomeric groups stretch, C_1-H-deformation, ring stretch
800	Pyran ring stretch

I.2.3.2. Nuclear magnetic resonance spectrometry (NMR)

In a nuclear magnetic resonance spectrometer, the sample is placed in electromagnet field, and a radio frequency field is applied, sending a current through a coil surrounding the sample. The magnetic field H_0 is slowly increased and the excitation or rearrangement of the nuclei, from one orientation to another, is detected as an induced voltage arising the energy of absorption from the radio frequency field. An NMR spectrum is the record of this induced voltage as a function of the magnetic field sweep. Only atoms with a magnetic moment in their nucleus give rise to the magnetic resonance phenomenon. The two most commonly used techniques for the structural analysis of polyoside are proton NMR (^{1}H-NMR) and carbon-13 NMR (^{13}C-NMR).

I.2.3.2.1. Proton nuclear magnetic resonance (^{1}H-NMR)

From a nuclear magnetic resonance spectrum, the number, nature and environment of the hydrogens in a molecule can be determined. This information can often be used to establish the skeletal structure of the molecule (Allinger et al. 1983). Although the NMR spectrum of a polysaccharide has a fairly large number of signals, NMR spectrometry is not a simple method of analysis. However, ^{1}H-NMR spectrometry is very useful in some cases, e.g. determination of branching type (anomeric bond) and polysaccharide-solvent interactions. To simplify the analyses, hydroxyls are transformed into deuteroxyls by an exchange in D_2O. Thus, only the proton signals appear, the deuterium nuclei resonating in a different frequency range. The residual water (HOD) signal is shifted to the gap between the anomeric protons and the backbone protons. Thus the ^{1}H-NMR spectrum of a water-soluble polysaccharide can be divided into 4 distinct regions as it is shown in Fig. 7. Going through this spectrum from weak to strong fields:

a) The region of anomeric protons
b) The HOD signal
c) The backbone protons
d) The protons of the non-carbohydrate substituents.

The characteristics of the polyoside ^{1}H-NMR spectrum are shown in table 3.

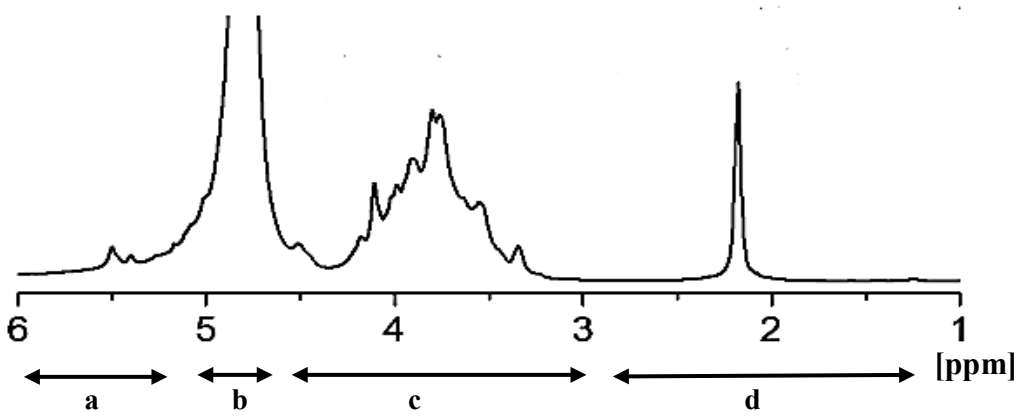


Fig. 7. ^{1}H NMR spectra in D_2O of native AcGGM of spruce (*Picea abies*), recorded at room temperature (Edlund and Albertsson 2011).

Table 3. Chemical shifts and corresponding carbon atoms for ^{1}H-NMR signal of polysaccharides (Heinze et al. 2006).

H atom (moiety)	*Chemical shift (ppm)*
H-1 (α)	4.9-5.8
H-1 (β)	4.4-4.9
H-2 to H-6	3.3-4.5
COOH	9-13
O-CH_3	3.3-3.5
O-(C=O)CH_3	2.0-2.2
CH_3	1.4-1.6

I.2.3.2.2. Carbon-13 magnetic resonance (^{13}C-NMR)

The ^{13}C-NMR allows a better study of the structure of organic and biochemical molecules because it provides information about the skeleton of the molecules rather than their peripheral structure. A clear separation of the signals from the different carbons is obtained. Furthermore, the chemical shift of ^{13}C in most organic compounds is around 200 ppm (Skoog et al. 2003). However, this technique requires more product (about 50 mg.ml^{-1}) than ^{1}H-NMR to obtain a correct spectrum.

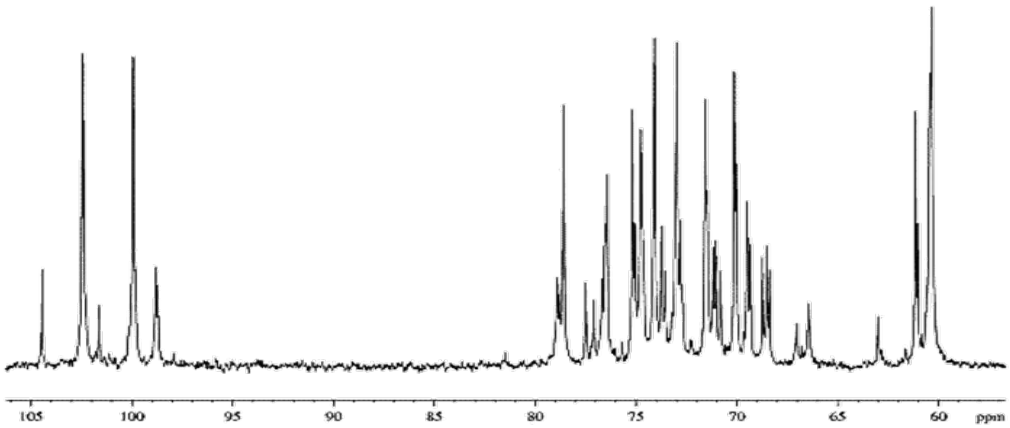


Fig. 8. ^{13}C NMR spectra of native GGM from the pulp of gabiroba fruits, in D_2O (Barbieri et al. 2017).

The main data provided by the chemical shifts of a ^{13}C-NMR spectrum are presented in table 4 (C. Jones and Mulloy 1993) and **Annex 12**. Meanwhile, Fig. 8 presents the ^{13}C-NMR spectrum of the GGM, isolated from the pulp of gabiroba fruit (*Campomanesia Xanthocarpa Berg*), that shows a high complexity of signals. The GGM composition was proposed as backbone of (1→4)-linked β-d-Glc*p* and β-d-Man*p* with side chains of d-Gal*p* residues (Barbieri et al. 2017).

Table 4. Typical Chemical Shifts in the Carbon NMR Spectra of Polysaccharides (Heinze et al. 2006)

	Chemical Shift. Ppm
Uronic acid C-6	170-180
N-acetylamino sugar carbonyl carbon	170-180
Furanose sugar C-1 (α, β)	101-111
Pyranose sugar C-1 (α)	91-101
Pyranose sugar C-1 (β)	95-105
Unsubstituted [a] furanose ring carbons	70-85
Unsubstituted [a] pyranose ring carbons	65-75
Glycosylated hydroxymethyl groups	65-68
Unsubstituted [a] hydroxymethyl ring groups	61-64
C-N in amino sugars	48-55
N-acetyl methyls	23-25
C-6 in 6-deoxyhexoses	15-19

[a] : substitution, e.g, with sulfate groups in the glycosaminoglycans, causes a downfield shift (of 2-7 ppm) of the resonance due to the carbon atom at the position of substitution.

There are four procedures used in ^{13}C NMR:

a) Complete proton decoupling (or proton noise decoupling): By irradiating at proton resonance frequencies, carbon-proton couplings are completely eliminated. The irradiation of the proton causes the nuclear Overhauser effect (NOE). This effect disturbs the intensity of the peaks and makes it impossible to know whether a signal represents one or more identical carbons.

b) Anti-gate technique: This consists of performing a total decoupling, as above, but without NOE. Rinaudo and Vincendon were able to determine the repeating unit of scleroglucan (*sclerotium rolfsii*) based only on the NMR spectrum data of this technique. It should be noted that some of the signals of the quaternary carbons (not carrying any proton) are not affected by NOE (Rinaudo and Vincendon 1982).

c) Partial decoupling (off resonance): Only the $_1J_{C,H}$ couplings remain. The NOE remains in part. The different types of carbons can be easily distinguished: quaternary carbons (appearing as singletons), tertiary carbons (doublets), secondary carbons (triplets) and primary carbons (quadruplets). This technique makes it possible to measure the $^1J_{C,H}$ coupling constants of carbons $^1(C_1)$, which have values characteristic of the anomer. Thus, these results can be compared with the results of ^{1}H-NMR.

d) Selective irradiation (heteronuclear or double): This is also a partial decoupling but the $^1J_{C,H}$ coupling of a particular carbon is removed. The signal of this carbon appears as a singlet and benefits completely from the NOE: its intensity is thus stronger than that of the other signals. This practice is particularly used in chemical synthesis.

I.2.3.3. Mass Spectroscopy

The concept of mass spectrometry is relatively simple: a compound is ionised (ionisation technique), the ions are separated according to their mass/charge ratio (ion separation technique) and the number of ions in each unit: mass, charge is recorded in the form of a spectrum. In a mass spectrum, fine peaks appear at the m/e values of the various cations. The charge of most cations is +1. The position of the peaks usually gives the mass of the cations. The highest m/e value corresponds to the molecular weight of the parent molecule.

This generalisation does not take into account the presence of natural isotopes (^{13}C,^{2}H) in the parent molecule. Various ionisation conditions can be used. The desorption ionisation technique incorporates four procedures (Silverstein et al. 2007). For example, matrix-assisted laser desorption, and ionisation (MALDI) is more versatile because most compounds give signals in their native state. Similarly, to other types of mass spectrometry, MALDI can provide valuable information on several aspects of structural analysis, such as the determination of sequence, branching, and linkage. MALDI mass spectrometry was developed for the analysis of molecules with large molecular weights. The pulsed nature of a laser ion source and its ability to ionise very large molecules is ideal for coupling to a time-of-flight (TOF) analyser (Harvey 1999).

I.3. Chapter III. Lactic acid bacteria

I.3.1. Generality

The group of lactic acid bacteria refers to a Gram-positive, catalase-negative, and non-spore-forming with a facultative aero-anaerobic or microaerophilic respiratory type, the common feature is their ability to produce lactic acid coming with the carbohydrate's fermentation (Stiles and Holzapfel 1997). Lactic acid bacteria are ubiquitous and found in different rich ecological niches such as milk products, plants, meat, fish, and in both human and animal mucosa. They have a chemoorganotrophic metabolism, so they use hydrocarbon substances such as sugars, alcohols and organic acids as energy sources. Roissart and Luquet (1994) elucidated that lactic acid bacteria have complex requirements for growth factors: amino acids, peptides, purine and pyrimidine bases, vitamin B, fatty acids and salts.

There are two distinguished fermentation pathways:

- The Embden-Meyerhof pathway: homofermentation, in which lactic acid is the main or only product of glucose metabolism.
- The Dickens-Horecker pathway: heterofermentation, producing lactic acid with other products such as CO_2, acetic acid and ethanol (Larpent and Larpent 1997).

I.3.2. Probiotics

The Food and Agriculture Organisation of the United Nations and the World Health Organisation defined probiotics as following "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Hill et al. 2014). However, Health Canada has accepted only a few bacterial species as probiotics when delivered in food at a level of 1×10^9 colony forming units (CFU) per serving, and they are Bifidobacterium (*adolescentis, animalis, bifidum, breve and longum*) and Lactobacillus (*acidophilus, casei, fermentum, gasseri, johnsonii, paracasei, plantarum, rhamnosus and salivarius*) (Canada Health 2009). To assure the probiotics' safety, functionality and survival; they should be characterised by some basis such as acid, bile and stress tolerance, carbohydrate metabolism (prebiotic), antibiotic resistance, no gene transfer potential and bacteriocin production.

I.3.3. Beneficial effect on human health

The lactic acid bacteria (LAB) have a positive effect on human health maintaining homeostasis along the length of the mammalian tract:

- Control some types of cancer, detoxifying the carcinogens by physical binding between the strains and toxins (Shoukat 2020).
- Reduce serum cholesterol levels by various mechanisms, including adsorption, co-precipitation, and enzymatic degradation of bile salt hydrolase (Negm El-Dein et al. 2022), that prevent the risk of heart attacks.
- Inhibit allergic response through modulating the Th1/Th2 balance and increasing T cells regulation (Ai et al. 2016).
- Stimulate the immune system by many complex interactions between the microflora, epithelial cells and immune cells, and then they send signals to activate immune cells through diverse mechanisms (Elmadfa et al. 2010; Perdigón et al. 2001).
- Manage irritable bowel syndrome, improve lactose digestion; produce lactic acid and decrease the associated symptoms of constipation, diarrhoea and cramps (K. and Thomas 2006).
- Promote colonisation resistance by maintaining the balance of microbial ecology in the intestinal tract (Lawley and Walker 2013).

I.4. Chapter IV. Health damage

I.4.1. Oxidative stress

Oxidative stress refers to a disturbance in the cellular metabolic balance while the generation of oxidants overwhelms the antioxidant defence system, either by an increase in the production of oxidants and/or by a decrease in the antioxidant defences. Unfortunately, the accumulation of reactive oxygen species results the appearance of cellular and tissue damage, which is often irreversible and contributes to many diseases. On the other hand, the gastrointestinal (GI) tract acts as a barrier between the gut contents and the rest of the body, allowing the absorption of essential nutrients only to the blood. Thus, the GI tract is more exposed to pro-oxidants at unusual levels than the whole other body tissues; this explains the alterations in its activities as it was reported by Bhor et al. In 2004 they demonstrated that the reactive oxygen species injured the small intestine in diverse conditions such as inflammatory bowel disease, radiation enteritis, iron supplementation and zinc deficiency (Bhor et al. 2004). The human body has defence mechanisms, such as enzymes that degrade peroxides and transition metals, proteins or other molecules which trap free radicals. However, in particular cases the defence system fails so, it needs external help to heal the damaged body.

I.4.2. Bacterial resistance

The microbiota which occupy the length of the intestinal tract, plays a central role in host development and basic physiology, including immune system development and digestion. Nevertheless, if intestinal bacteria have developed antibiotic resistance and multiplied in large numbers, then evolved into super-bugs, the diseases caused by these bacteria will lead to death due to incurability. Unfortunately, antibiotic-resistant infections have become increasingly prevalent. There are three principal types of antibiotic resistance, named: intrinsic, acquired, and adaptive. The intrinsic resistance comprises all the properties provided by microorganisms limiting antimicrobial action. The second type, acquired resistance, is an originally susceptible strain which can become resistant as a result of mutations or by incorporating new genetic material. Eventually, adaptive resistance is a temporary increase in the bacterium's ability to survive an antibiotic, due to alterations in gene and/or protein expression. Versus intrinsic and acquired stable resistance mechanisms, that can be transmitted vertically to subsequent generations, the adaptive resistance has a transient nature and usually reverts once the inducing condition is removed. Ben et al. (2019) assume that this bacterial resistance risk is not only on human individuals but to the global human population.

I.5. Chapter V. Biological activities

I.5.1. Antioxidant activity

Several methods are used to evaluate the antioxidant activity of foods and biological systems. According to their mechanisms, they can be classified into two groups.

Hydrogen atom transfer group: the techniques are used to assess lipid peroxidation using a lipid or lipoprotein substrate and the quantification of this property is expressed by measuring the degree of oxidation inhibition.

Single electron transfer group: the methods are based on the ability to trap free radicals' measurement. They include the scanning of hydrogen peroxide (H_2O_2), hydroxyl (HO), superoxide anions (O_2^-), peroxide (ROO) and nitric oxide (NO). Among these techniques, we mention the FRAP method; DPPH (2,2- diphenyl-1-picrylhydrazyl) radical method and the β-carotene bleach test.

Polyphenols can act by two mechanisms of action: the first consists of inhibition of pro-oxidant enzymes and chelation of metal ions and the second one consists of the protection of biological antioxidant defence systems. Halliwell assumed that the antioxidant activity of polyphenols is determined by their structures, in particular, the position of the hydroxyl groups on the aromatic rings and the ability of the aromatic compounds to support electron delocalisation (Halliwell 1994). Many recent studies highlighted the significant antioxidant activity of Cistaceae plants (Mohammed et al. 2021; Hitl et al. 2022; Rebaya et al. 2015; Benabdelaziz et al. 2017). The leaves of diverse Cistaceae plants, a source for water-soluble ellagitannins-enriched polyphenolic extracts, had considerable antioxidant and antimicrobial activities (Barrajón-Catalán et al. 2010). Alsabri et al. (2013) highlighted the anti-ulcer, anti-microbial, antioxidant and anti-inflammatory activities of *Helianthemum lippii* (Cistaceae). Moreover, the study of Pinela et al. on *Tuberaria lignosa* samples revealed an interesting antioxidant property, particularly the infusion of the shade-dried wild samples that showed the highest DPPH radical scavenging activity and reducing power compared to decoctions, commercial and freeze-dried samples. However, some investigators have questioned the ability of ingested dietary polyphenols to affect systemic antioxidant capacity, because of the poor intestinal absorption, while large quantities of polyphenol compounds are delivered to the colon, where many undergo extensive metabolism via colonic flora. Although, high levels of dietary polyphenols actually alter colonic flora (Gee and Johnson 2001).

It has been widely reported that the fruit polysaccharide has distinct antioxidant activity. Previous studies on some Algerian fruits, which are commonly consumed, confirm the magnitude of discover

and value this field. Some of the evaluated polysaccharides were from dates; *Phoenix dactylifera L.* (Djaoud et al. 2022), Jujube; *Zizyphus lotus L.* (Berkani et al. 2021) and myrtle; *Myrtus communis L.* (Chidouh et al. 2014). Various studies aimed to better understand the relationship between antioxidant activity and polysaccharides' chemical structure. Meng et al. (2015) suggested that the monosaccharide composition had a significant influence on polysaccharide activity, and the results presented a high correlation between the mannose, glucose contents and the radical scavenging activity. Similarly, to the rhamnose contents, that was previously proven by (Zhang et al. 2021; Liu et al. 2022; Luo et al. 2010). Liu et al. (2022) concluded that the polysaccharide with the highly branched region, high content of rhamnose and low molecular weight exhibited better antioxidant bioactivities.

In vitro laboratory experiments, confirmed that the Hawthorn polysaccharides (from *Crataegus pinnatifida Bunge* berry fruit) are a free radical scavenging agent aimed at DPPH·, OH· and Fe^{+3}· free radicals (X. Chen et al. 2019; Sun et al. 2020). Those latter researchers had no detailed structural study on the polysaccharides of red hawthorn fruit but they assumed it is pectin rich in galacturonic acid. According to our knowledge, no previous research studied either the structure or the biological activities of yellow hawthorn polysaccharides (*Crataegus azarolus).*

I.5.2. Antimicrobial activity

Microbial resistance has become an increasing global problem, therefore there is a mandatory need to find out potential novel antimicrobial agents as an alternative to antibiotic therapy. Firstly, it is important to understand that the mode of action of antimicrobial agents is a very complex phenomenon where different mechanisms may be involved. Rabea suggested an interaction between the positively charged bioactive compounds and negatively charged microbial cell membranes, which leads to the leakage of proteinaceous and other intracellular constituents; inhibition of the production of toxins and microbial growth and inhibition of mRNA synthesis binding with DNA sequence (Rabea et al. 2003); those are some of the possible modes of action of antimicrobial agents. Meanwhile, plant extracts rich in polyphenols has been frequently investigated as an antimicrobial agent against a wide range of microorganisms. Recent studies tested the antimicrobial ability of phenolic extracts from Algerian Cistaceae plants, *Cistus salviifolius L.* (Boubekeur et al. 2022) and *Cistus munbyi Pomel* (Benbelad et al. 2021), which showed moderate results. The MICs value went up to 4 and 5 $mg.ml^{-1}$, however, that won't be considered a problem as long as it has been proved it is safe by the pharmaco-toxicological tests, and that was the case of the aqueous leaf infusion of *Cistus salviifolius*. Generally, the most active polyphenols are flavonoids or hydrolysable tannins;

the latter is divided into gallotannins and ellagitannins. But the extracts with antimicrobial potential contain mostly ellagitannins (Manso et al. 2022). In this context, the ellagitannin fraction extracted from *Tuberaria lignosa* has been proven to exhibit antiviral activity against HIV (Bedoya et al. 2010). Lavoura research was the first published work evaluating *Tuberaria lignosa* antimicrobial activity, he tested the fresh leaves of *Tuberaria lignosa* comparing hydrodistillation, infusion, decoction, 100% methanol, 96% ethanol, 50% ethanol and n-Hexane extracts. The leaves hydrodistillation extract performed the best antimicrobial ability; the lowest MIC was 0.0195 $mg.ml^{-1}$ against *C. albicans* ATCC 90028 and *C. tropicalis* ATCC 750 (Lavoura 2018).

Also, there is a new alternative to antibiotics therapy, it is the bioactive compounds produced by *lactobacilli spices,* the latter were recently used to control the propagation speed of pathogenic intestinal bacteria; lactic acid bacteria exert inhibitory activity through a variety of its products: bacteriocins, organic acids (lactic acid and ethanol or acetic acid), hydroxy fatty acids, hydrogen peroxide and carbon dioxide (heterofermentative). According to the experimental data *Lactobacillus rhamnosus* and *Lactobacillus plantarum* are the most active strains against many pathogens. Senouci et al. (2021) evaluated the antifungal and antibacterial activity of *lactobacilli* strains isolated from camel's milk (south Algeria), the results revealed that *L. brevis, L. rhamnosus, L. plantarum* and *L. paracasei subsp*. *Paracasei* showed a strong inhibitory ability against both Gram-positive and Gram-negative bacteria and inhibit the mycelial growth of all tested fungi. Moreover, Anas et al. (2008) investigation tested the antimicrobial activity of eight *Lactobacillus species* isolated from Algerian raw goat's milk; the dominant species, able to inhibit *Staphylococcus aureus'* growth, were *L. plantarum, L. paracasei subsp. paracasei* and *L. rhamnosus*. In order to understand this strong ability, there are new findings focused on characterising the bacteriocin produced by *Lactobacillus rhamnosus*, Xu et al. (2021) found out that the purified bacteriocin 1.0320 was able to form pores on the surface of the cell membrane leading to cell death. Similarly, it has been proved that bacteriocin produced by *L. rhamnosus* exhibited strong antibacterial activity against Gram-positive and Gram-negative bacteria (Zhao et al. 2020; Guo et al. 2020).

So, it is interesting to define local safe compounds acting as new agents and replacing antibiotic consumption, because antibiotics became less effective against clinical multi-resistance pathogens.

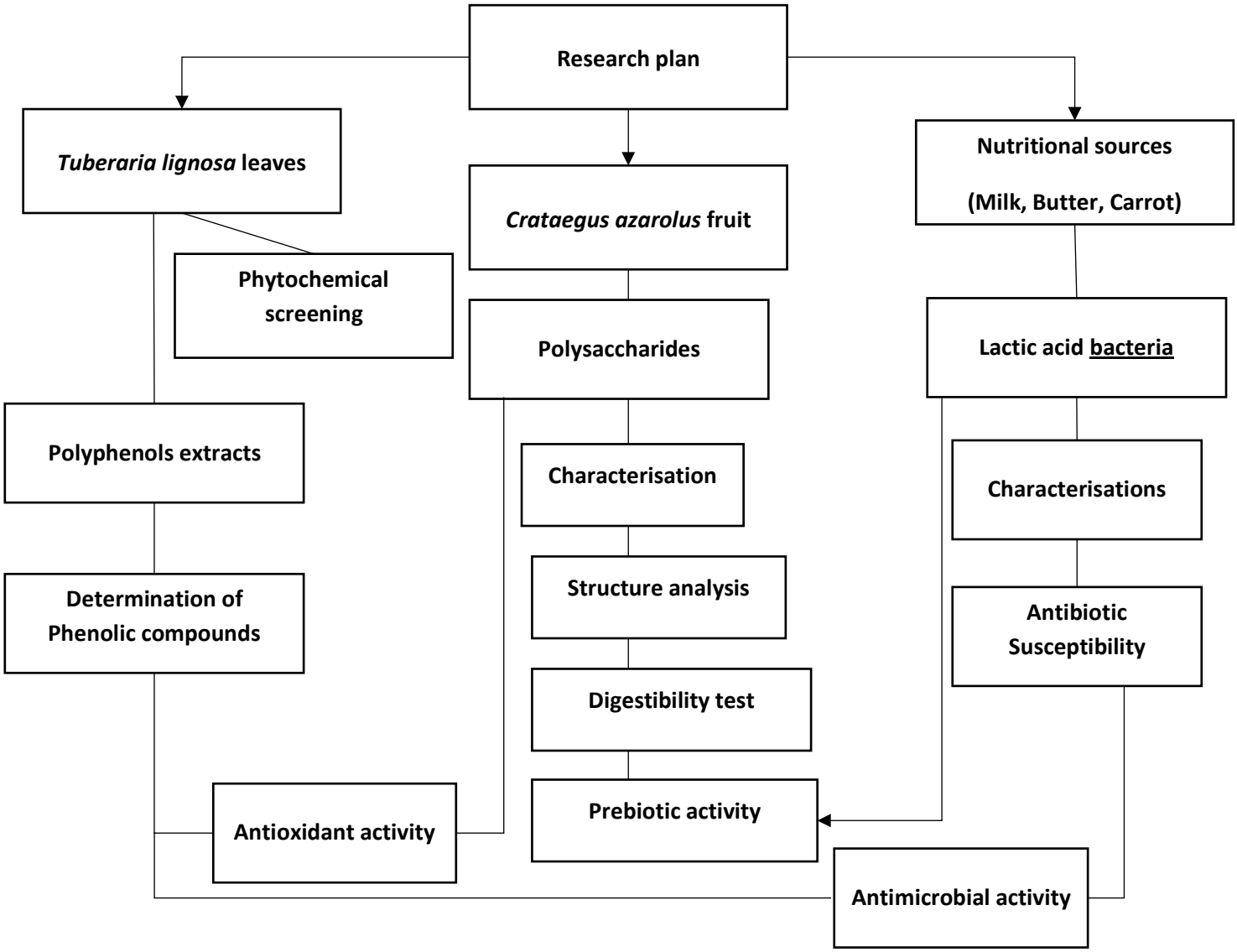
I.5.3. Prebiotic activity

Modulation of the gut microbiota through the consumption of prebiotic ingredients is an emerging concept for promoting gut health. The term prebiotic was introduced by Gibson and Roberfroid (1995) who defined a prebiotic as "a non-digestible food ingredient that improves host health by selectively stimulating the growth and/or metabolic activity of one or a limited number of beneficial bacteria in the colon". This definition partially overlaps with the fibre dietary, except the digestibility by some probiotic strains. The prebiotics concept is defined by certain criteria such as resistance to gastric acidity, hydrolysis by mammalian digestive tract enzymes and gastrointestinal absorption. These prebiotics stimulate the growth of beneficial bacteria (probiotics), furthermore, they limit the development of pathogenic or potentially pathogenic bacterial strains, as *Salmonella* or *Escherichia coli* strains (Singdevsachan et al. 2016). Thus, they promote the host ‘s well-being.

The prebiotic activity of KGM has been proven by many studies (Hayeeawaema et al. 2020; Du, Liu, and Ding 2021; Wan et al. 2022). The KGM can selectively ferment beneficial intestinal microorganisms and regulate the balance of intestinal flora (Chen et al. 2006). It was found that hydrolysed mannans with a short degree of polymerisation could significantly promote the secretion of Short-chain fatty acids (SCFA) and branched-chain amino acids by *lactobacilli* to form biofilms (Du et al. 2021). Moreover, the SCFAs produced by polysaccharides in fermentation could lower the pH of gut surrounding and inhibit the proliferation of some pathogens and colon cancer, maintaining human safety (Xiaofei Xu et al. 2013). An interesting investigation by Zhou et al. (2022) proved that Hawthorn polysaccharide HPS, extracted from fruits (*Crataegus pinnatifida*), maintain intestinal health by regulating the composition and abundances of intestinal microbiota; and significantly increased the abundance of *Firmicutes* at the phylum level, whereas *Bacteroides*, *Proteobacteria*, *Escherichia, shigella* and *Fusobacterium* significantly decreased. More recent investigations confirmed the beneficial effect of isolated fruit *Crataegus pinnatifida* polysaccharides on promoting probiotics growth and maintaining intestinal homeostasis (Zhu et al. 2022; Guo et al. 2020). However, no research has been done to evaluate the prebiotic potential of the polysaccharide extracted from *Crataegus azarolus* fruits.

MATERIAL & METHODS

II. Material and Methods



The materials and methods of the present work are divided into three parts. The first part deals with *Tuberaria lignose* leaves, where we could evaluate their phytochemical, antimicrobial activities. The second part deals with *Crataegus azarolus L*. fruits; studying their potential abilities of the extracted polysaccharide and uncovering for the first time its structure. The third part deals with the characterisation of isolated lactic acid bacteria. This study was carried out mainly in the laboratory of Biochemistry and Applied Microbiology, Biochemistry Department at Badji Mokhtar University Annaba and in the Department of Chemistry at Gebze Technical University Turkey.

II.1. *Tuberaria lignosa* leaves

II.1.1. Plant material

Tuberaria lignosa Sweet Samp. (خياطة الجراح) is a perennial plant in the rockrose Cistaceae family, native to Western and Southern Europe, occurs mostly in dry, stony places of the Mediterranean area. The leaves are in rosettes at the base of the plant, yellow flowers are organised in relaxed determinate inflorescences (Castroviejo and Botánico 1986) as shown in Fig. 10. *T. lignosa* was hand-harvested from Chetaibi (state of Annaba, Algeria; 37° 02′ 00″ N latitude and 7° 27′ 48″ E longitude, Fig. 9) in the early flowering season of May 2018.

Fig. 9. Geographical situation of Chetaibi region (Google Earth)

The plant was identified at the Department of Biology, Faculty of Sciences, Badji Mokhtar University, Annaba, Algeria, by Dr. Hamel (Fig. 10[a]). The leaves were separated from the aerial part, shade dried, being stored in a dark and dry place in paper bags kept at room temperature (24±3ºC) for almost 30 days, simulating general conditions of traditional plant-use. Then crushed with a grinder to obtain particles between 1 and 3 mm in size.

a b

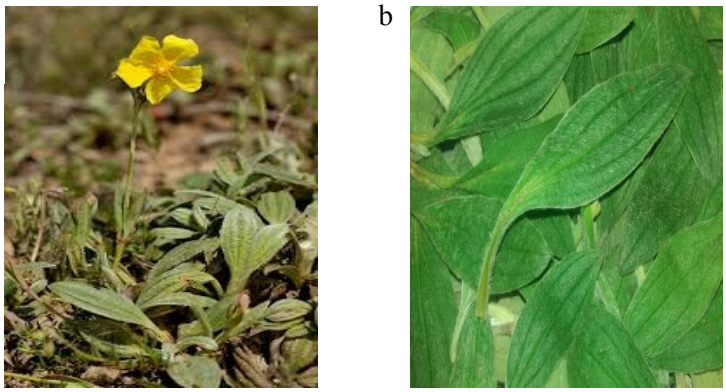


Fig. 10. [a] *Tuberaria lignosa* plant. [b] Recolted leaves left to air drying

II.1.2. Phytochemical screening:

Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive compounds present in medicinal plants. Phytoconstituents individually or in combination, determine the plant's pharmacological value. The conventional qualitative tests are still necessary for the preliminary phytochemical screening of plants in the presence of various modern identification tools and techniques. The characterisation and evaluation of the plant phytoconstituents were carried out using standard methods as described (Shaikh and Patil 2020).

II.1.2.1. Detection of alkaloids Mayer's test

A maceration of 5 g of dried and crushed leaves in 50 ml of diluted hydrochloric acid (1%) and filtered. The filtrate was carefully tested with drops of Mayer's reagent. The appearance of cream to white coloured precipitate shows the presence of alkaloids.

II.1.2.2. Detection of steroids and terpenoids Salkowski's test

The sample powder (5g) was macerated in 20 ml of petroleum ether, after filtration, evaporation of the organic phase was in a water bath at 90°C, the residue is dissolved in acetic acid, and then 1 ml volume of concentrated sulphuric acid was added and shacked gently. The presence of steroids and terpenoids is indicated by the upper layer turning brownish red and the lower layer showing yellow green fluorescence.

II.1.2.3. Detection of flavonoids Ammonia test

The search begins with a maceration of 10 g of plant powder in 150 ml of 1% hydrochloric acid for 24 hours. After filtration, 10 ml of the filtrate is added to a basic solution of Ammonium Hydroxide. After 3 hours, the appearance, of a light-yellow colour in the upper part of the tube, indicates the presence of flavonoids.

II.1.2.4. Detection of saponins Foam test

A decoction solution is prepared: 2 g of dry and crushed plant material mixed in 100 ml of water (boiling for 30 min). After cooling and filtration, the volume is readjusted to 100 ml. In a series of 10 test tubes, 1 ml of the extract is placed in tube N°1, 2 ml in tube N°2, ..., 10 ml in tube N°10. The final volume in each tube was readjusted again to 10 ml with distilled water. The tubes are stirred strongly in a horizontal position for 15 seconds. After a rest of 15 minutes in an upright position, the heights of the persistent foam were measured in cm. If it is close to 1 cm in the X^{th} tube, then the foam index (I) is calculated according to the following formula (1):

$$I = \frac{The\ foam\ height\ (cm) in\ the\ X\ tube\ \times 5}{0.0X} \quad (1)$$

0.0X : if the X^{th} tube is , for example, the 3^{th} tube then 0.0X in formula is 0.03;

So, the presence of saponins is determined quantitatively by the calculation of the foam index and confirmed with an index higher than 100.

II.1.2.5. Detection of tannins Braymer's test

Taking 5 ml of the 10% plant infused, to which we add drop by drop 1 ml of 1% ferric chloride solution. The appearance of green colour indicates the presence of catechic tannins and dark blue from gallic tannins. A confirmation test comes next by adding 15 ml of Stiasny's reagent (30% Formaldehyde / concentrated hydrochloric acid: 3/1 v/v) to 30 ml of the infused. After heating for 30 minutes in a water bath, an observation of an orange precipitate indicates the presence of gallic tannins.

II.1.2.6. Detection of anthocyanins HCl test

The search is based on the change in colour of the 10% infused with the change in pH. Add a few drops of pure hydrochloric acid, then a few drops of ammonia (25%). The colour change indicates the presence of anthocyanins.

II.1.2.7. Detection of leuco anthocyanins Isoamyl alcohol test

A 5 ml volume of the infused is mixed with 4 ml of hydrochloric alcohol (Ethanol/ pure hydrochloric acid: 3/1 v/v). After heating in a water bath at 50 ° C for a few minutes, the appearance of a cherry red colour indicates the presence of leuco anthocyanins.

II.1.2.8. Detection of carbohydrates Fehling's test

A 1 ml of Fehling's solution A (Copper Sulfate Heptahydrate) and 1 ml of Fehling's solution B (potassium sodium tartrate) were added to 2ml of infused extract. They were heated in a boiling water bath for 5 min and observed the blue colour turning to red forming a precipitate.

II.1.3. Extraction for phytochemical analysis and antimicrobial screening

The preparation method had a higher influence on bioactive properties than the processing treatment, being infusions preferable over decoctions for shade-dried wild samples, as indicated (Pinela et al. 2012) in their investigation about the effect of *Tuberaria lignosa* drying and oral preparation techniques. The air-dried powdered leaves (7.5 g) were added to 500 ml of boiling

distilled water, left to stand at 25 ±1°C for 5 min, and then filtered under reduced pressure. The obtained filtrate was oven-dried at 45 ±5°C until a constant weight was reached.

Methanol organ extract was obtained by magnetic stirring for 30 min of 2.5 g of dry leaves with 25 ml of methanol solvent. The extract was kept at 4 °C for 24 hours, filtered through a Whatman No° 4 filter paper, evaporated to dryness and stored at 4 °C until analysis (Falleh et al. 2008; Bougandoura and Bendimerad 2013). According to Falleh et al. (2008) pure methanol yielded the highest extracting power (%) as compared to the other solvents (n-hexane, acetone and water), so methanol was used to obtain an alcoholic extract.

The yield (%) of evaporated dried extracts was calculated according to the following equation (2):

$$Extraction\ yield\ (\%, {}^{w}/_{w}) = \frac{weight\ of\ dried\ extraction\ (g)}{weight\ of\ plant\ powder\ (g)} \times 100 \quad (2)$$

II.1.4. Phenolic compounds extraction for HPLC analysis

The powdered leaves (1 g) were extracted by stirring with 30 ml of methanol: water 80:20 (v/v), at room temperature,150 rpm, for 1 hour. The extract was filtered through Whatman n°4 paper. The residue was then re-extracted twice with additional portions (30 ml) of methanol: water 80:20 (v/v). The combined extracts were evaporated at 45-50°C using a rotary evaporator to remove methanol. The aqueous phase was dried in incubator at 45°C and the extracts were re-dissolved in 20% aqueous methanol at 1 mg.ml^{-1} and filtered through a 0.2 µm disposable LC filter disk for high performance liquid chromatography analysis (Chahdoura et al. 2015; Gori et al. 2016).

II.1.5. Determination of total antioxidant compounds

II.1.5.1. Total polyphenol content

The total phenolic content was estimated by Folin Ciocalteu method as described by Singleton and Rossi (1965) with slight modifications. The 1 mg.ml^{-1} extract (50µl) was mixed with 250 µl of Folin Ciocalteu reagent, left in dark at ambient temperature for 5 min, and then 500 µl of sodium carbonate (20%) was added. The mixture was allowed to stand at 22°C for 30 min. The final volume of the mixture was adjusted to 5000 µl with distilled water. The content of total phenolic compounds was expressed as mg of gallic acid equivalents per g of extract (mg GA eq. g^{-1} extract), the standard curve was given in **Annex3**. The absorbance was measured at 727 nm using a UV-Vis spectrophotometer.

II.1.5.2. Total flavonoid content

The flavonoid content was determined by aluminum trichloride method previously described by Zhishen et al. (1999) using Quercetin as reference compound. A volume of 1 ml of extract (50 $mg.ml^{-1}$) is added to 4 ml of distilled water. An aliquot of 300 µl of a sodium nitrate solution (50%) was added at t_0. The mixture was allowed to stand for 5 min, followed by the addition of 300 µl of aluminum trichloride (10%), then at the exactly 6^{th} min (t_6) 2 ml of sodium hydroxide (1M) was added. The final volume of the solution was adjusted to 10 ml with distilled water. The mixture turned to pink after 15 min of incubation and the absorbance was measured at 510 nm. The total flavonoid content was expressed as mg QE. g^{-1} extract (standard curve; **Annex 4**).

II.1.5.3. Total condensed tannin contents

Condensed tannin quantification was determined using the vanillin assay based on the method outlined by Butler et al (1982) with slight modifications, using catechin as a reference compound. An aliquot (50 µl) of the extract is added to 3 ml of a vanillin solution (4%; prepared in methanol) and 1.5 ml of concentrated hydrochloric acid. After 20 min of incubation in dark at 30°C, the absorbance was read at 500 nm. Concentration was calculated by subtracting the background read from the final read. The condensed tannin was expressed as mg CE . g^{-1} Extract (standard curve; **Annex 5**).

II.1.5.4. HPLC

Polyphenol analysis by HPLC was realised according to Pinela et al. (2012). The *T. lignosa* extract (1 mg) was dissolved in water: methanol (80:20 v/v), filtered through 0.2 µm nylon filters from Whatman and analysed by HPLC (HPLC 1100) with a diode array detector (DAD). A C18 column was used,5 µm (4.6 mm × 250 mm). The solvents used were: (A) 0.1% formic acid in water, (B) acetonitrile. The elution gradient established was 10% B to 15% B over 5 min, 15–25% B over 5 min, 25–35% B over 10 min, isocratic 50% B for 10 min, and re-equilibration of the column, using a flow rate of 0.5 $ml.min^{-1}$. Double detection was carried out in the DAD using 280 nm and 340 nm as preferred wavelengths. The phenolic compounds present were characterised according to their retention times, and comparison with the data reported in the literature (Salles et al. 2021; Pinela et al. 2012; Sandhu and Gu 2010; Toku 2003; Gaynor et al. 1988).

II.1.6. Antioxidant activity

II.1.6.1. DPPH radical scavenging assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH radical) scavenging effect was evaluated according to the method employed by Bouaziz and Sayadi (2005). A 2 ml of sample extract at different concentrations (10 to 500 $\mu g.ml^{-1}$) were added to 5 ml DPPH ethanol solution (1.5×10^{-4} M). After mixing the solutions gently, it was left at room temperature for 30 min, the optical density was measured at 520 nm against ethanol as blank. Both Aq-E and MeOH-E sample extracts and positive control BHT were tested over the same range of concentrations. The antioxidant activity was expressed in terms of concentration required to inhibit 50% DPPH radical formation (IC_{50} $\mu g.ml^{-1}$) and calculated from the inhibition percentage curve.

II.1.6.2. Ferric-reducing ability power (FRAP)

The FRAP assay was made according to the method of Oyaizu (1986) with a slight modification. The sample extracts in a different concentrations ranging were mixed with a 2.5mM phosphate buffer and 2.5ml, 1%, w/v potassium ferric cyanide, then the mixture were incubated at 50°C for 20 min. Afterwards, 2.5ml of 10%, w/v trichloroacetic acid and the mixture was centrifuged at 3000 rpm for 10 min. A supernatant volume of 2.5 ml was mixed with 2.5 ml of distilled water and 0.5 ml 0.1% ferric chloride. Finally, the absorbance was measured at 695nm against a blank of the reagent. FRAP results were expressed as mM equivalent to Fe (II) per gram of extract, using Ferrous sulfate heptahydrate calibration as standard.

II.1.6.3. Total antioxidant activity

The total antioxidant capacity (TAC) of the plant extracts was evaluated by the phosphomolybdenum method of Prieto et al. (1999). A 0.1 ml aliquot of the plant extract was mixed with 1 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). After 90 min of incubation at 95°C, the absorbance of the mixture was measured at 695 nm against the blank (1 ml of reagent mixture plus 0.1 ml of extraction solvent: water or methanol). A standard curve was performed with the ascorbic acid solution, and then the antioxidant activity of the sample was expressed as milligrams of ascorbic acid equivalents per gram of extract.

II.1.7. Evaluation of antibacterial activity

II.1.7.1. Bacterial strains

In order to evaluate the antibacterial activity of *T. lignosa* extracts, thirteen pathogenic microorganisms isolated in CHU Ibn Rochd Annaba Algeria were used (table 5). After checking the purity of strains, microscopic examination and Gram strain were tested. API 20E test was used in order to identify the type of Enterobacteriaceae and the API Staph was used as a standardized system to identify the *Staphylococcus* strains. The antibiotic susceptibility has been evaluated.

II.1.7.2. Inoculum preparation

Each pure culture was activated by subculture using the streak method on Mueller Hinton medium, then incubated at 37°C for 24 hours. One or more colonies of each pure culture are removed and transferred to physiological water at a turbidity equivalent to 0.5 McFarland, which translates into an absorbance of 0.08 to 0.13 at 625 nm.

II.1.7.3. Determination of zone of inhibition method (Disc assay)

In vitro antibacterial activity of *T. lignosa* leaves Met.E and Aq.E was conducted using Kirby–Bauer technique (Bauer 1966). The 0.5 McFarland inoculums of the different bacterial species were used to inoculate new Petri dishes containing the Mueller Hinton medium by the swab technique. The sets of three dilutions (50,100 and 150 $mg.ml^{-1}$) of *T.lignosa* leaves extracts were selected based on Doughari et al. (2008); Adesokan et al. (2007); Ali et al. (2020); Atya et al. (2018) and Zubair et al. (2017) researches, which were reconstituted in DMSO for organic extract and distilled water for aqueous extract. Sterile Wattman n°1 discs 6 mm in diameter were aseptically put on agar surfaces and immediately impregnated with 15 µl of various concentrations of Aq.E or Met.E on each of the plates containing cultures of the different test organisms. Spread plates were then kept at ambient temperature for 30 min to allow diffusion of extracts. Positive Control experiments were carried out under similar conditions by using Gentamicin as standard drugs and negative control discs are soaked in distilled water and DMSO were used to prepare extracts solutions. Petri dishes are incubated at 37°C for 24 hours. The sensitivities of the microorganism species to *T. lignosa* extracts were determined by measuring the diameters in millimeter of inhibitory zones (including the disk diameter and around it). The values ≤8 mm was considered nonactive extracts against bacteria.

II.1.7.4. Determination of Minimum Inhibitory Concentration (MIC)

The minimal inhibition concentration (MIC) values were studied for the bacterial strains which were sensitive to the extracts in the disc diffusion assay. The bacterial inocula were prepared from 12 hours of broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The *T. lignosa* extracts were dissolved in distilled DMSO 10% or water. It was diluted to the lowest effective concentration 50 $mg.ml^{-1}$ to make serial two-fold dilutions. The 96-well plates were prepared by dispensing into each well 95 µl of Mueller Hinton broth (MHB) and 5 µl of the inoculum. A 100 µl aliquot from the stock solutions of *T. lignosa* extracts initially prepared (50 $mg.ml^{-1}$) was added into the first wells. Then, 100 µl from their serial dilutions were transferred into eight consecutive wells. A negative control was containing 195 µl of MHB without compound and 5 µl of the inoculum on each strip. A second negative control was containing only MHB so the contamination of the medium wasn't questioned. The positive control was 100 µl of extracts prepared with MHB. The final volume in each well was 200 µl. The plate was covered with a sterile parafilm. Contents of each well were mixed on a plate shaker at 300 rpm for 20 seconds and then incubated at appropriate temperatures (37 °C) for 24 hours. Microbial growth was determined by phenolsulfonphthalein (PSP) indicator. A 10 µl of 0.044 % of PSP was added to each well, this pH indicator was incorporated in viability assays studies (Chu et al. 2000; Welch et al. 2012). The rise in pH can be observed as a change in colour from yellow to purple. This colour-change is used to determine the inhibition effects; microorganisms' viability or death (Sanjotha 2017). The MIC is indicated by the lowest extract concentration which there is no bacterial growth.

Table 5. List of pathogenic bacteria used in the study

Strain	Bacteria	Family
Gram-negative	*Escherichia coli ATCC 8739* *Escherichia coli ATCC 25922* *Escherichia coli 1* *Escherichia coli 2* *Escherichia coli 3* *Proteus mirabilis 1* *Proteus mirabilis 2* *Proteus mirabilis 3* *Proteus vulgaris* *Salmonella typhimurium ATCC 14028* *Salmonella enterica* *Klebsiella pneumoniae* *Pseudomonas aeruginosa ATCC 27853*	Enterobacteriaceae
Gram-positive	*Enterococcus faecalis ATCC 29212*	Enterococcaceae
	Staphylococcus aureus ATCC 43300 *staphylococcus albus ATCC 8799* *Staphylococcus aureus ATCC 25923* *Staphylococcus aureus 1* *Staphylococcus aureus 2* *Staphylococcus aureus 3* *Staphylococcus aureus 4*	Staphylococcaceae

II.1.7.5. Determination of Minimum Bactericidal Concentration (MBC)

From each well that does not show bacterial growth a 100 µl was seeded on a nutrient agar medium then incubated at 37 °C for 18 to 24 hours. The MBCs are interpreted as the lowest concentration of extract, which has shown a clear liquid without the development of turbidity or any bacterial growth on the Petri dish.

The MBC/MIC ratio is calculated, to determine the antibiotic power of the tested extracts. If the ratio is less than or equal to 4, the extract is said to be bactericidal, however, if it is higher than 4, the extract is qualified as bacteriostatic (Joubert et al. 1958).

MBC/MIC ratios are calculated. The extracts with ratios greater than 1 are considered as microbiostatic, while the others are considered microbicide (Karou et al. 2005). All tests are performed in three replicates.

II.1.8. Evaluation of Antifungal activity

To examine the antifungal potential of *Tuberaria lignosa* extracts, the direct contact technique was used (Singh et al. 2008; Labiod 2016) against the Candida albicans strain.

The aqueous and methanolic tested extracts were prepared by solubilising dried extract to final concentrations of 50, 25, 12.5 and 6.25 $mg.ml^{-1}$ then 0.5 ml of each concentration was added to a sterile tube containing 9.5 ml of PDA medium. After homogenisation with a vortex, the contents of the tubes were poured into Petri dishes, then left to dry for 15 to 20 min. A 6 mm diameter mycelial disk cut from a 5-day-old culture was placed in the centre of the Petri dish and incubated at 25±3 °C for 8 days under darkness. One Petri dish containing only PDA medium and without any extract was used as a negative control. And one Petri dish with PDA medium containing mandipropamid (1000 ppm) was used as positive control. The MIC was defined as the lowest extracts concentration that inhibited 100% of the fungal growth. All experiments were evaluated in triplicate in this assay.

II.2. *Crataegus azarolus* fruit

II.2.1. Plant Material

In October 2016, the fresh fruits of *Crataegus azarolus* (الزعرورة الصفراء) were hand-harvested from Chetaibi (state of Annaba, Algeria; 37° 01′ 43″ N latitude and 7° 22′ 40″ E longitude).

II.2.2. Polysaccharides extraction, separation and purification process

The pulps were separated from the seeds, dried then crushed to obtain a fine powder. The extraction was performed using a method described elsewhere by Diaz (1981); 5 g of the powder were magnetically stirred over a night with 100 ml distilled water (1:20 m/v). Followed by filtration on gauze then centrifugation at 4500 rpm for 30 min, the separated supernatants were mixed with ethanol (until 60% of final ethanol contents), then it was left at 4°C overnight. The precipitate was collected by simple centrifugation and dissolved in distilled water, reprecipitated a second time with ethanol (60%), then centrifuged to obtain precipitation of crude AZWSP (*Azarolus* water-soluble polysaccharides), dried in incubator 40 - 55 °C to constant weight.

The crude polysaccharide was dissolved in water (10 $mg.ml^{-1}$) and steadily stirred for 24 hours. The obtained solution is vacuum filtered. An insoluble complex is formed by adding Fehling liqueur (Jones and Stoodley 1965). It is transferred into a porcelain mortar to be mixed with 5% hydrochloric acid (in 96% ethanol v/v). The precipitated regained its fibrous character as the solution changed its colour. 4 volumes of ethanol 80% were added, followed by centrifugation to isolate the resulting precipitate (Haworth et al. 1937). Repeated wash was performed several times using ethanol. Finally, the purified fruit polysaccharide was dried. The dry powder was stored at room temperature and protected from light and humidity until later use. The AZWSP extraction yield percentage (%) was calculated by Eq (3):

$$Extraction\ yield\ (\%, {}^{w}/_{w}) = \frac{weight\ of\ dried\ extraction\ (g)}{weight\ of\ fruit\ pulps\ powder\ (g)} \times 100 \qquad (3)$$

II.2.3. Characterisation of AZWSP

II.2.3.1. Chemical analysis

II.2.3.1.1. Total carbohydrate contents

The quantification of the total carbohydrate content of the extracted polysaccharide was based on Dubois method using sulfuric acid phenol as reagents (Dubois et al. 1956). Under the action of concentrated and hot mineral acids, the hexoses and pentoses undergo extensive internal

dehydration, followed by cyclisation leading to the formation of furfural derivatives and 5-hydroxymethylfurfural, reacting with phenol. The formation of a yellow-red complex makes it possible to follow the concentration of total sugars of the sample by reading the absorbance at 485 nm.

In glass test tubes, we introduced 1 ml of the sample and place the tubes in an ice bath. Then added 1 ml of the aqueous solution of phenol 5%. After homogenisation, 5 ml of sulfuric acid (96%) was carefully added. The tubes are then incubated in the dark for 30 min. Absorbance is measured at λ=485 nm. The glucose was used to produce a calibration curve **(Annex 1).**

II.2.3.1.2. Protein contents

The protein content was determined by the Bradford method (Bradford 1976). It is based on the colour change of Coomassie blue in contact with basic amino acids (arginine, histidine and lysine) plus hydrophobic amino acids present in proteins. The cationic (free) forms of the dye are red-brown. The anionic form of the reagent corresponds to the form bound with proteins. The absorbance of this complex is measured at 595 nm.

The "micro-Bradford" method is more sensitive than the standard method, and it is used on samples containing a protein concentration less than 20 µg. In a test tube, 800µl of sample solution was added to 200µl of Bradford reagent. The mixture was homogenized and left in the dark for 30 min then the OD was detected at λ=595 nm. Absorbances (OD) are proportional to the amount of protein in the sample. The concentration was measured based on the bovine serum albumin calibration curve **(Annex 2).**

II.2.3.1.3. Total phenolic compounds

Total phenolic compounds were estimated by the Folin-Ciocalteu method using gallic acid as the standard (Thetsrimuang et al. 2011).

The Folin-Ciocalteau reagent containing phosphomolybdate and sodium tungstate oxidizes the polyphenols and reduces to tungsten and molybdenum oxides (blue) which absorb at 750 nm.

Diluting 0.5 ml of the sample solution in 10 ml of distilled water was followed by adding 0.5 ml of the Folin-Ciocalteu reagent, and left to stand for 3 min. One ml of a saturated solution of Sodium carbonate was added and mixed for 10 seconds. Left in the dark for 1 hour until a blue colour appears and the OD detection was at λ=750 nm. Gallic acid was used as calibration curve **(Annex 3).**

II.2.3.2. Monosaccharides composition

The purified AZWSP was hydrolysed according to the method described elsewhere (Adams 1965). The 0.1 g of polysaccharide was added to 1 ml of 72% sulfuric acid in a sealed tube, the mixture was left for 20 min at room temperature, then 6 volumes of distilled water were added and put in the oven at 100 ° C for almost 18 hours. After cooling, it was neutralised with barium hydroxide, and filtered through glass wool. The filtrate was deionised by adding silica then left to dry. Thin layer chromatography (TLC) was performed as a screening analysis of hydrolysed compounds; the used stationary phase was silica gel plates and the mobile phase mixture consisted of n-butanol /acetone/water (4:5:1) (Ghebregzabeier et al. 1976). The plate was visualized by spraying 70° of sulfuric acid and heating at 100 °C- 10 min.

II.2.4. Structure analysis

II.2.4.1. Fourier-transform infrared spectroscopy

To analyse the functional groups of *azarolus* polysaccharides, the technique of FT-IR was used. The polysaccharide was directly placed on the ATR plate for measurement on a Perkin Elmer Spectrum 100. The recorded spectrum was in the wavelength ranging of 500- 4000 cm^{-1}, and the infrared spectrum was analysed using OriginPro 8,5.

II.2.4.2. Nuclear magnetic resonance spectroscopy

The proton and carbon NMR are usually chosen to determine the anomeric configurations fine structure of polysaccharides. The extracted polysaccharide was dissolved in 0.5 ml deuterium oxide (D_2O) into 10% and 60% (w/v) concentration to record ^{1}H /^{13}C-NMR spectra, respectively. The mixture was stirred overnight at room temperature and then sonicated with an ultrasonic homogeniser sonicator at one power level (400 W) in regular pulse mode (10 s on, 10 s off) for 30 min. During this process, the reaction vessel containing the AZWSP solution is placed in an ice bath. NMR spectra were recorded using a Varian 500 MHz spectrometer equipped with a 5 mm broadband probe operating at 500 MHz for ^{1}H and 150 MHz for ^{13}C. Chemical shifts are expressed in ppm. Spectra were processed and analysed using MestReC software.

II.2.4.3. MALDI-TOF- mass spectrometry

The structure of yellow hawthorn polysaccharide is determined by MALDI-MS as described by Ropartz et al. (2011). The mass measurements are carried out on a Bruker Microflex LT MALDI-TOF MS instrument, equipped with a nitrogen laser emitting at 337 nm in positive ionisation mode, and with linear mode detection. The polysaccharide solubilised at 1 $mg.ml^{-1}$ in water is mixed with the DHB matrix (10 $mg.ml^{-1}$ 2,5-dihydroxybenzoic acid in acetone) in a 1:1 (v/v) ratio. A 1 µL of the mixture is deposited on a polished steel plate for MALDI analysis. The *azarolus* polysaccharide spectra were recorded in a range 0 to 12,000 m/z (mass/charge), and fragments adduct ions $[M + Na]^+$ or $[M + K]^+$ were assigned. The intensity of the laser is fixed at 500 U.A., with an acquisition time of 5 s. The software GlycoWorkbech 2.1 was used to match the mass specter with databases structures. The software was downloaded on Download GlycoWorkbench 2.1 Build 146 (softpedia.com).

II.2.5. Antioxidant activity of *Crataegus azarolus* polysaccharide

II.2.5.1. DPPH free radical scavenging activity

The scavenging capacity of *azarolus* polysaccharide against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was carried out according to the method described by Bersuder et al. (1998). Aliquots of the tested solution (250 µl) at various concentrations were mixed with 1,25 ml of DPPH solution (0.2 mM) freshly prepared in ethanol. The reaction mixture was shaken with vortex and kept in the dark for 30 min, at room temperature. The absorbance was measured against ethanol as blank, using a spectrophotometer at 517 nm.

The positive standards Ascorbic acid and butylated hydroxytoluene were tested respecting the same process.

The results were expressed as a percentage of eliminated DPPH radicals. It was calculated according to eq (4).

$$Percent\ Inhibition\ \% = [\text{A Control} - \text{A Sample } p)/\ \text{A Control}] \times 100 \qquad (4)$$

Where A control is the absorbance of the DPPH solution without sample *p*

A sample *p* is the absorbance of the sample solution without DPPH

Antiradical DPPH activity is denoted as IC_{50} in ($mg.ml^{-1}$) referring to the required AZWSP sample concentration which scavenges 50% of DPPH free radicals.

II.2.5.2. Hydroxyl radical-scavenging assay

Hydroxyl radicals scavenging ability were performed according to a protocol adapted by Jin et al. (1996) with slight modifications. The polysaccharide sample was dissolved in distilled water at

different concentrations. A volume of 0.5 ml of each dilution was mixed with 0.5 ml of 7.5 mM Ferrous sulfate solution. After homogenisation, a 0.5 ml volume of 1% Hydrogen peroxide was added. The mixtures were incubated at 37 C for 30 min. Then 3 ml of distilled water was added before detecting the absorbance at 510 nm. Ascorbic acid (AA) is used as a positive control.
The percentage scavenging activity of hydroxyl radicals was calculated according to the following equation (6);

$$Scavenging\ activity\ \% = \left(1 - \frac{A1-A2}{A0}\right) \times 100 \qquad (6)$$

where A 0 is the control group absorbance in the hydroxyl radicals generating system (water instead of polysaccharide sample), A1 is the absorbance of the tested polysaccharide and A2 blank is the absorbance of the polysaccharide only (water instead of 1% Hydrogen peroxide solution).

II.2.5.3. Linoleic acid peroxidation assay

Linoleic acid peroxidation was performed according to the described conjugated diene method (Lingnert et al. 1979). The AZWSP sample was dissolved in 0.2% acetic acid solution at different concentrations, a 100 µl of each one was added to 2 ml of 10 mM linoleic acid emulsion containing 200 mM sodium phosphate buffer (pH 6.5), to achieve the oxidation phenomenon, incubation at 37 °C in the dark for 15 hours was made. Then, 6 ml of 60% methanol was added, the absorbance of the solutions was measured at a wavelength of 234 nm. The antioxidant activity is calculated according to the formula (5).

$$Antioxydant\ Activity\ \% = \left(1 - \frac{A\ Sample\ p}{A\ Control}\right) \times 100 \qquad (5)$$

A Control is the absorbance of the control (methanol and reagent solution without the sample), and A sample p is the absorbance of the polysaccharide sample (the reagent solution containing AZWSP). Anti-lipid peroxidation activity expressed as IC_{50} value ($mg.ml^{-1}$) represents the half maximal inhibitory concentration. Ascorbic acid (AA) was used for comparison.

II.2.5.4. Ferric-reducing antioxidant power (FRAP) activity

The reducing power was determined according to the process described elsewhere by Oyaizu (1986) respecting some appropriate modifications (Hammi et al. 2016). From various concentrations of the sample, a volume of 250 µl was mixed with 625 µl of sodium phosphate buffer (0.2 M, pH 6.6), then 625 µl of Potassium ferricyanide (1%, w/v) was added. The mixture was incubated for 20 min at 50°C. The reaction was stopped by adding 625 µl of trichloroacetic acid (10%, w/v), the tested

samples were centrifuged at 2000 rpm for 10 min. Then, 625 µl of the upper layer was mixed with 625 µl of distilled water and 125 µl of Ferric chloride (1% w/v). The absorbance was measured after 10 min against the reaction mixture as blank (adding water instead of sample) at 700 nm. Ferrous sulfate heptahydrate standard curve was used to estimate the AZWSP capacity as µm Fe^{2+}. g^{-1} E.

II.2.6. In vitro digestibility test of AZWSP

The digestibility of AZWSP is studied by calculating the degree of their hydrolysis after incubation with artificial human gastric juice. Inulin was used as a reference and dissolved in distilled water in a 1% (m/v). The digestibility was tested according to the method described by Korakli et al. (2002). An artificial gastric juice was prepared by a hydrochloric acid buffer containing the following reagents: sodium chloride (8 $g.l^{-1}$), sodium phosphate (14.35 $g.l^{-1}$), disodium hydrogen phosphate dihydrate (8.25 $g.l^{-1}$), calcium chloride dihydrate (0.1 $g.l^{-1}$) potassium chloride (0.2 $g.l^{-1}$), and magnesium chloride hexahydrate (0.18 $g.l^{-1}$). The pH of the buffer is adjusted into 1, 2, 3, 4 and 5 using a 5 M hydrochloric acid solution. One mL of the sample is mixed with 5 mL of artificial gastric juice for all pHs and the mixtures reaction were incubated in a water bath at a temperature of 37 °C for 6 hours. 500 µL of the reaction mixture are taken periodically after 0, 0.5, 1, 2, 4 and 6 h of incubation in incubator Hood. The content of reducing sugars was determined by the dinitrosalicylate method (DNS) described by Miller (1959) and total sugars were determined as described above (section II.2.3.1.1.). The percentage of hydrolysis of the sample is calculated based on the content of sugars reducing agents released and the total sugar content of the extracted polysaccharide sample according to equation 7.

$$\%Hydrolysis = [reducing\ sugars\ released / (\text{total sugars-initial reducing sugars})] \times 100 \qquad (7)$$

II.2.7. In vitro evaluation of the prebiotic activity of AZWSP

Selected isolated strains were used to determine the ability of *azarolus* polysaccharide to induce the growth of the beneficial bacteria *lactobacillus sp*. The Man Rogosa Sharpe (MRS) broth composition was modified to perform the growth experiment with the extracted polysaccharide. The modified Man Rogosa Sharpe (m-MRS) did not contain the usual carbon source (glucose), which was provided by the *azarolus* polysaccharide. The prebiotic activity evaluation was tested using 1% (w/v) of AZWSP in m-MRS. The positive growth control was performed using m-MRS containing 0.5% glucose. The m-MRS negative control was without adding any sugar. The medium containing AZWSP is prepared as follows: protease peptone (1g), beef extract (1 g), yeast extract (0.5 g), polysorbate 80 (0.1 g), sodium acetate (0.5 g), ammonium citrate (0.2 g), dipotassium phosphate

(0.2 g), magnesium sulphate (0.01 g), manganese sulphate (0.005 g) and the tested carbon source AZWSP (1 g) were weighed in a flask, suspend all ingredients in distilled water and boil until completely dissolved. Those components were for 100 ml of m-MRS;

The sterilisation was at 120°C for 15 minutes. Next, the pH was lowered to 5.8±0,1 and 0.1% sorbic acid was added, this latter makes the medium selective to lactic acid bacteria (Corry et al. 2003). Young bacterial cultures with an absorbance of 0.08 to 0.1 at 625 nm (0.5 McFarland) were inoculated at 2% (v/v) into a flask containing m-MRS medium under anaerobic conditions at 37°C for 48 hours. At different times of incubation (from t_0 to t_{48}), the lactic acid bacteria growth and pH variations were systematically pursued. Finally, the acidity was determined by titration using sodium hydroxide.

II.3. Lactic acid bacteria

II.3.1. Lactic acid bacteria isolation

II.3.1.1. From milk

Milk samples were collected under hygienic conditions from healthy animals by hand milking. Cleaning the udder of animals before milking is one of the most important hygienic required practices to ensure having clean milk. Moreover, during the milking process, the first quantities of milk weren't collected. Aliquots of 200 ml per animal were saved into sterile laboratory bottles then transported immediately to LBMA laboratory for analyses.

1 ml of each milk sample was aseptically added into 9ml of sterile saline solution and mixed thoroughly to a homogenous suspension. Serial dilutions (10^{-1} to 10^{-7}) were performed and 1ml aliquots from each dilution was plated out onto MRS agar plates by spread plate method in triplicates, the agar plates were incubated in anaerobiosis for 48 hours at 37°C for isolation of lactobacilli (Makete et al. 2017).

II.3.1.2. From butter

An amount of 10 g of traditional butter (Zebda Beldia) were homogenized in 90 ml of sterile physiological water (0.85% Sodium chloride), then peptone was added (0.1%), and from this dilution of 10^{-1}, decimal dilutions (from 10^{-2} to 10^{-8}) have been prepared, a volume of 0.1 ml of each dilution was spread on the surface of MRS media. The Petri dishes were incubated for 72 hours at 37°C in darkness (Abdelmoumene 2015).

II.3.1.3. From carrot

The carrot (1 g) was added to 9 ml of MRS broth, the set was incubated in anaerobiosis in jars at 37 °C for 24 hours to isolate the lactic acid bacteria. Successive decimal dilutions were carried out in 9 ml of sterile physiological water until a dilution factor of order 10^{-6}. 1 ml was taken from each dilution 10^{-4}, 10^{-5} and 10^{-6} then seeded deep into Petri dishes containing warm MRS* (*: Calcium carbonate was added to MRS; $5g.l^{-1}$); Pour plate method was used. After incubation of 72 hours at 37°C, the colonies surrounded by a clear halo were selected and purified on MRS using the streak plate method (Laref 2014).

II.3.2. Purification

After isolating colonies of different morphological aspects (size, colour and surface) each isolate was transplanted on MRS medium and incubated at 37°C in order to ensure the purity of cultures.

Purity was checked by light microscopy examination and it was confirmed by the presence of only one cell type.

II.3.3. Preservation

The pure cultures are transferred periodically onto the slope of a slanted MRS agar (subculturing) in a hermetically sealed tube allowing the continuous growth and viability of microorganisms. The transfer is always subject to aseptic conditions to avoid contamination. The tubes are kept cold at 4°C in darkness.

The purified colonies were also stored in MRS broth supplemented with glycerol, mixing 0.6 ml of overnight culture with 0.4 ml of sterile 50% glycerol and stored at -4°C.

II.3.4. Characterisations of isolated lactobacilli

Selected isolates of lactobacilli were identified by phenotypic and biochemical tests. These included Gram reaction; oxidase and catalase reaction; bacterial growth and gas production from glucose in MRS broth tubes; the sugar fermentation pattern was determined by using the API 50 CH according to the manufacturer's instructions (API-BioMerieux, France) (Hébert et al. 2000).

II.3.4.1. Tolerance to Inhibitory Conditions

Some technological characteristics were detected. The LAB isolates were checked for their ability to survive in growth-inhibitory conditions like growth ability at 15 and 45 °C, in presence of Sodium chloride and at low pH. Tolerance studies were done by growing the selected isolates in MRS broth containing varying amounts of Sodium chloride (4% and 6.5%). Survival in low pH was determined by incubating the isolates in MRS media with pH adjusted to 4 and 9.5. After 48 hours of incubation at 37 °C, the turbidity of each culture was noted as a simple indication of growth or no growth (Mami 2012; Mohd et al. 2007).

II.3.4.2. Hemolytic activity

All the isolated strains of lactobacilli with inhibitory power were tested in order to verify their harmlessness. Fresh overnight bacterial cultures were streaked in triplicates on Columbia agar plates, containing blood and incubated at 30°C for 48 hours. Hemolytic activities of the bacterial culture were examined for signs of β-hemolysis (a clear zone around the colonies), a partial hydrolysis and greening zone (α-hemolysis) or no reaction (γ-hemolysis) (De Vuyst 2003). Only the strains without hemolytic capacity were taken for the rest of the probiotic tests (section II.2.7).

II.3.5. Antibiotic Susceptibility

The test was performed based on Divya research (2012). A culture (50 µl) of each of the LAB isolates (OD $_{625}$ adjusted to 0.8) was plated evenly over the entire MRS plates' surface using a sterile cotton wool swab. Antibiotic disks: CD clindamycin (2 µg), E erythromycin (15 µg), Te teicoplanin (10 µg), Ox oxacillin (1 µg), P penicillin (10 units) and SXT Cotrimoxazole (25 µg) were applied to the surface of the agar plates and incubated at 37 °C for 48 hours, and diameters of the zone of inhibition around the disks were measured.

II.3.6. Antimicrobial activity against bacterial pathogens

The antimicrobial activity of the isolated lactic acid bacteria was determined by the well method as described by Talon et al. (1980) against six strains (*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Proteus mirabilis*, *Salmonella typhimurium* and *candida albicans*). These assays were performed in triplicate. The plates were poured with 20 ml of MRS agar (soft agar with 9 $g.l^{-1}$ agar) at 45°C mixed with 200 µl of young culture in bacterial nutrient broth or Sabouraud's yeast broth at 37ºC (OD $_{625}$ = 0.08 - 0.1). After solidification, wells were made with a sterile cone (Alioua 2016).

The lactobacillus strains were grown in 10 ml of MRS broth for 24 hours at 37ºC. After incubation, a cell-free supernatant (CFS) was obtained by centrifuging the culture (8000 × g / 15 min), followed by filtration of the supernatant through a 0.2 µm cellulose acetate filter (Kermanshahi and Peymanfar 2012; Alioua 2016).

Testing the bacteriocins' inhibitory power, CFS was treated with the enzyme catalase (1 $mg.ml^{-1}$) to rule out the inhibitory effect of hydrogen peroxide. Neutralisation of CFS with Sodium hydroxide (0.1N) was performed to rule out the inhibitory effect due to acidity (Kermanshahi and Peymanfar 2012; Alioua 2016).

Treated CFS was deposited in the wells at 100 µl/well. After incubation at 37°C for 24 hours, the zones of inhibition were measured (mm).

The reading is done by measuring the diameter of the inhibition zone around the disc, the size of the inhibition diameter will be indicated as follows: + + +: 15mm (strong inhibition), + +: 14mm 10mm (medium inhibition), +: 9mm 7mm (weak inhibition), -: = 6mm (no inhibition).

II.4. Statistical analysis

The experiments were performed independently in triplicate and the data were expressed as the mean ± SD (standard deviation) values. In each experiment, the treatment effect was determined by an analysis of variance (ANOVA) followed by student's t-test, with a confidence interval of 95%. Comparisons of means were analyzed by Tukey's test. All statistical analysis is carried out through GraphPad Prism 7.00 (statistical software) and the values with $p < 0.05$ were considered significantly different.

RESULTS & DISCUSSION

III. Results and Discussion

III.1. *Tuberaria lignosa* leaves

III.1.1. Phytochemical screening:

As shown in Table 6, the preliminary phytochemical tests revealed that the main active constituents of *Tuberaria lignosa* are polyphenols including flavonoids, tannins, anthocyanins, reducing sugars and saponins, while leuco anthocyanins, steroids, terpenoids and alkaloids were absent. This is in accordance with the findings of Alsabri et al. (2013) evaluating the aerial part of *Helianthemum lippii,* Cistaceae family.

Table 6. Phytochemical Screening of *Tuberaria lignosa* leaves

Chemical croups	***Reagents***	***Positive results indicator***	***Tuberaria lignosa results***
Alkaloids	Mayer	Cream/white precipitate	-
steroids and terpenoids	acetic acid/ sulphuric acid	upper layer turning brownish red and lower layer showing yellow green fluorescence.	-
Flavonoids	1%HCl / ammonia	light-yellow color	+
Saponins	Foam index	I >100	+
Tannins	ferric chloride	Green or blackish blue color	+ (catechic tannins)
Anthocyanins	HCl/25% ammonia	color change	+
leuco anthocyanins	hydrochloric alcohol	cherry red color	-
Carbohydrates	Fehling liqueur	Red precipitate	+

III.1.2. Extraction yield

The *Tuberaria lignosa* leaves dry residue obtained from both aqueous and methanolic extracts yielded 5,12% and 6,55%, respectively (Fig. 11). It is well known that methanol was identified as the most effective solvent for extraction, and commonly resulting the highest extraction yield. However, it should be highlighted that drying was done using incubator at 45 -50°C, so the methanolic extract took generally one day to catch the constant weight meanwhile aqueous extract took 2 to 3 days, which probably led to the loss of some compounds.

III.1.3. Phenolic compounds

III.1.3.1. Colorimetric estimation

The total phenolic content of *Tuberaria lignosa* leaves was evaluated spectrophotometrically using gallic acid as the standard. The highest contents of phenolic compounds were present within the methanolic extract (130,96 ±3,28 mg GA eq. g^{-1} extract), in contrast to a previous study using absolute methanol as an extraction solvent (Ayad et al. 2022; Ahmed et al. 2020; Ammar et al. 2018). This may be due to the fact that phenolics are often extracted in higher amounts in polar solvents such as methanol and ethanol as compared with those having absolute polarity (Sultana et al. 2007). Other studies also reported that methanol is an effective solubilising agent for phenolic compounds from different plant matrices (Anwar et al. 2010). The aqueous extract (105,65 ± 2,3 mg GA eq. g^{-1} extract), was in agreement with the findings of *Cistus salviifolius* by Lukas et al. (2021). Similarly, the phenolic content of Cistaceae leaves extracts also varied significantly from 50.72 to 138.45 mg GAE. g^{-1} in the previous report by Rubio-Morage et al. (2013). Both *T. lignosa* extracts showed TPC greater than one hundred milligrams GA eq per gram of extract (>100 mg GA eq. g^{-1} extract) (Fig. 12), it is higher than aqueous and methanolic extracts of *C. ladaniferus* reported by Benali et al. (2020), as of *Cistus creticus* and *Cistus salvifolius* reported by Waed et al. (2016).

The total flavonoid contents of methanolic and aqueous extracts were 12,68± 1,9 and 6,29± 1,46 mg QE. g^{-1} extract, respectively (Fig. 12). Our findings were in the range of 5.85- 71.27 mg QE. g^{-1} of *Cistus ladanifer* and *C.libanotis arial* parts (Zidane et al. 2013). The extracts were higher than TFC obtained from both Algerian *Arthrophytum scoparium* (Benslama and Harrar 2016) and *Thymelaea hirsuta L.* (Bouzouina et al. 2016). Our methanolic extract had lower TFC than the one obtained from *Cistus Ladanifer* ;15.2 mg QE. g^{-1} extract (Tavares et al. 2020). Moreover, the aqueous extract showed total flavonoid contents comparable to the TFC estimated of Tunisian *Cistus* species 5,27 mg QE.g^{-1} (Mahmoudi et al. 2016), and higher than Algerian *Ruta chalepensis* aqueous extract 1.96 ± 0.12 mg QE.g^{-1} (Terkmane et al. 2018).

The determination of catechin tannins revealed that the methanolic extract had the highest content, which contains 23.73 ± 2;49 mg CE. g^{-1} extract, while aqueous extracts contain almost similar TCT around 23.14 ± 2,01 mg CE.g^{-1} extract (Fig. 12). It was higher than cathechic tannin content in aqueous and methanolic extracts of the aerial part of *Anabasis aretioïdes* (0.70 - 4.10 mg CE. g^{-1} extract) (Senhaji et al. 2020). Although, it was lower than 56.36 mg CE. g^{-1} extract of methanolic leaves extracts from *Cistus salviifolius* reported by El Euch et al. (2015).

These differences between the present study and the literature may be caused by the difference in cultivar type, climate, edaphic condition, maturity, and affected by the determination methods used. Ali et al. (2020) findings confirm that the extraction method and the solvent ratio also affect yields, total phenol content and total flavonoid content.

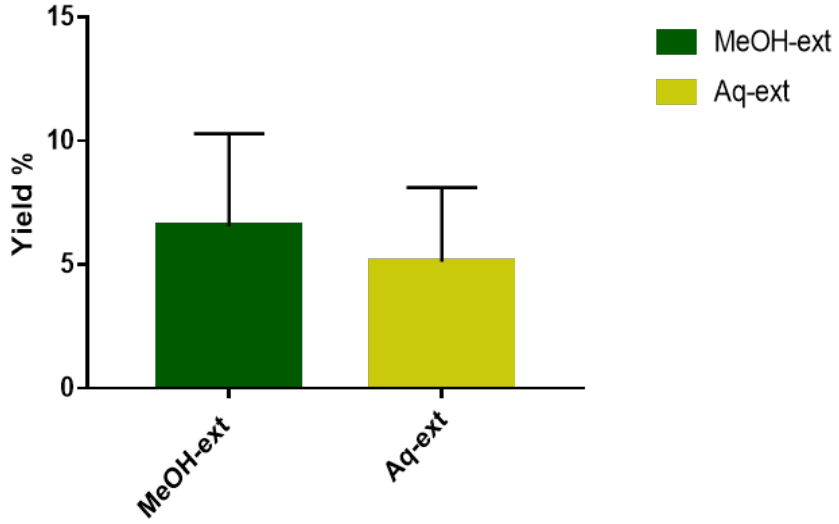


Fig. 11. Aqueous and methanolic yields of *Tuberaria lignosa* leaves extracts. Data are represented as mean ± SD of multiple replicates

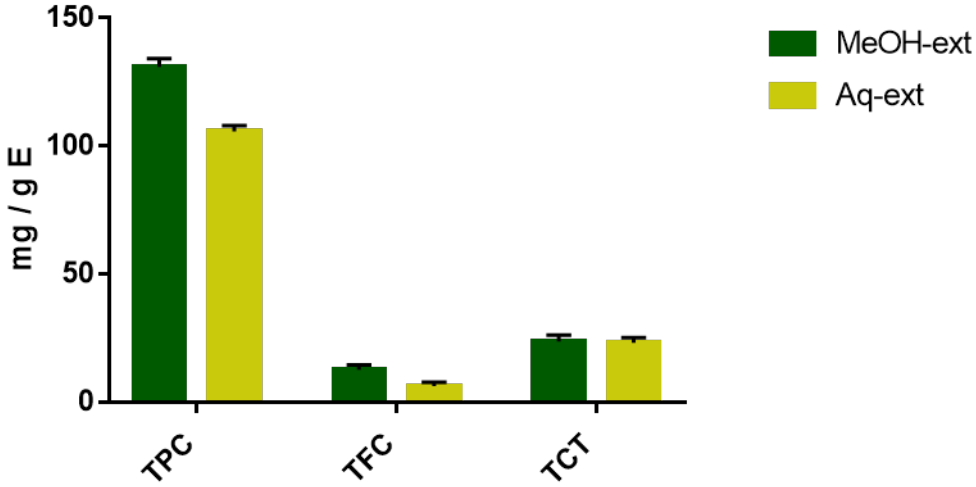


Fig. 12. Mean values of total phenolic, total flavonoid and tannins contents of *Tuberaria lignosa* aqueous and methanolic extracts. Data are represented as mean ± SD of three replicates.

III.1.3.2. HPLC analysis

Sajid et al. in 2012 study appraises the antioxidant and antimicrobial attributes of various solvent extracts (absolute methanol, aqueous methanol, absolute ethanol, aqueous ethanol, absolute acetone, aqueous acetone and deionized water) from different parts of *Pongamia pinnata (L.) Pierre* , the HPLC analysis was only performed for aqueous methanol which was established to be the most effective solvent to recover higher amounts of phenolics compared with other solvents.

The phenolic profile of wild *Tuberaria lignosa (sweet sample)* is shown in table 7. The data obtained from HPLC-DAD analysis (retention time) was used for the identification of fifteen phenolic compounds, including ten hydrolysable tannins and five flavonoids. Ellagitannins were the major compounds found in the *T. lignosa* sample as previously reported by Pinela et al. (2012). Both categories of hydrolysable Tannins (gallotannins and ellagitannins) were present in the sample extract. Gallotannins consist of a glucose molecule in which hydroxyl groups are partly or completely substituted with galloyl groups, or form an ester with hexahydroxydiphenoyl (HHDP) group (Sandhu and Gu 2010), and ellagitannins were consisting of Punicalagin derivates. As it is shown in Fig. 13 & 14, peaks 1 and 2 (Rt; 3.84 ; 4.59) were assigned to hexahydroxydiphenoyl-(HHDP)-glucose (Sandhu and Gu 2010), whereas the ellagitannins were characteristic of peaks 3, 9, 10. Peaks 4, 5, 7, 8, and 13 were tentatively identified as derivatives of gallotannins. Flavonoids were also found in the studied samples. Peak 6 might correspond to the 3,5-diglucoside peonidin, peaks 11, 12, 14 and 15 were respectively identified as epicatechin gallate, kaempferol rutinoside, Kaempferol-p-coumaroylglucoside-glutarate and Kaempferol-p-coumaroylglucoside. Benoudina et Harieche (2018), investigation about Algerian *Tuberaria lignosa* plant, proved the presence of the Kaempférol 3-O-(3",6"-di-O-E-p-coumaroyl)- β-D-glucopyranoside in the hydroalcoholic extract of the areal part.

Barros et al. (2013), by using HPLC, determined that *C. ladanifer* fresh leaves contain similar phenolic compounds of Galloyl glucose, Punicalin, Punicalagin gallate and Kaempferols. While Lukas et al. (2021), found that *Cistus creticus L.* extract contain also the two punicalagin derivatives (punicalagin and punicalagin gallate). The obtained results were comparable with those reported by Barrajón-Catalán et al. (2011), who determined the polyphenolic composition in several *cistus* genus species; *Cistus ladanifer, C. salviifolius , C. populifolius , C. laurifolius ,C. libanotis and C. clusii,* were the species showing four ellagitannins in commun with our findings (punicalin; punicalagin; punicalagin gallat and Hexahydroxydiphenoyl-glucose). Epicatechin derivates were present only in *C. albidus, C. salviifolius* and *C. incanus* samples, while kaempferol 3-O-rutinoside was only detectable in the *C. incanus* sample. Generally, *T. lignosa* polyphenol profile was similar

to those of *C. ladanifer* and *C. populifolius* which exhibited low flavonoids content, but were very rich in ellagitannin.

Differences in chemical profiles of *T. lignosa* comparing to Pinela et al. (2012) estimation could be justified by ElNaker et al. (2021) findings which explain the lyophilisation and oven-drying effect on phytochemical compounds abundance and chemical transformations of metabolites in plant extracts. The metabolites most involved in discriminating between samples were 10 to 20-fold more abundant in the lyophilized extracts compared to the oven dried extracts, suggesting that prolonged exposure to moderate temperatures has been shown to cause thermal degradation leading to reduced phenolic content and the antioxidant activity. In addition to heat, the chemical transformation of metabolites can be promoted by humidity and oxygen. Open-air-drying leads to moisture absorption, which directly affects the plant metabolites degradation rate (Cortés-Rojas et al. 2016). Open-air-drying also leads to the formation of free radical oxygen species that can interact with electron donors (phenolic compounds) leading to oxidative degradation (Damak et al. 2008). Furthermore, oven drying resulted generally decrease the glycosylated phenolics and flavonoids.

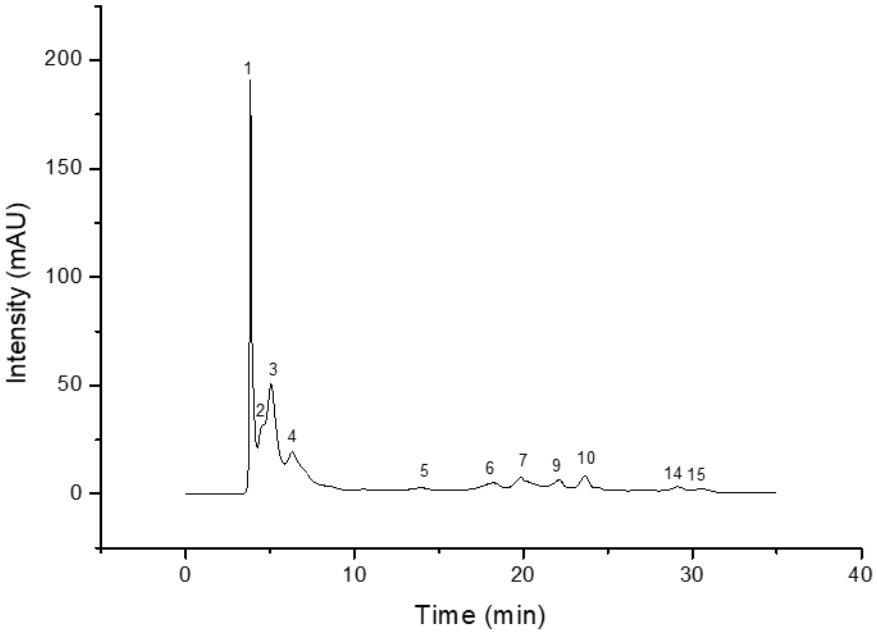


Fig. 13. HPLC profile of phenolic compounds in the *Tuberaria lignosa* sample, recorded at 280 nm

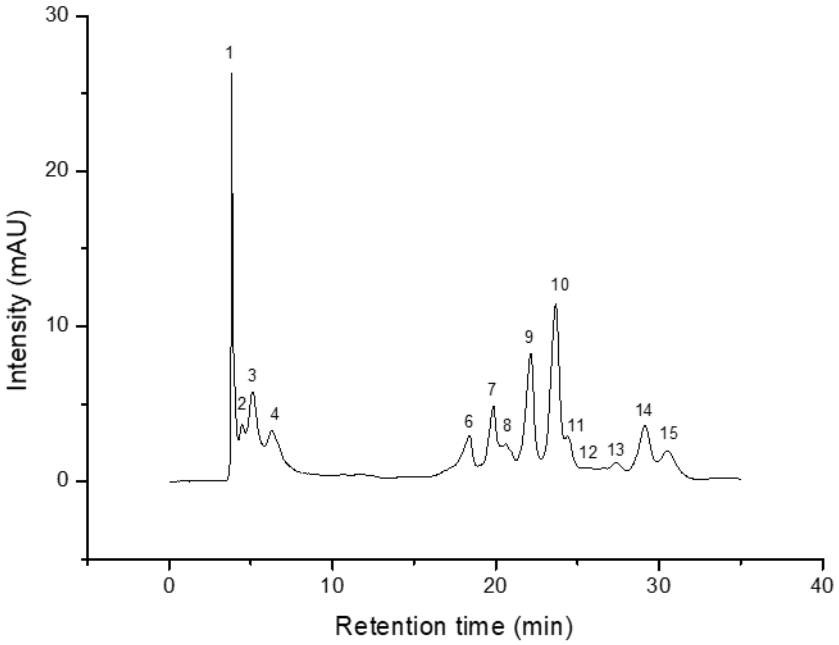


Fig. 14. HPLC profile of phenolic compounds in the *Tuberaria lignosa* sample, recorded at 340 nm

Table 7. Retention Times data of phenolic compounds in *Tuberaria lignosa* leaves determined by HPLC-DAD-UV

Peak	*Rt (min)* *340/28*	*Peak area*	*Tentative identification*	*Class*
1	3.8416	1980480	HHDP-glucose	Tannins
2	4.5916	644060	HHDP-glucose	
3	5.1166/5.066	2297563	Punicalin	
4	6.28333/6.31667	1427084	Monogalloylglucose	
5	13.975	74852	trigalloylglucose	
7	19.858/19.85	449126	Trigalloylglucose	
8	20.625	94647	HHDP-digalloylglucose	
9	22.1333/22.125	449126	Punicalagin isomer 2	
10	23.666/23.6666	269048	Punicalagin gallate isomer 2	
13	27.4	56997	Pentagalloylglucose Gallotannins	
6	18.4/18.2166	273697	3,5-diglucoside peonidin	Flavonoids
11	24.40	2697	epicatechin gallate	
12	25. 37	668	kaempferol rutinoside	
14	29.1333/29.1166	119543	Kaempferol-p-coumaroylglucoside-glutarat	
15	30.48333/30.51166	102382	Kaempferol-p-coumaroylglucoside	

III.1.4. Antioxidant activity

The results for the antioxidant potential of aqueous and methanolic *T. lignosa* leaves extracts, using TAC, FRAP and DPPH assays are summarised in table 8. The DPPH radical scavenging activity is reported in terms of IC_{50} and EC_{50} (µg extract.ml^{-1}). Thus, lower values represent higher antioxidant activity. In this sense, the most active extract was the methanolic extract, followed by BHT, then the aqueous infusion (IC_{50}/ EC_{50}: 21,31/20,01; 36,17/32,92; 182,86 / 169,13 µg.ml^{-1}, respectively).

Comparing these results with that described by Lavoura (2018), which an EC_{50} value was around 141,77 µg.mL^{-1} obtained from 5 min infusion and 308,19 µg.ml^{-1} of methanolic extract, using fresh *T. lignosa* leaves. It can be concluded that the dried leaves used in this work resulted a lower EC_{50} value in methanolic extract. However, the aqueous extract for fresh leaves indicated a greater power of free radical inhibition compared to our dried leaves. This difference can be explained by the fact that the content of water in fresh leaves acts as a co-solvent extracting a portion of polar antioxidants in the solvent bulk and thus leading to better anti-radical activity (Bampouli et al. 2014).

Moreover, Benoudina et al. (2018), used dried Algerian T. *lignosa* ariel parts, they reported an IC_{50} of 11,93 µg.ml^{-1} testing the ethanolic crude extract capacity.

Comparing with the same family species such as *Helianthemum* genus, the aqueous leaves extract has shown a higher antioxidant potential (EC_{50}; 29,88 to 44,01 µg.ml^{-1}) than our *T. lignosa* aqueous leaves extract. However, the methanolic extracts presented EC_{50} ranges from 29,76 to 66,2 µg.ml^{-1} under the tested conditions by Rubio-Moraga et al. (2013). Furthermore, Chouikh et al. (2015) showed that the methanol extracts of *H. lippii* during flowering had lower significant efficacy on DPPH radicals (IC_{50}; 61,49 µg.ml^{-1}).

The reducing power of methanol extract increased from 0,17 at 60 µg.ml^{-1} to 1,83 at 1,000 µg.ml^{-1}. These values (OD) were significantly higher than those of the aqueous extract (0,03 at 60 µg.ml^{-1} to 0,62 at 1,000 µg.ml^{-1}).

As shown in table 8, the aqueous extract showed a ferric ion reducing capacity of 0,31 mM Fe^{2+}.g^{-1} and the methanolic extract showed the highest value of 2,63 mM Fe^{2+}.g^{-1}. As for our *T. lignosa* leaves extracts, showed a reducing power confirming the previous studies of (Pinela et al. 2012) on *Tuberaria lignosa* sample, as well as the Cistaceae samples capacities reported by Rebaya et al. (2015) and Amensour et al. (2010).

The valuation of the total antioxidant capacity of the *T. lignosa* extracts from the leaves using the phosphomolybdenum process was expressed in mg AA. g^{-1} of extract (Table 8). The methanolic

extract showed a higher TAC (with 128,84±12,32 mg AA.g^{-1}) than the aqueous extract (62,64± 12,27 mg AA.g^{-1}). When compared with Moroccan *Halimium halimifolium* leaves TAC values (with 289.85 mg AA. g^{-1} of aqueous extract and 280.22 mg AA g^{-1} ethanolic extract) (El gamouz et al. 2022), our results showed a lower capacity. The TAC values would be validated by the content of the major antioxidant molecules; polyphenols, tocopherols, ascorbate of samples. Accumulating evidence has demonstrated that the antioxidant capacity *in vitro* testing a food component, occurs through highly specific mechanisms rather than direct antioxidant effects. Polyphenols are a very important example of these indirect antioxidant effects, and their antioxidant action measured with TAC methods has driven various misleading health claims (Fraga et al. 2014).

Table 8. The phenolic contents and antioxidant activity of aqueous and methanolic extracts of *T. lignosa* leaves

	TPC mg GAeq.g^{-1}E	TFC QE. g^{-1}E	TCT mgCE.g^{-1}E	TAC mgAAE.g^{-1}E	FRAP MmFe^{2+}.g^{-1} E	DPPH IC_{50} µg.ml^{-1}	DPPH EC_{50} µg.ml^{-1}
Aq-E	105,65 ± 2,3	6,29 ±1,46	23,14 ± 2,01	62,64 ± 12,27	0,31 ±0,30	182,86 ±1,7	169,13 ±1,65
MeOH-E	130,96 ± 3,28	12,68±1,9	23,73 ± 2,49	128,84 ±12,32	2,63±0,18	21,31 ±1,67	20,01 ±1,53
BHT				-	-	36,17±0,72	32,92±0,73
AA				-	1,74±0,60	-	-

III.1.5. Antimicrobial activity

III.1.5.1. Strain identification

The strains used in the antimicrobial activity were isolated at Ibn Rochd hospital CHU- Annaba. However, the identification using standard system **(Annex 6)** and antibiogram had to be confirmed. So, *E coli* strains were sub-cultured on EMB agar **(Annex 8)** , *Staphylococcus sp* were sub-cultured on Chapman agar **(Annex 7)**. The Hektoen agar was used to culture *Salmonella sp* (green colonies with a dark black centre), MacConkey agar was used to subculture *Proteus sp* (beige colonies) and *Klebsiella sp* (pink colonies).

The determination of biochemical characteristics is essential for the accurate identification of bacterial genera and species, so two API: API 20E and 20 staph were used. The strains were identified by Excel identification software; API 20E+ and API 20 Staph (version 4.1). The test results are reported in tables 9 & 10. Three *Proteus mirabilis sp* were purified and identified by Mm GHENAIET Khaoula (PhD student in LBMA laboratory, biochemistry department. University of Badji Mokhtar ANNABA).

III.1.5.2. Antibiotic susceptibility test

Antibiotic sensitivity of the tested pathogenic isolates revealed them as sensitive or resistant (Tables 11 & 12). As it is observed for *Staphylococcus sp*; 100% were resistant to Tetracycline, Erythromycin, Oxacillin, Penicillin G and Fusidic acid, while 75% were susceptible to Clindamycin and Pristinamycin. Thus, we assume the tested *Staphylococcus sp* are multidrug-resistant**.** As it is shown in table 12, *E coli* strains were 100% susceptible to Trimethoprim, 66,6% susceptible to both Fosfomycin and Ofloxacin, also 66,6% resistant to Cefalotin, Cefazolin and Imipenem**.** *Proteus sp* isolates were susceptible to Fosfomycin (75 %), Ofloxacin and Trimethoprim (50%) but highly resistant to Cefalotin, Cefazolin and Imipenem (100%). *K. pneumoniae* was susceptible only to Ofloxacin and Trimethoprim. And *S. choleraesuis* was highly resistant to all test antibiotics.

Table 9. Results of identification tests with the API 20E gallery

	E.coli			*Proteus sp*	*Salmonella sp*	*Klebsiella sp*
ONPG	+	-	+	-	+	-
ADH	+	-	-	-	+	-
LDC	+	+	+	-	+	+
ODC	+	-	+	-	+	-
CIT	-	-	-	+	+	+
H2S	-	-	-	+	+	-
URE	-	-	+	-	+	+
TDA	+	+	-	+	+	-
IND	+	+	+	+	-	+
VP	-	-	-	-	-	+
GEL	+	+	-	+	+	+
GLU	+	+	+	+	+	+
MAN	+	+	+	-	+	+
INO	-	-	-	-	-	+
SOR	+	+	+	-	+	+
RHA	+	+	-	+	+	+
SAC	-	+	-	+	+	+
MEL	+	+	+	-	+	+
AMY	-	-	-	-	-	-
ARA	+	+	+	+	+	+
OX	-	-	-	-	-	-
NO2	+	+	+	+	+	+
ident	*Escherichia coli 1*	*Escherichia coli 1*	*Escherichia coli 1*	*Proteus vulgaris*	*Salmonella choleraesuis ssp arizonae*	*Klebsiella pneumoniae ssp pneumoniae*
Prob%	*0,989*	*0,996*	*0,999*	*0,987*	*0,996*	*0,935*

Table 10. Results of identification tests with the API 20Staph gallery

Tests	*Staphylococcus sp*			
GLU	+	+	+	-
FRU	+	+	+	+
MAN	+	+	+	+
MAL	+	+	+	+
LAC	+	+	+	-
TRE	+	+	+	-
MAN	+	+	+	+
XLT	-	-	-	+
MEL	-	+	-	-
NIT	-	-	+	-
PAL	+	+	+	-
VP	+	+	+	+
RAF	-	-	-	-
XYL	-	-	-	-
SAC	+	+	+	-
MDG	-	-	-	+
NAG	+	+	+	-
ADH	+	+	+	+
URE	-	-	-	+
LSTR	+	-	-	+
ident	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus capitis*
Prob%	*0,966*	*0,953*	*0,971*	*0,901*

Table 11. Antibiogram of *Staphylococcus sp* used in the antimicrobial activity.

ATB	*Symbol*	*Disk Content µg*	*Critical diameters* R <(mm)*	*S. aureus 1*	*S. aureus 2*	*S. aureus 3*	*S. capitis*
Fusidic acid	FA	10	24	R	R	R	R
Clindamycin	CD2	2	19	S	S	S	R
Penicillin G	P	6	18	R	R	R	R
Pristinamycin	PT	15	19	S	S	S	R
Tetracycline	TE	30	20	R	R	R	R
Erythromycin	E	30	19	R	R	R	R
Oxacillin	OXC	1	20	R	R	R	R

Table 12. Antibiogram of bacterial pathogens isolated at Ibn Rochd hospital CHU- Annaba

ATB	*Symbol*	*Disk Content µg*	*Critical diameters* R <(mm)*	*E. Coli 1*	*E. Coli 2*	*E. Coli 3*	*P. mirabilis1*	*P. Mirabilis2*	*P. Mirabilis3*	*P. vulgaris*	*S. choleraesuis*	*K. pneumoniae*
Fosfomycin	FOS	200	24	R	S	S	R	S	S	S	R	R
Cefalotin	CF	30	15	S	R	R	R	R	R	R	R	R
Cefazolin	CZ	30	15	S	R	R	R	R	R	R	R	R
Ofloxacin	OFX	5	22	S	R	S	R	R	S	S	R	S
Imipenem	IMI	5	20	S	R	R	R	R	R	R	R	R
Trimethoprim	TMP	5	14	S	S	S	R	R	S	S	R	S

[T]According to ('CASFM2019_V2.0_MAI.Pdf' n.d.; 'WDI_1993_7_n2_p68-78.Pdf' n.d.)

Table 13. Antibacterial activity of *T. lignosa* aqueous extract against pathogenic Gram positive and negative bacteria

Gram	*Test organism*	*Positive control Gentamicin*	*Inhibition Zone (mm)*			*MIC*	*MBC*	*MBC/ MIC*
			50 mg/ml	100 mg/ ml	150 mg/ml			
Negative	*Escherichia coli ATCC 8739*	33±2	R	11±1	13,00±2	0,78125	3,125	4
	Escherichia coli ATCC 25922	31,43±1.15	10±1.36	11,67±0.58	11,33±0.58	1,5625	6,25	4
	Escherichia coli1	30±0.87	13±1	12,33±2.08	15,33±1.53	0,78125	1,5625	2
	Escherichia coli 2	25±1.36	R	9,67±0.58	10,33±1.53	1,5625	1,5625	1
	Escherichia coli 3	30±0.90	10±0.58	12,00±1	15,00±1	3,125	6,25	2
	Proteus mirabilis1	23±2.65	15,67±1.15	17,00±1	18,33±1.53	1,5625	1,5625	1
	Proteus mirabilis2	35±0.58	13±1	14,00±	15,67±0.58	1,5625	3,125	2
	Proteus mirabilis3	38,67±1.53	16±1	18,00±1	18,00±1	1,5625	3,125	2
	Proteus vulgaris	36,67±1.53	17±0.5	18,00±1	18,00±1	3,125	6,25	2
	Salmonella typhimurium ATCC 14028	30±1	8±1.36	9,33±0.58	9,67±0.58	1,5625	1,5625	1
	Salmonella enterica	32±0.58	12±1	12,00±2	15,00±0.58	1,5625	6,25	4
	Klebsiella pneumoniae	32±0.90	R	9,00±1.36	14,00±1.15	1,5625	3,125	2
	Enterococcus faecalis ATCC 29212	32±1	12±1.36	9,00±1.53	15,33±0.58	0,78125	3,125	4
	Pseudomonas aeruginosa ATCC 27853	21,5±1.48	R	9,00±2	11,00±1	0,78125	3,125	4
Positive	*Staphylococcus aureus ATCC 43300*	20±0.87	17±1	19,00±2.65	13,67±0.58	1,5625	1,5625	1
	Staphylococcus albus ATCC 8799	30±1.15	9±1,36	10,33±0.58	19,33±2.52	1,5625	6,25	4
	Staphylococcus aureus ATCC 25923	30±1.53	15±1.36	12,00±2.65	14,33±1.15	1,5625	6,25	4
	Staphylococcus aureus 1	33,67±1.73	14±1	17,00±2.65	16,00±2	1,5625	3,125	2
	Staphylococcus aureus 2	32±0.5	13,67±0.58	14,00±1	14,33±1.53	1,5625	6,25	4
	Staphylococcus aureus 3	32±0	9,33±1.15	11,33±0.58	12,33±1.15	1,5625	3,125	2
	Staphylococcus aureus 4	30±1	10,67±0.58	10,67±0.58	9,00±1.15	1,5625	6,25	4

Table 14. Antibacterial activity of *T. lignosa* methanolic extract against pathogenic Gram positive and negative bacteria

Gram	*Test organism*	*Inhibition Zone (mm)*			*MIC mg/ml*	*MBC mg/ml*	*MBC/MIC*
		50 mg/ml	100 mg/ ml	150 mg/ml			
Negative	*Escherichia coli ATCC 8739*	10,00±1	11,67±0.58	14,33±0.58	0,78125	0,78125	1
	Escherichia coli ATCC 25922	9,33±0.58	10,33±0.58	10,67±0.58	0,78125	0,78125	1
	Escherichia coli 1	12,67±0.58	15,33±1.53	13,67±2.08	1,5625	1,5625	1
	Escherichia coli 2	10,33±0.58	10,00±1	12,00±1.73	3,125	3,125	1
	Escherichia coli 3	9,00±1.15	11,00±2	11,33±0.58	0,78125	1,5625	2
	Proteus mirabilis 1	15,00±1	16,77±0.68	23,33±1.53	0,78125	0,78125	1
	Proteus mirabilis 2	15,00±1.73	18,00±1	22,33±1.15	0,78125	0,78125	1
	Proteus mirabilis 3	18,67±0.58	21,00±2.65	20,33±1.53	0,78125	0,78125	1
	Proteus vulgaris	19,67±0.58	22,00±1	22,00±2	3,125	3,125	1
	Salmonella typhimurium ATCC 14028	R	10,33±1.53	13,00±1	1,5625	1,5625	1
	Salmonella enterica	R	11,33±0.58	13,00±0.58	1,5625	1,5625	1
	Klebsiella pneumoniae	9,00±1	11,00±1	12,33±1.53	1,5625	6,25	4
	Enterococcus faecalis ATCC 29212	12,00±0	12,67±0.58	15,67±0.58	1,5625	1,5625	1
	Pseudomonas aeruginosa ATCC 27853	9,00±1	12,00±1.15	15,00±1.53	0,78125	0,78125	1
Positive	*Staphylococcus aureus ATCC 43300*	14,00±1	17,33±0.58	15,33±1.15	1,5625	1,5625	1
	Staphylococcus albus ATCC 8799	9,83±0.29	16,00±1.73	12,33±2.08	0,78125	1,5625	2
	Staphylococcus aureus ATCC 25923	18,00±5.57	16,33±0.58	15,67±3.79	1,5625	1,5625	1
	Staphylococcus aureus 1	18,67±0.58	19,33±1.15	22,67±0.58	1,5625	3,125	2
	Staphylococcus aureus 2	17,67±0.58	18,67±0.58	21,00±1	0,78125	0,78125	1
	Staphylococcus aureus 3	8,67±1.15	10,33±1.53	15,33±2.08	1,5625	1,5625	1
	Staphylococcus aureus 4	11,33±1.15	12,33±0.58	17,67±0.58	1,5625	1,5625	1

The antibacterial capacity of the tested extracts is reflected in the appearance or absence of an inhibition halo around the discs. The largest inhibition zone was observed with the methanolic extract of *T. lignosa* leaves (150 mg.ml^{-1}) against *Proteus mirabilis 1* with an inhibition zone of 23 mm as shown in Table 14. The tannin-rich plant extracts have attributed the inhibition of microbial growth due to their content in gallotannins and related compounds, which mainly act on the membranes of the bacteria and/or their ability to complex metal ions (Maisetta et al. 2019). Gallotannins have been reported to inhibit bacterial growth through a variety of mechanisms including binding cell surface molecules including lipotoichoic acid and proline-rich cell surface proteins and inhibiting glucosyltransferase enzymes (Hutchings and Cock 2018). Meanwhile, the aqueous extract (150 mg.ml^{-1}) showed the highest efficacity against *staphylococcus albus* ATCC 8799 with an inhibition zone of 19 mm (Table 13). This can be justified by the hypersensitivity of the *Staphylococcus aureus* strain, the Gram-positive bacteria are devoid of an outer membrane making the organism more sensitive to external environmental changes, such as temperature, pH and natural extracts (Balentine et al. 2006; Labiod 2016).

At the lowest concentrations of 50 mg.ml^{-1}, it has been noted resistance from *Salmonella typhimurium* ATCC 14028 and *Salmonella enterica* against Met. E. Furthermore, *Escherichia coli* ATCC 8739, *Escherichia coli* 2, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* were not sensitive to the Aq. E neither. These results confirmed the evidence that methanol is a better solvent for more efficient extraction, this behavior is probably due to the capacity of methanol to solubilise bioactive compounds contained in tested plant.

The Gram$^+$ bacteria were more sensitive to the antimicrobial action of *T. Lignosa* extracts than Gram$^-$ strains, which is in agreement with the majority of previous works. In fact, Gram$^-$ bacteria have an intrinsic resistance to biocides agents, which is related to the nature of their bacterial wall. In Gram$^+$ bacteria, the peptidoglycan is very thick and associated with exposed parietal proteins and polysaccharide structures (lipoteichoic acids and teichoic acids). However, in Gram$^-$ bacteria, the peptidoglycan is very thin and associated with a complex outer envelope defining a periplasmic space. This outer membrane is an asymmetric hydrophobic lipid bilayer made up of phospholipids, proteins (porins) and lipopolysaccharides (LPS). The periplasmic space is filled with enzymes that degrade complex substances so they can cross the cytoplasmic membrane, and inactivate toxic chemicals (antibiotics). The resistance of Gram$^-$ bacteria to glycopeptides and macrolides is due to the inability of these molecules to cross the outer membrane (Boukhatem et al. 2014; Labiod 2016).

Aligiannis et al. (2001) classified the antibacterial activity of plants extracts based on them

minimal inhibitory concentrations: for a MIC up to 0.5 $mg.ml^{-1}$, it is considered as strong activity, a MIC between 0.6 and 1.5 $mg.ml^{-1}$ it is a moderate activity or weak activity if the MIC is above 1.6 $mg.ml^{-1}$.

The MIC results showed that the *T. lignosa* extracts generally have a moderate capacity. Except the weak effect of Aq.E against *Escherichia coli* 1 and *Proteus vulgaris*, as for Me.E against *Escherichia coli 2* and *Proteus vulgaris*. These differences in sensibility could be due to the nature and level of the antimicrobial agents present in each extract and their mode of action on the tested bacteria. As shown in table 13 and 14, *T. lignosa* extracts are generally qualified as microbiostatic so it is able to inhibit the growth of bacteria.

III.1.6. Antifungal activity

The antifungal activity of *Tuberaria lignosa* was not yet been reported. Thus, direct contact assay was performed to determine the sensitivity of *Candida albicans* strain to the aqueous and methanolic extract of *Tuberaria lignosa* leaves by measuring the growth inhibition (%) as shown in table 15. Both *T. lignosa* extracts at concentrations of 6.25, 12.5, 25 and 50 $mg.ml^{-1}$ showed antifungal activity and exhibited a total inhibition of *Candida albicans* mycelial growth (Fig 15). The minimal inhibitory concentration is noted in table 15 (MIC of $\leq$ 6.25 $mg.ml^{-1}$). These findings concurred with those of Karim et al. (2016), who reported that phenolic extracts of various *Cistus* species (Cistaceae) had distinctive fungistatic effect (100% germination inhibition) at a concentration of 5 mg/ ml. Also, the *C. ladanifer* extract acted as inhibitor against four Candida species, the MIC values varied from 0.5 to 5 mg. ml^{-1} (Barros et al. 2013). Domenico Rongai et al. (2019) showed that there is a correlation between punicalagins content and the percentage of mycelial growth. This is in accordance with D. Rongai et al. (2018), who reported that punicalagins can form pore-like structures in which the inner wall is formed by the glucoside unit, and the outer wall is formed by the lasting moieties including the ellagic acid, all of which may alter the physiological transmembrane gradients and lead to cell death. In another study of Barros et al. (2013) showed that hydrolysable tannins tri and di-galloyl HHDP glucose were the main groups of phenolic compounds, which might be related to the stronger fungicide effect. According to Fadda et al. (2021) the inhibition of fungi growth was positively correlated with galloyl-HHDP glucose. Furthermore, kaempferol was found to have high antifungal potential as reported by Rocha et al. (2019). This can justify the successful growth inhibition in our test.

This result shed light on the great ability of *Tuberaria lignosa* and provides only a direction for further researches to evaluate the antimicrobial activity of its extracts and compounds.

Table 15. The inhibition percentage at various concentrations and the minimum inhibitory concentrations of aqueous and methanolic extracts of *T. lignosa* leaves against Candida albicans

Concentrations (mg/ml)	*6.25*	*12.5*	*25*	*50*	*MIC (mg/ml)*
Methanolic Extract	100	100	100	100	≤6.25
Aqueous Extract	100	100	100	100	≤6.25

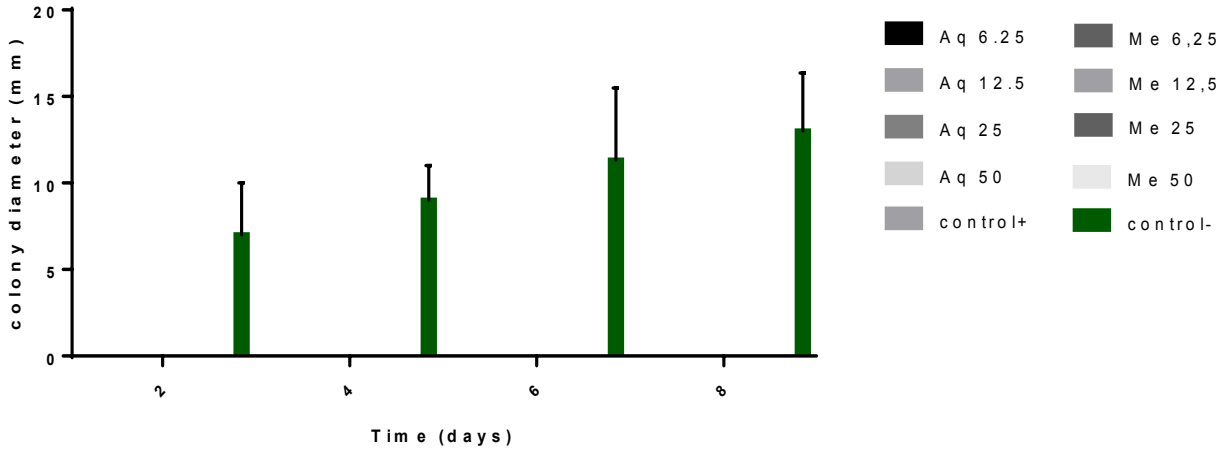


Fig. 15. Effect of aqueous and methanolic extracts at different concentrations from *T. lignosa* leaves on Candida albicans fungal colony growth. Results are presented as the growth of colony (diameter in mm) during 8 days plants. Data are represented means ± SD of 3 independent experiments. Control - : Untreated control , Control + : Synthetic fungicide

III.2. *Crataegus azarolus* Polysaccharides:

III.2.1. Chemical analysis

The fruit water-soluble polysaccharide yielded a fraction of about 2,19 %, composed of 85.58% total carbohydrates, 4.26% of protein content and also contained polyphenols, which represented 5.34% (Fig. 16). The values were in the range observed in other water-soluble polysaccharides extracted from *Artocarpus heterophyllus Lam*. Pulp (Zhu et al. 2017). However, our results are in discrepancy with the reported levels of *Crataegus azarolus* polysaccharide composition (Rjeibi et al. 2020), due to the different extraction processes that have been used.

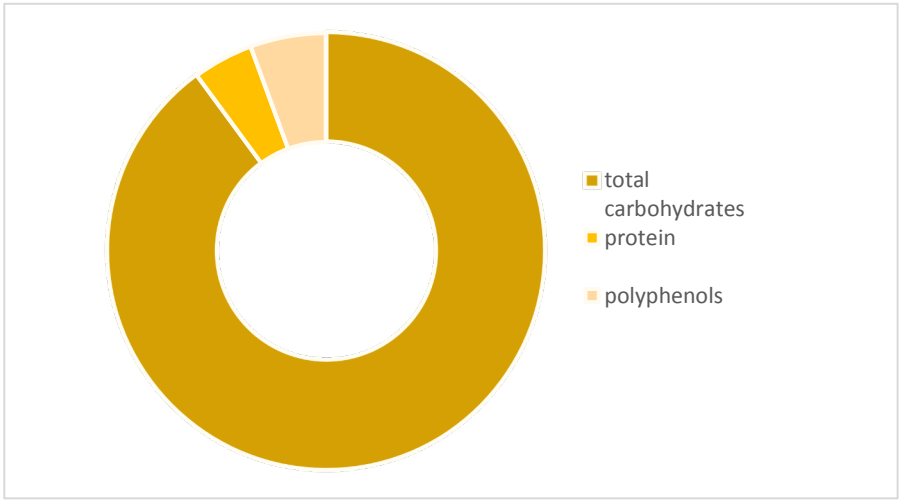


Fig. 16. Chemical composition of AZWSP

III.2.1. 1. AZWSP monosaccharides composition

The pure AZWSP hydrolysates were analysed by thin-layer chromatography (TLC), the results indicated that it was an heteropolysaccharides, composed of glucose, galactose, mannose and rhamnose as shown in Fig. 17.

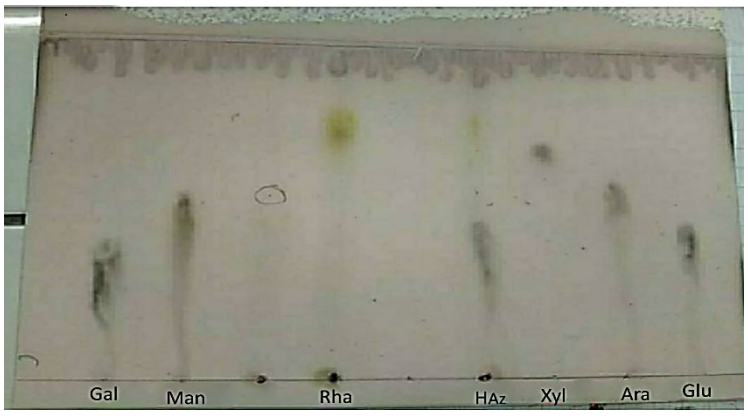


Fig. 17. TLC analysis of 1% AZWSP hydrolysate (HAz: polysaccharide hydrolysate; as controls. Gal: galactose, Man: mannose, Rha: rhamnose, Xyl: xylose, Ara: arabinose Glu: glucose).

III.2.2. Structural characterisation

III.2.2.1. Fourier transform infra-red characterisation

The FT-IR profile of *C. azarolus* polysaccharides illustrated the characteristic functional chemical groups (Fig. 18) showing peaks at 3285, 2930, 2855, 1735, 1604, 1418, 1333, 1243, 1145, 1012, 863,817, and 777 cm^{-1}. Similar findings were observed, although not identical results to *Taxus Chinensis var. mairei* (Wu et al. 2015) and *Crataegus pinnatifida Bunge* (Chen et al. 2019) IR analysis. The broadly-stretched intense characteristic peak at 3285 cm^{-1} was assigned to O-H stretching vibration of bonded and non-bonded hydroxyl groups and water (Chen et al. 2019), the peaks at approximately 2930 were related to the C–H unsymmetrical Stretching vibration (Wu et al. 2015; Xiaojuan Xu et al. 2012). Each specific polysaccharide has a particular specific band in the 1,800–800 cm^{-1} region (Szymanska-Chargot and Zdunek 2013). According to Wu et al. (2015), Sara et al. (2020) and Silverstein et al. (2007); the peak spectrum 2855 cm^{-1} corresponds to -CH3 stretching. The absorbance peaks at 1735 and 1604 cm^{-1} correspond to acetyl groups indicating C=O stretching. Three intense bands at 1418, 1333 and 1245 cm^{-1} correspond to C-O stretching and C-H or O-H bending. The bands between 1145 and 1012 cm^{-1} correspond to C-O-C and C-OH stretching. The second important area for the preliminary examination of a spectrum is the region between 900 and 650 cm^{-1}. The bands at 863 cm^{-1}, 817 cm^{-1} and 777 cm^{-1} are respectively attributed to β-configuration (axial bonding of C-H of carbon-1) and α-configuration (equatorial bonding of C-H of carbon-1) and pyranose-ring stretching. These results indicated that AZWSP possessed a typical polysaccharide pattern behaviour.

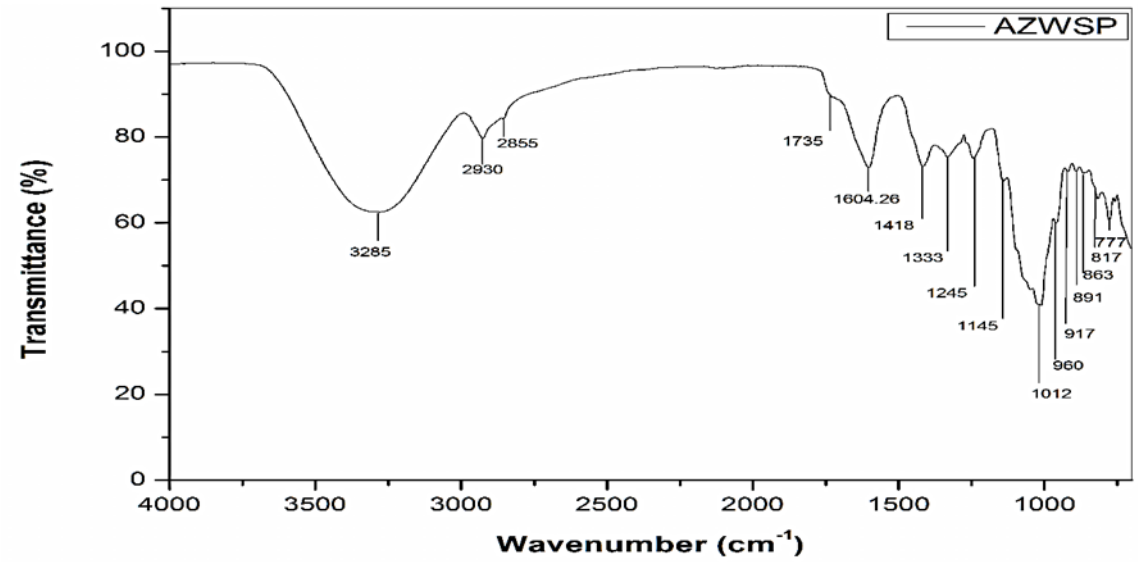


Fig. 18. FTIR Spectrum of the AZWSP

III.2.2.2. Nuclear Magnetic Resonance

The chemical shifts of NMR were calculated by MestReNova (version 5.3.1). The ^{1}H and ^{13}C NMR spectrums were shown in Fig.19 [a] and [b]. The peak at around δ 4.66 ppm was attributed to the chemical shifts of HOD. The anomeric proton signals at δ 5.30, 5.12, 5.04, and 4.98 were, respectively, attributed to α-D-Gal*p*, α-L-Rha*p,* 1,4 linked β-D-Man*p*;1,4,6 linked β-D-Man*p*. Meanwhile, the peak at σ=4.5 ppm, interferes with the HOD signal, so it can be attributed to the residues 1,4 linked β-D-Glc*p* and/or 1,4,6 linked β-D-Glc*p* because the protons are not fully disclosed in this region of the spectrum. This result was consistent with literature data (Jones and Mulloy 1993; Habibi et al. 2004). The middle region of the spectrum (3.3 – 4.5 ppm) identifies the signals of the protons of the carbon skeleton. In the high fields, the signal at σ=1.94 ppm indicates the presence of the acetyl group in ionic form ($O\text{-}AC^{-}$) and the peak at σ 1.17 ppm refer to the rhamnose methyl. Furthermore, interference may exist between the anomeric proton signal of the 2-O-acetyl 1,4 linked Man*p* residue and that of the α-L-Rha*p*. Thus, the recording conditions of this spectrum do not allow the H-2 peak of 2-O-acetyl 1,4 linked β-D-Man*p* detection (Hua et al. 2004). Signals in 13C NMR spectra of *C. azarolus* were assigned and identified as possible, based on literature values (Bock and Pedersen 1983; Wang et al. 2010) **(Annex 12)**. For technical reasons, the ^{13}C NMR spectrum of AZWSP emerged between 58 and 110 ppm (Fig. 19 [b]). The resonance of the non-substituted D-mannosyl unit of C-4 is sensitive to the nearest neighbour plus more structural details (Manzi et al. 1986). This situation appears on our NMR ^{13}C spectrum in the region 63-63.5 ppm by the presence of a shoulder. This indicates that the studied polymer has an irregular structure in the distribution of the substituents on the main chain.

Also, the signals from the anomeric carbons are of weak intensity, except the signal at σ= 96.5 ppm (C-1 of 2-o-acetyl 1,4 linked Man*p*) and the signal at σ=99 ppm 1,4,6 linked β-D-Man*p*. This signal weakness has been observed often with macromolecules, representing irregular side chain distribution. This is the case of certain β-D-glucans that consist of a main chain β-1,3 with β-1,6 branches; in hairy zones, the bonds of certain residues tend to disappear from the spectrum (Aouadi et al. 1992). Overall, the ^{13}C NMR spectrum of *Crataegus azarolus* shows similarities with the spectrum of *Dendrobium nobile Lindl* (Wang et al. 2010).

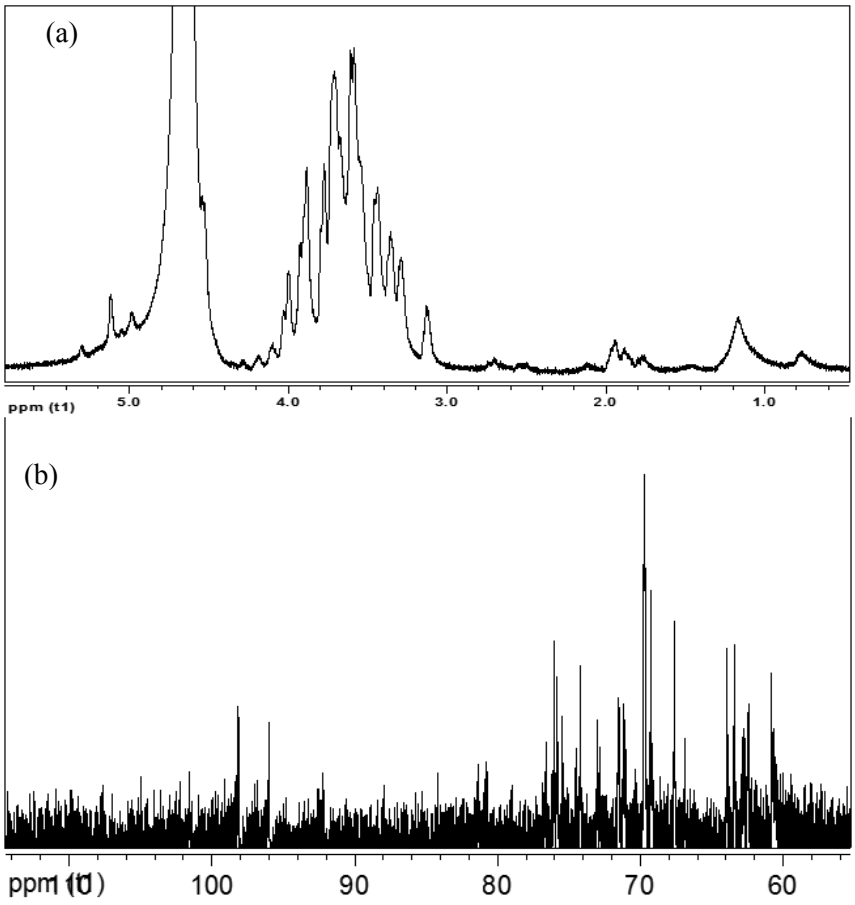


Fig. 19. NMR spectra of AZWSP in D_2O. (a) ^{1}H NMR spectra, (b) ^{13}C NMR spectra.

III.2.2.3. MALDI-TOF-MS spectroscopy

MALDI-TOF-MS spectroscopy is a convenient tool for polysaccharide structure analysis (Ding et al. 2016) and has been used to detect the main connectivity of AZWSP in this study. As seen in Fig. 20, the oligosaccharide composition is represented by Hex for anhydrohexose residues (m/z = 162.14) or Pent for anhydropentose residues (m/z = 132). Considering that oligosaccharides will exist in the form of sodium (m/z = 22.99) or potassium (m/z = 38.96) cationized substances such as $[M + Na]^+$ and $[M + K]^+$(Mele and Malpezzi 2000). Additional molecular weight of acetyl group (m/z = 42) can be obtained (Nakahara et al. 2014), the peak at 815.8 m/z is derived from the dominant ion corresponds to Hex_4Ac_3 as [m/z = 815.6 = (162.14 × 4 + 18) + 42×3+22.99].Peaks at 2540.7, 1659.8 and 546.8 can correspond to Hex_{15} Ac_2 [m/z = 2539.1 = (162.14 × 15) + 42 × 2 + 22.99], Hex_{10} [m/z = 1660.4 = (162.14 × 10) + 38.96], and Hex_3 [m/z = 543.4 = (162.14 × 3 + 18) + 38.96], respectively. Therefore, the glucomannan of *C. azarolus* presents an irregular structure of the acetyl group distribution.

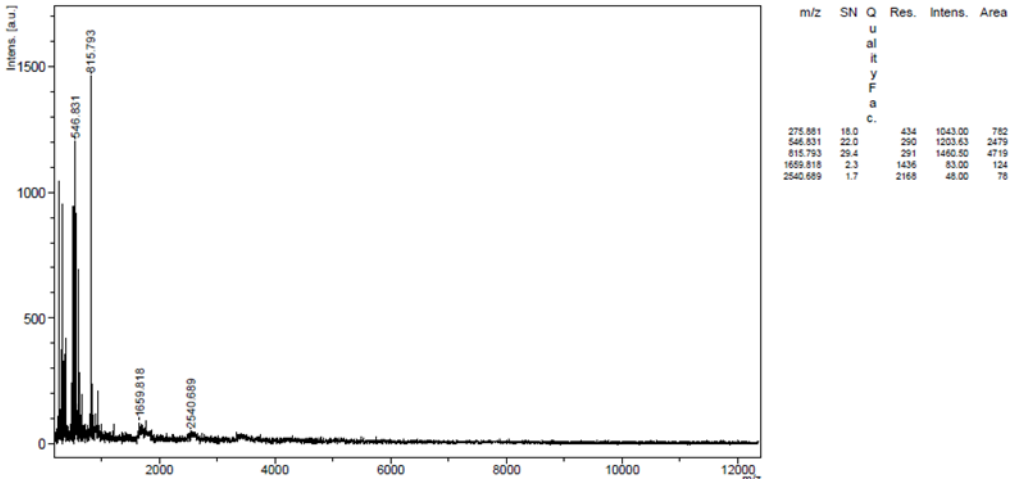


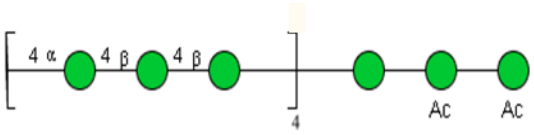
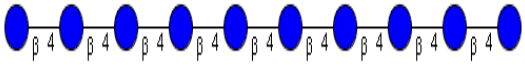
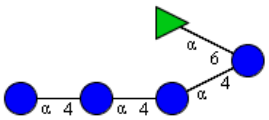
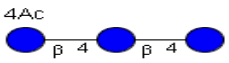
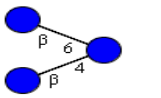
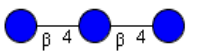
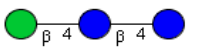
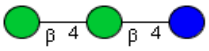
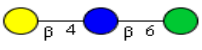
Fig. 20. MALDI TOF of the AZWSP

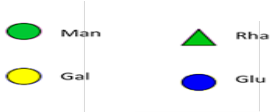
III.2.2.3.1. Glycoworkbench:

Glycoworkbench is a software tool designed for glycan structure determination based on MS data, moreover, it can be used for rapid structure drawing too. Comparing mass to charge of each peak and fragments intensity. Each mass spectre was matched to the structure in GlycomeDB database as it is presented in table 16, except the peak at 2540 m/z which was drawn and mass to charge estimated using Glycoworkbench.

Based on the above comprehensive analysis by TLC, MALDI-TOF MS, FT-IR and NMR, the structure of AZWSP is a novel heteropolysaccharide; It was mainly a glucomannan with a backbone consisting of 1,4-β-Man*p*, 1,4,6-β-Man*p*, 1,4-β-Glc*p* and 1,4,6-β-Glc*p* branched at C-6 substituted by T-α-Gal*p*. To the best of our knowledge, the structure of the fruit polysaccharide reported in this paper is completely different from the previous studies.

Table 16. Structure compositions matchings AZWSP SM peaks using Glycoworkbench softwar

Mass to charge	Ion m/z	Intensity	Ion		Structure
2540.689	2539.830	48.0	Ac2Hex15	Na	
1659.818	1663.543	83.0	Hex10	Na	
815.793	815.302	1460.5	Hex4dHex	H	
546.831	549.202	1203.63	Ac1Hex3	H	
275.881	276.081	1043.0	Hex3	2Na	
					
					
					
					
					



III.2.3. Antioxidant properties

The DPPH scavenging capacity of yellow hawthorn polysaccharide is presented in Fig. 21 using butylated hydroxytoluene (BHT) and ascorbic acid (AA) as positive controls. The results revealed a noticeable effect on scavenging DPPH free radicals in a concentration-dependent manner. At 0.1 $mg.ml^{-1}$, the scavenging activity of ascorbic acid was the strongest around 91,15 %, followed by butylated hydroxytoluene which was 72,09%, while AZWSP's scavenging ability was only 54,24%. Previous studies of the crude polysaccharide extracted from *Crataegus azarolus* pulps indicated that the DPPH radical scavenging rate was 83.2% at 4 $mg.ml^{-1}$ (Rjeibi et al. 2020). While our results attained maximum values of 73,82% at 2 $mg.ml^{-1}$. The experimental DPPH scavenging ability values (IC_{50}) for the tested substances AA, BHT and AZWSP were 3.48 ± 0.262 (Justino et al. 2018), 36.17 ± 0.727 (Xia et al. 2014) and 99 ± 2 $\mu g.ml^{-1}$ (Lin et al. 2015; Surin et al. 2020; Wang et al. 2012), respectively.

The scavenging ability of AZWSP on hydroxyl radicals were in the range of 32,19– 87,54 % for the concentration range of 0,025 – 0.4 $mg.ml^{-1}$ as demonstrated in Fig 21. The IC_{50} value of extracted polysaccharide was 0,106 ±0,003 $mg.ml^{-1}$, in agreement with previous studies (Tian et al. 2020). However, AA reached a better inhibition rate; where the IC_{50} was 0,015±0,001 $mg.ml^{-1}$. This result illustrated that *C. azarolus* polysaccharide exhibited a hydroxyl radical scavenging activity.

Lipid peroxidation is an oxidation of polyunsaturated fatty acids, which results in the formation of Lipid free radicals, it may affect the quality of all food containing lipids on storage (Duthie, 1993). Hence, it is significant to determine the effect of polysaccharides on the lipid peroxidation phenomenon. The extract and ascorbic acid abilities increased with increasing concentrations. The ascorbic acid had a strong anti-lipid peroxidative effect, while the sample exhibited moderate activity (IC_{50} value of 1,219 ± 0,226 $mg.ml^{-1}$). Therefore, the polysaccharides in *Crataegus azarolus* fruit break the chain reaction of lipid peroxidation.

The antioxidant potential of the polysaccharide was estimated from their ability to reduce the ferric ion Fe (III) to the ferrous ion Fe (II). The sample showed steadily increasing reducing activities as the respective increased concentrations. The ferric reducing ability of AZWSP was 592,58 ± 3,32 $\mu m\ Fe^{2+}.\ g^{-1}$ E, similar to IOP4 polysaccharide fraction from *Inonotus obliquus* (Huang et al. 2012)

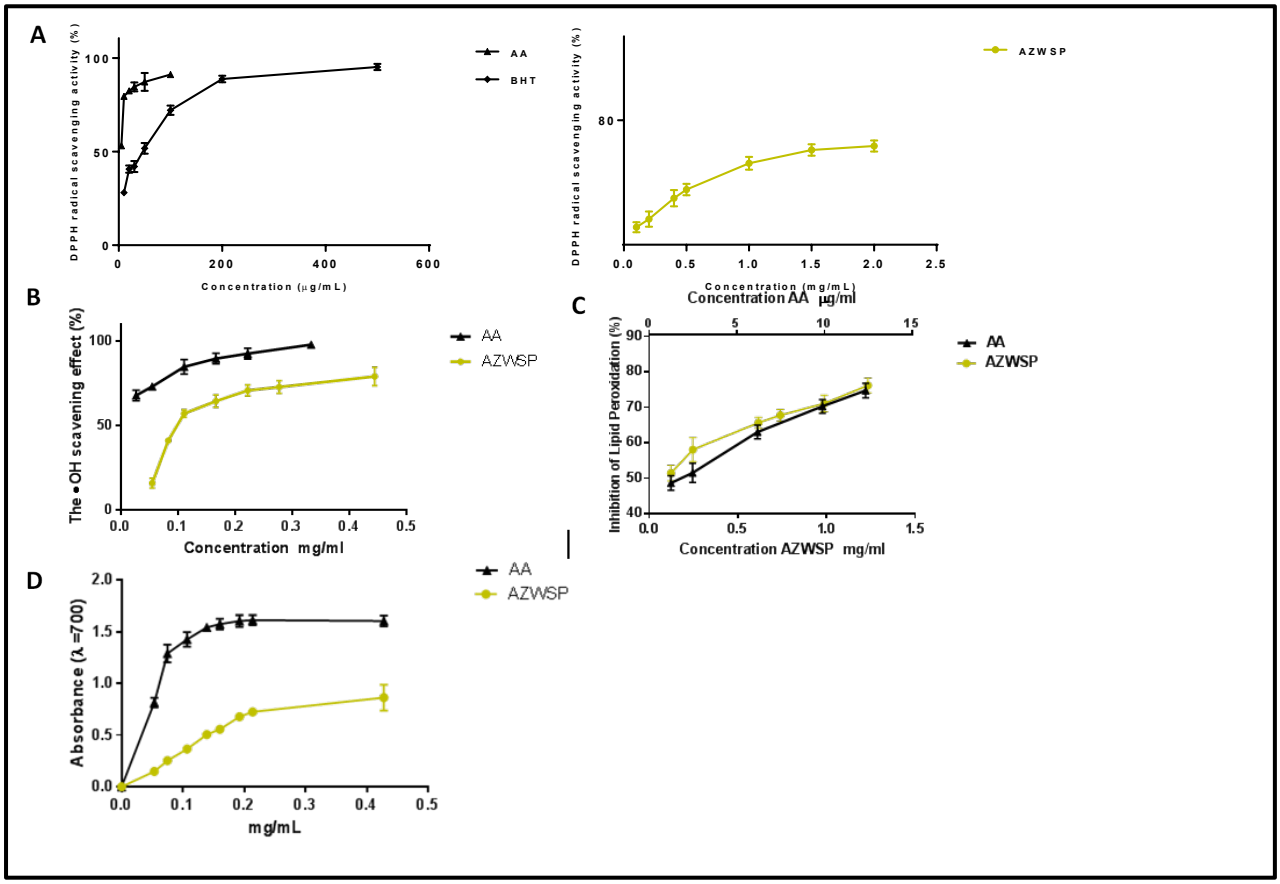


Fig. 21. Antioxidant activity analysis of AZWSP with various methods: (A) DPPH radical scavenging activity of AZWSP; (B) hydroxyl radical scavenging activity of AZWSP; (C) Inhibition of lipid peroxidation of AZWSP; (D) Correlation between the AZWSP concentrations and absorbance of reducing power. Each value represents the mean ± SD (n = 3).

These findings implied that *C. azarolus* fruit polysaccharides might behave as electron or hydrogen donators to scavenge free radicals. It is well known that polysaccharides' biological activity is affected by their structural characteristics, such as chemical composition, molecular weight, type and conformation of glycosidic bonds. The differences in sources, extraction procedures and drying techniques affect the physicochemical properties, structure or polysaccharides conformation. That may lead to differences in antioxidant activity (Wang et al. 2016). Thus, the high antioxidant abilities may be due to the presence of mannose, this latter exhibits a quite high scavenging activity. Kozarski et al. (2012) evidenced that antioxidant ability increases with mannose and rhamnose increased concentrations. Furthermore, Wang et al. (2007) found that water-soluble heteropolysaccharides composed of Ara, Glc, Gal and GlcA may be associated with strong reactive oxygen species scavenging ability. Methylation and acetylation have been proven to enhance the antioxidant capacity of polysaccharide, it exhibits significantly stronger scavenging activity and

reducing ability compared to unmodified polysaccharides. The introduction of these substituents in polymer results in weaker dissociation energy for hydrogen bonds. So, the hydrogen donating ability of the polysaccharide derivative is increased (Wang et al. 2016). Our results are in accordance with Yuanfeng et al. (2012) studies, shown a high antioxidant potential of tea polysaccharide fraction, which had low protein and polyphenol content, so other factors than the polyphenolic compounds were concerned; like carboxyl groups. Overall, the antioxidant capacity of polysaccharides is not determined by a single factor, but by a combination of distinct related factors.

III.2.4. Prebiotic properties

III.2.4.1. Resistance to digestion

The non-digestible carbohydrates, due to their chemical structure, are not absorbed in the upper gastrointestinal tract or hydrolysed by human enzymes. That ensures they reach the colon and continue throughout the large intestine, to stimulate mainly the probiotics in the gut (Gibson and Roberfroid 1995). The resistance of yellow hawthorn polysaccharide to hydrolysis in artificial human gastric juice was shown in Fig. 22, the inulin was used as a reference showing higher acid resistance abilities and gave maximum hydrolysis of 0,95%, 1,19%, 1,37%, 1,52%, 2,53% at pH 5, 4,3,2 and 1 , respectively after 6h (Nithya Bala Sundari et al. 2020). While the degree of hydrolysis for AZWSP was 3,58%, 3,89%, 7,10%, 13,70% and 16,45% at the same pH order. The hydrolysis degree increased with the decreased pH values of gastric juice. Usually, food remains in the human stomach under acidic conditions for approximately 2 hours. The pH rises to around 5 in a further section of the gastrointestinal tract (Wang 2009). Therefore, when AZWSP are ingested, more than 83% of it would reach the intestine. Our findings are consistent with that represented by Kimteck (2016) who reported that more than 85 % of mushroom water-soluble polysaccharide was resistant to acid hydrolysis and reached the colon.

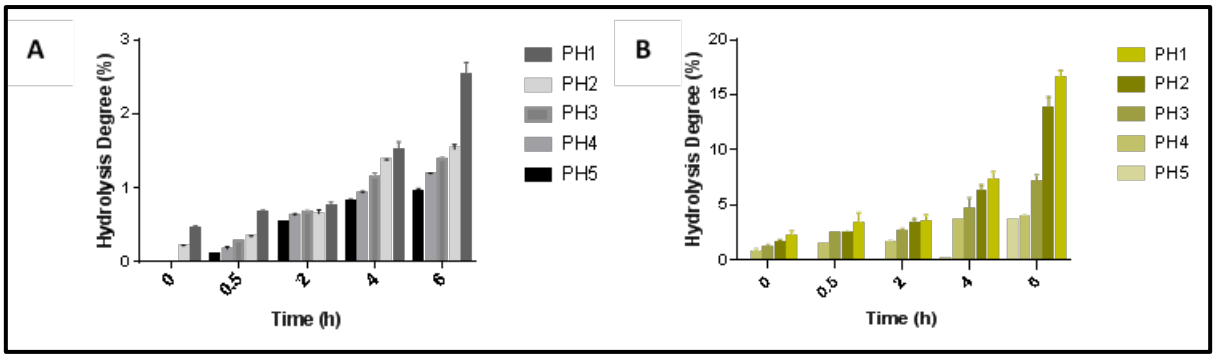


Fig. 22. Hydrolysis degree (%) of (a) Inulin and (b) AZWSP by artificial human gastric juice; incubated at 37°C for 6 hours. Each data point is an average of three independently replicated experiments. Error bar represents standard deviation of the mean (n=3).

III.2.4.2. Growth stimulation of probiotics

The fermentation of AZWSP as a prebiotic candidate was studied *in vitro* anaerobic growth assays, the effect on the selected safe *Lactobacillus sp* (ϒ hemolysis) proliferation was performed for 48h of incubation at 31 ±1°C. As indicated in Fig. 23, the growth of all tested strains was almost not observed in a sugar-free medium (negative control), whereas it was significantly improved in the presence of glucose (positive control). The growth of all tested *Lactobacillus sp*; *Lactobacillus paracasei* V1, *Lactobacillus rhamnosus* V2, *Lactobacillus plantarum* V3, *Lactobacillus paracasei* C, *Lactobacillus rhamnosus* E and *Lactobacillus pentosus* Rc, in 1% AZWSP medium varied between 0,19 and 1,24 (OD_{625}). These results are comparable to those measured with already reported polysaccharides as prebiotics; Galactoglucomannan from Spruce (Polari et al. 2012), homogeneous glucan from *Gastrodia elata* (Huo et al. 2021) and Galactoglucomannan from Pinewood (Rajani et al. 2016).

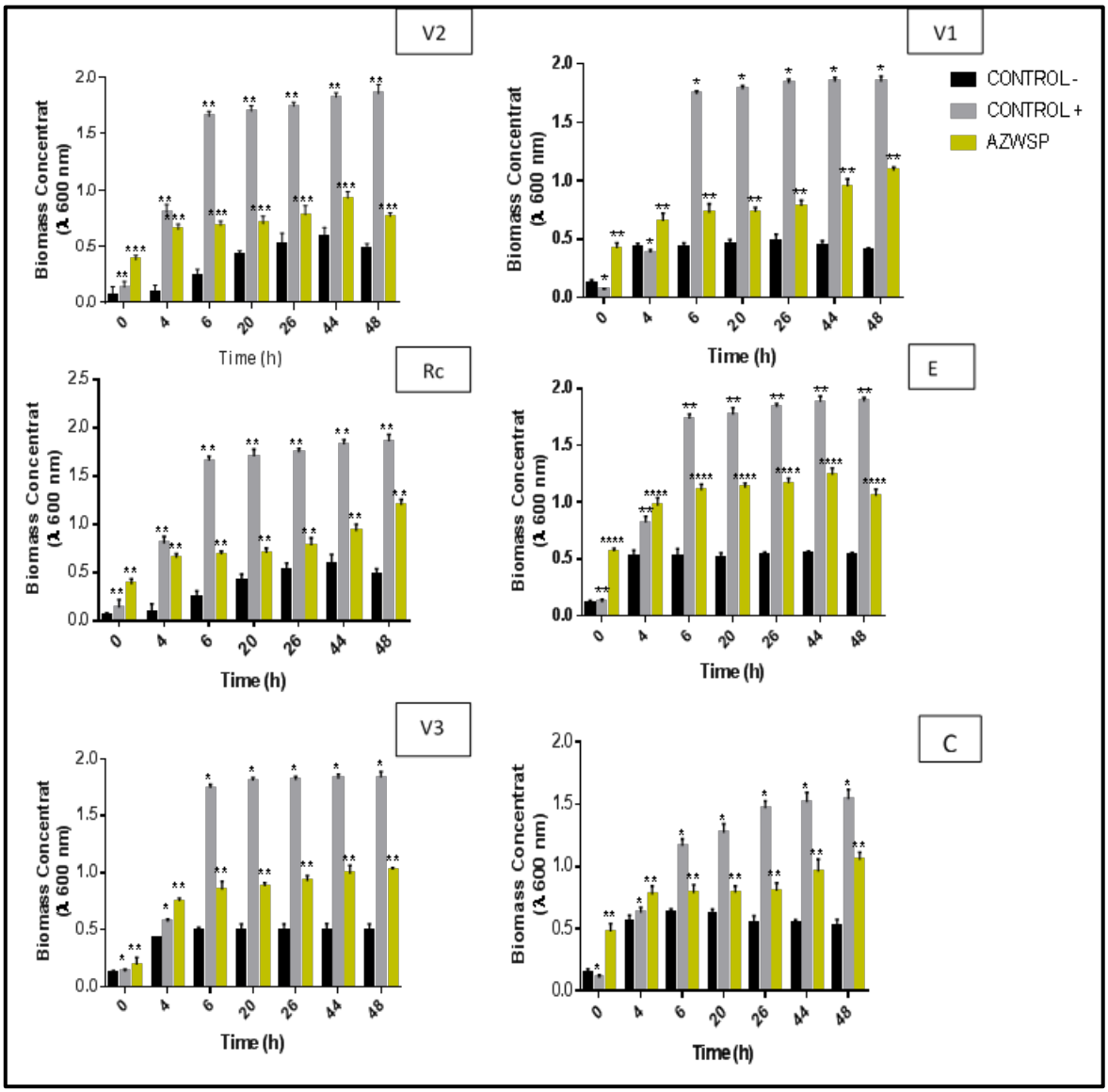


Fig. 23. Growth of selected *Lactobacillus* bacteria in 1% AZWSP medium during 48h incubations. Bars represent the average of tree replicate samples (mean ± SD) based on OD_{625} values, blank value subtracted. Glucose as positive control and the AZWSP are compared to negative control (sugar-free medium). For statistical analysis, a significant difference: AZWSP and positive control versus negative control. α= 0.05 (95% confidence interval): **** p<.0001; *** p<.001; ** p<.005; * p <.05 and no*: not significant.

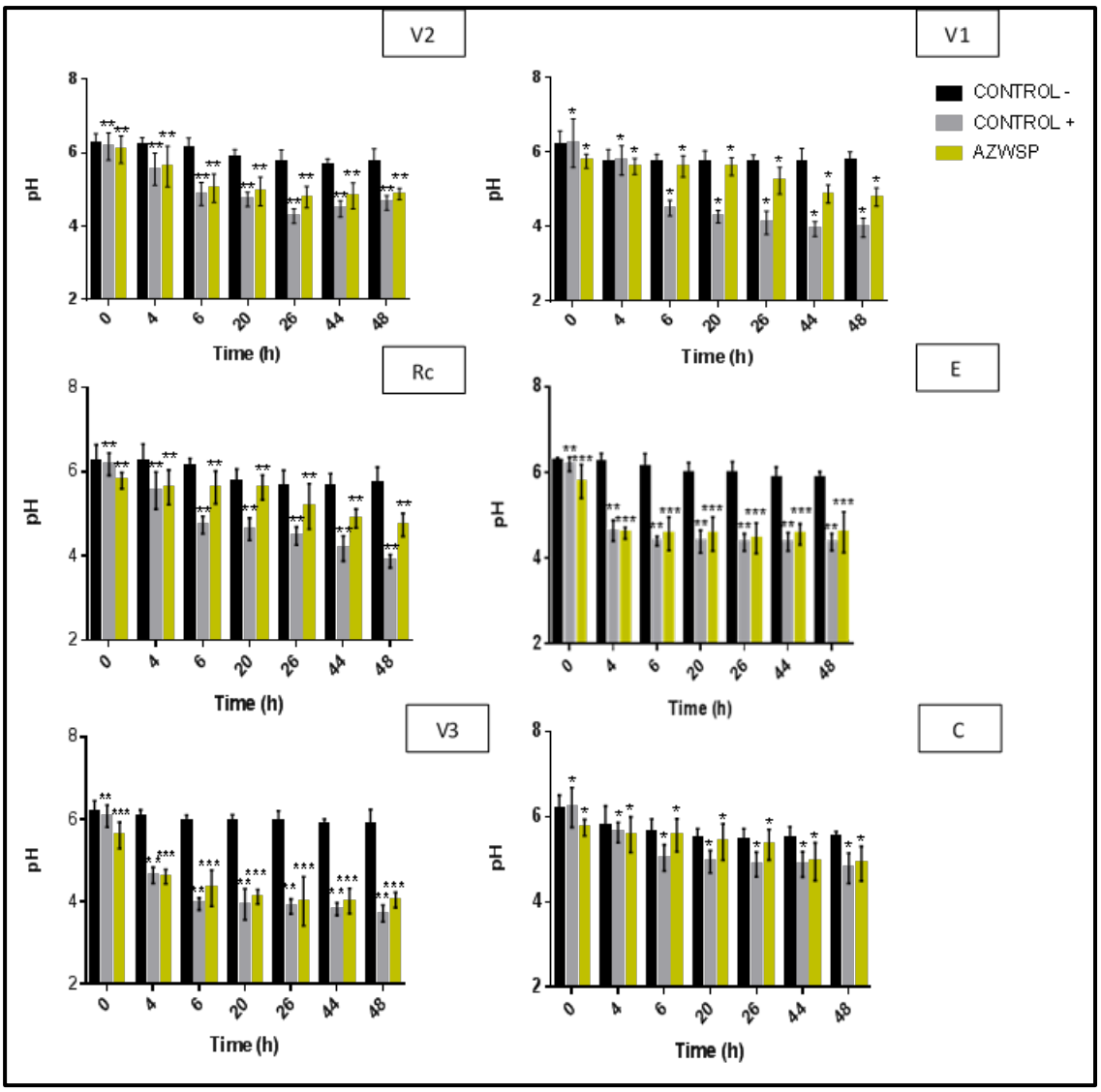


Fig. 24. The pH changes during 48H fermentation with selected Lactic acid bacteria. Bars represent the average of three replicate samples (mean ± SD). Glucose (positive control) and AZWSP are compared to negative control. For statistical analysis, AZWSP and positive control versus negative control. Significant difference: AZWSP and positive control versus negative control. α= 0.05: *** p<.005; ** p<.01; * p<.05; and no*: not significant.

Moreover, the organic acid produced during the fermentation lowered the pH levels and increased the titratable acidity of the fermenting medium. The highest absorbance reading had the lowest pH value. For example, the pH fell from 5,79 to 4,60 for *L. rhamnosus* E (Fig. 24) and the same was noticed with the rest of strains. So, we assume that the acidic side products of glucomannan fermentation affect pH values of in vitro assays and prevent the growth of specific bacteria.

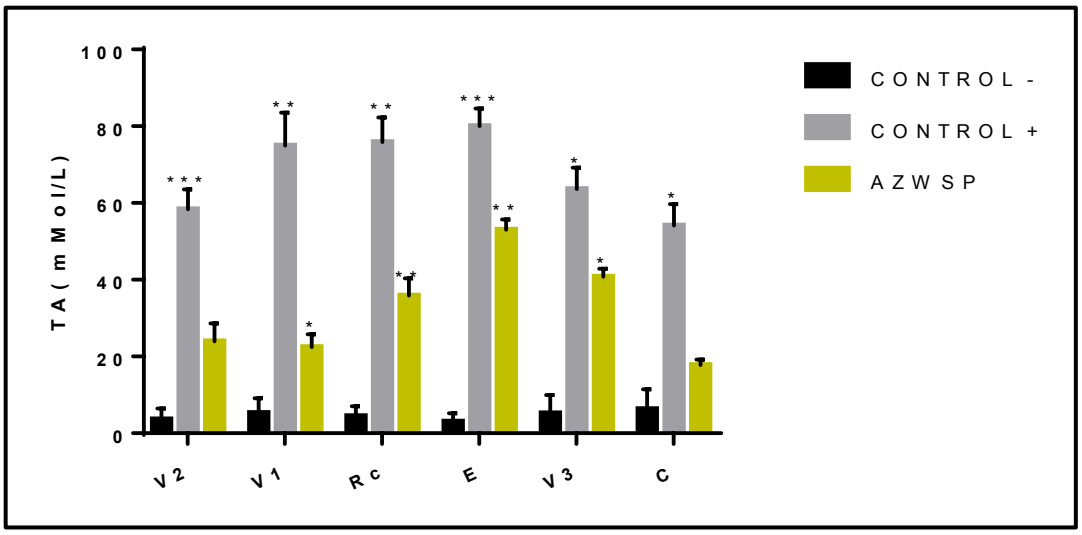


Fig. 25. Titratable acidity changes (mMol.l^{-1}) after 48 h fermentation. Significant differences versus negative control (***p<.0002; ** p<.001; * p<.002).

Fig. 25 shows that the titratable acidity value increased substantially at the end of the 48h fermentation, the highest TA value in the AZWSP fermentation was obtained with *Lactobacillus rhamnosus* E which has been deemed to have the better sensory ability. Similar pH variations related to LAB's produced acidity were reported by Thakur et al. (2018).

Generally, the media with AZWSP supported the growth of all tested strains. Hence, it seems that yellow hawthorn polysaccharide was a suitable substrate as a carbon source for the growth and development of *Lactobacilli sp*. Various *in vitro* experiments suggested that reduction in pathogenic colonisation may be related to a possible reduction in intestinal pH due to intestinal fermentation of supplemented treatments. The combination of these probiotic bacteria with AZWSP as symbiotic food and the use of their synergistic effects may have interesting prospects.

III.3. Lactic acid bacteria

III.3.1. Phenotypic and biochemical identification

Seven lactic acid bacteria obtained from various food sources were isolated on MRS agar, they were all Gram-positive, catalase and oxidase negative **(Annex 9)**. Purified LABs were identified by API 50 CHL (API system BioMérieux, France) assay **(Annex 6)**. Using Identification2011 Excel (API50 CHL 5.1) the isolates were identified as *Lactobacillus paracasei spp paracasei3* (V1), *Lactobacillus rhamnosus* (V2), *Lactobacillus plantarum 1* (V3), *Lactobacillus rhamnosus* (B), *Lactobacillus paracasei spp paracasei 3* (C), *Lactobacillus rhamnosus* (E), *Lactobacillus pentosus* (Rc).

This group of bacteria is divided into homofermentative and heterofermentative types according to the end products from glucose metabolism. Homofermentative strains convert glucose to lactic acid primarily via the Embden-Meyerhof pathway, while heterofermentative LABs transform glucose into lactic acid, carbon dioxide and ethanol or acetic acid via the 6-phosphogluconate pathway. As shown in table 17, all the isolates were heterofermentative except E strain.

The growth ability is an important physiological characteristic of LAB. All tested strains exhibited good growth at 37 and 45°C but did not grow at 15°C. Overall, the isolated strains were thermophilic and had very good growth characteristics over a wide temperature range, consistent with previous findings (Gupta 1996; Toba et al. 1991; Hébert et al. 2000; Svetoslav Dimitrov Todorov et al. 2017). According to Coulibaly et al. (2008), the bacterial capability to grow at high temperature indicate an increased rate of growth and lactic acid production, at the same time the high fermentation temperature decreases contaminations.

In order to exert their beneficial effects on the host, LAB must remain alive during ingestion and reach the large intestines. They have to pass through the stressful conditions of a low pH gastric acid environment as well as the exposure of high sodium chloride concentrations. All the isolates could grow at pH 4, this ability was attributed to the high tolerance of Lactobacillus strains to free acid (H^+). LABs are acidophilic but while that means a tolerance to low pH, it should be differentiated from a condition of high concentration of free acids (H+), because the latter may cause growth inhibition (Amrane and Prigent 2001). On the other hand, all the isolates, except B, showed high alkali tolerance (pH 9.5). The observed results correlate with reports of Todorov et al. (2007). Moreover, the B strain could be survived under the alkaline pH without any visible growth. We assume that there is a correlation between the plant environment (lactobacilli origin) and the strain's alkali tolerance.

Lactic acid bacteria are generally tolerant to high salt concentrations, which help initiate acid-producing metabolism (Aswathy et al. 2008) and further inhibit the growth of non-desirable organisms in pickled food products. Sodium chloride tolerance is an indicator of the strain's osmotolerance level. During industrial fermentation, when the cells produce lactic acid, alkali is pumped into the broth to prevent the pH from dropping too much. Therefore, the free acid will be converted to its salt form, which in turn will increase the osmotic pressure of the cell. Therefore, LAB strains with high osmotolerance would be interesting industrial strains (Adnan and Tan 2007). All seven LAB isolates could carry on well through a sodium chloride concentration of up to 4%. A further increase in Sodium chloride concentration resulted a marked reduction in the growth and survival of the isolates V2, B, and E (*L. rhamnosus*). The rest tested strains could tolerate up to 6.5% Sodium chloride.

Although these assays cannot predict patterns of behaviour in the human body, the results are valuable in demonstrating that *Lactobacillus sp* could survive in a variety of stressors, similar to other potential extraintestinal probiotic bacteria. The results of the tolerance studies are shown in Table 17.

The hemolytic activity of isolated strains was based on red blood cell lysis, a green zone was noticed around the B colonies (α-hemolysis). However, none of the other strains showed α- nor β-hemolysis so they showed ϒ hemolytic activity and were considered safe (to be used on test II.2.7).

III.3.2. Antibiotic Susceptibility

Antibiotic resistance in microbial communities has become a health concerning issue even for beneficial bacterial species. The genetic elements carrying resistance genes are mobile and transferable to pathogens. Moreover, exposure to antibiotics may allow bacteria to develop different mechanisms to counteract the bactericidal effect, including an intrinsic (innate) and acquired resistance mode (Imperial and Ibana 2016). Imperial and Ibana reported that LAB strains have intrinsic resistance to bacitracin, vancomycin, kanamycin, teicoplanin and quinolones. Office of Food Additive Safety document (Smith 2017) mentioned that the acquired clindamycin resistance in *lactobacillus* has been demonstrated to be transferable but mediated by conjugation of mobile elements encoding the resistance, and only when resistance is encoded within the erythromycin resistance gene (Wang et al. 2006). So, based on this finding it is important to determine if the LAB resistance is intrinsic or extrinsic (acquired). As shown in table 18, a susceptibility against six antibiotics was checked **(Annex10)** , our results were in agreement with (Wang et al. 2022) assays showing that all tested probiotics were susceptible to erythromycin. The Oxacillin (1 µg) was

effective against all strains except *L. pentosus* RC. Consistently, no resistance to Penicillin (10 units) was detected except with *L. plantarum* V3. According to Halder et al. (2015) the chances of transferring a low level of resistance (intermediate susceptibility) are limited because such resistance is intrinsic and not plasmid mediated, thus, Clindamycin (2 µg) intermediate resistance observed with *L. rhamnosus* V2, *L. plantarum* V3, *L. paracasei* C, *L. rhamnosus* E and *L. pentosus* Rc doesn't carry on any safety concerns. In the case of Teicoplanin (30 µg), resistance was observed by *L. plantarum* V3, *L. paracasei* C, *L. rhamnosus* E and *L. pentosus* Rc; it is also considered an intrinsic resistance. Furthermore, and similarly to *L. paracasei* and *L. rhamnosus* reactions, *Lactobacillus* resistance to Cotrimoxazole (25 µg) have been reported by Yi Wang et al. (2022) as well. Co-trimoxazole is commonly used to treat a broad range of bacterial infections, since it acts on the biosynthetic pathway of the vitamin folic acid that is lacking in most *lactobacilli*, resistance to co-trimoxazole is considered intrinsic (Rossi et al. 2011).

According to our findings, there is no isolated strain that showed resistance to both Clindamycin and erythromycin, so all isolates can be considered safe without any potential for carrying transferable antibiotic resistance genes. Generally, it is desirable that probiotics are sensitive to commonly prescribed antibiotics at low concentrations. But the resistance of the probiotic strains to some antibiotics could also be used in the treatment of intestinal disorders and/or in preventing antibiotic-induced diarrhoea, in a situation when resistance genes present in the probiotic strains are silent (El-Naggar 2004).

III.3.3. Antimicrobial activity

The antimicrobial activity of LABs isolates was studied against gastrointestinal pathogens (table 19) including *Escherichia coli, Staphylococcus aureus* and *Salmonella typhimurium* **(Annex 11)**, furthermore against *Klebsiella pneumoniae, Proteus mirabilis* and *candida albicans*. The results showed variable effects (from medium to weak inhibition); Subsequently, the cell-free supernatants from *L. rhamnosus* "B", treated with catalase neutralised and tested by well diffusion assay, were found to maintain the most powerful antimicrobial activity against both Gram-positive and Gram-negative bacteria as well as of Candida albicans. Similar results were found by Srinivasan et al. (2013), Gurban oglu Gulahmadov et al. (2006) and Tkhruni et al. (2020). The efficacy of *Lactobacillus paracasei sp* "C" was the lowest. The bacteriocin displays weak antibacterial activity against *Escherichia coli, Proteus mirabilis, Salmonella typhimurium* and *Candida albicans* exhibiting an antibacterial activity in accordance with Bartkiene et al. (2018) and Lele et al. (2018) reports. However, it did not cause any effect on both *Klebsiella pneumoniae* or *Staphylococcus*

aureus. Manzoor et al. (2016) revealed that all the *Lactobacillus* including *L.paracasei* had no inhibition effect on the indicator strain *S. aureus* DPC 6867. Moreover, Mogna et al. (2016) obtained similar results testing inhibitory activity of *L. paracasei* against *K. pneumoniae* ATCC 10031 with an inhibition zone around 6 mm.

Defining the correlation between the lactic bacteria diversity and their capacities is crucial, so more tests are required to elicit phenotypic and molecular characterisations of the selected probiotic strains (using PCR, electrophoresis…).

Table 17. Biochemical and morphological characterisation of isolated *Lactobacilli sp*

Isolates	**V1**	**V2**	**V3**	**B**	**C**	**E**	**Rc**
Origin	Cow milk	Cow milk	Cow milk	Ewe milk	Goat milk	traditional butter	Carrot
Bacterial Shape	bacilli	bacilli	bacilli	bacilli	bacilli	bacilli	bacilli
Gram	+	+	+	+	+	+	+
Catalase	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-
Gas production	+	+	+	+	+	-	+
15°C	-	-	-	-	-	-	-
45°C	+	-	+	+	+	+	+
pH 4	+	+	+	+	+	+	+
pH 9.5	+	+	+	-	+	+	+
NaCl 4%	+	+	+	+	+	+	+
NaCl 6.5%	+	-	+	-	+	-	+
strain	***Lactobacillus paracasei spp paracasei 3***	***Lactobacillus rhamnosus***	***Lactobacillus plantarum 1***	***Lactobacillus rhamnosus***	***Lactobacillus paracasei spp paracasei 3***	***Lactobacillus rhamnosus***	***Lactobacillus pentosus***
Hemolysis	**ϒ**	**ϒ**	**ϒ**	**α**	**ϒ**	**ϒ**	**ϒ**

Table 18. Antibiogram of the isolated *Lactobacillus sp* and the *E coli* strain as control;

Antibiotic agent	Symbol	Disc content	Interpretative criteria[1]			*V1* *L. paracasei*	*V2* *L. rhamnosus*	*V3* *L. plantarum*	*B* *L. rhamnosus*	*C* *L. paracasei*	*E* *L. rhamnosus*	*Rc* *L. pentosus*	*E coli* *ATCC*
			Sensitive	Intermediate	Resistant								
Clindamycin	CD	(2 µg)	≥21	15–20	≤14	R	I	R	S	I	I	I	R
Erythromycin	E	(15 µg)	≥23	14-22	≤13	S	S	S	S	I	S	S	I
Teicoplanin	Te	(30 µg)	≥14	11–13	≤10	S	S	R	S	R	R	R	S
oxacillin	Ox	(1 µg)	≥13	11-12	≤10	S	S	S	S	S	S	R	R
penicillin	P	(10 units)	≥15	-	≤14	S	S	R	S	S	S	S	R
Cotrimoxazole	SXT	(25 µg)	≥16	11–15	≤10	R	R	S	R	R	R	S	S

[1]Zone size interpretative criteria is as per CLSI standard according to Antimicrobial Susceptibility Systems in https://www.himedialabs.com/HML/images/literature/pdf/100000027/68.pdf

Table 19. Antimicrobial activity of treated cell-free culture supernatants of lactic acid bacteria isolates against pathogenic bacteria

Strains	**V1**	**V2**	**V3**	**B**	**C**	**E**	**Rc**
	L. paracasei	*L. rhamnosus*	*L. plantarum*	*L. rhamnosus*	*L. paracasei*	*L. rhamnosus*	*L. pentosus*
Escherichia coli	+	++	+	++	+	+	-
Klebsiella pneumoniae	+	+	+	++	-	+	+
Staphylococcus aureus	+	++	++	+	-	-	+
Proteus mirabilis	+	+	+	++	+	++	+
Salmonella typhimurium	+	+	+	+	+	+	++
Candida albicans	+	+	+	++	+	+	++

Interpretation of zone inhibition diameter.

+ + +: 15mm (strong inhibition).

+ +: 14mm 10mm (medium inhibition).

+: 9mm 7mm (weak inhibition).

-: = 6mm (no inhibition).

CONCLUSION & PERSPECTIVE

Conclusion and perspective

The Algerian local knowledge has great cultural significance and refers to the use of many wild resources and natural plants. So, the present study aimed to analyse the ethnobotanical knowledge about *Tuberaria lignosa* and *Crataegus azarolus* medicinal plants, harvested from Chetaibi (Annaba). In order to improve and maintain the host's health, more precisely the gastrointestinal tract.

In the first part of the thesis work, the investigations were on *Tuberaria lignosa* dried leaves. The phenolic profile of constituents is mainly part of the Tannin class in addition to some Flavonoids. The aqueous and methanolic extracts of *T. lignosa* leaves differed in their contents of phenolic compounds; the methanolic extract contained a higher level of total phenolics 130,96 mg GA eq. g^{-1} E, total flavonoids 12,68 mg QE. g^{-1} E and tannins contents 23.73 mg CE. g^{-1} E. Therefore, the methanolic extract exhibited the highest activities; the antioxidants activity screening showed that the extracts are a good hydrogen donator, had an interesting reducing power and total antioxidant capacity. The preliminary antibacterial study revealed that the $Gram^+$ bacteria were more sensitive to the antimicrobial action of *T. lignosa* extracts than $Gram^-$ strains. The resistance was noted only at the lowest concentrations of 50 $mg.ml^{-1}$, from *Salmonella typhimurium* ATCC 14028 and *Salmonella enterica* against Met. E. Additionally, to the Aq. E resistance of *Escherichia coli* ATCC 8739, *Escherichia coli 2*, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae*. The MIC values varied between 0,781 and 3,125 $mg.ml^{-1}$ and the *Tuberaria lignosa* extracts are qualified as microbiostatic. The direct contact assay used to determine the sensitivity of *Candida albicans*, revealed a great antifungal activity where both Aq. E and Met. E had a successful growth inhibition effect.

In the second part, seven lactic acid bacteria were isolated then the candidates with probiotic potential were selected. In order to evaluate their ability to ferment indigestible polysaccharides. Regarding the probiotic potential, all isolates were able to resist low pH conditions (pH 4), and could carry on well through a sodium chloride concentration of up to 6.5%, except *L. rhamnosus* strains. Six isolates were considered safe (ϒ hemolytic activity), and found to possess antimicrobial activity against pathogens causing common diseases. Further studies, concerning the extraction of polysaccharides from *Crataegus azarolus* fruits, were aimed to illustrate the fruit polysaccharide structural characterisation and valorise its antioxidants and prebiotic potentials. Our data suggest that AZWSP is mainly a galactoglucomannan, but to confirm the structural hypothesis, more

analysis should be carried out, such as methylation analysis, and 2D NMR spectra including COSY, HSQC, HMBC. This water-soluble polysaccharide showed high resistance to hydrolysis in artificial human gastric juice, and can be attributed to hydrogen donating ability, reducing power, metal chelating ability and other free radicals. Our results demonstrated clearly that the addition of *C. azarolus* galactoglucomannan to the growth medium increased the concentration of *Lactobacilli sp*. However, the viability of the bacteria needs to be assayed because the optical density of the growth medium does not decrease notably when the viable bacteria amount starts to decrease. And during the 48H fermentation probably some bacteria would become dormant or die due to lack or depletion of substrate. So, further investigations to use plate counts from the growth assay seem to be necessary. Our results confirm previous findings indicating that medicinal plants can be used in the treatment of gastrointestinal disorders; thus, the *Tuberaria lignosa* extracts (polyphenols) may treat the digestive tract by decreasing infections, primary inflammatory disorders and peptic ulcer disease. Similarly, the *Crataegus azarolus* fruits (polysaccharides) is suggested as promising prebiotics with a beneficial effect for the intestine-health-promoting.

These results significantly increase the current knowledge of the key bioactive and nutritional components of both *Tuberaria lignosa* leaves and yellow hawthorn fruits and provide a basis for further experiments on the management of gastrointestinal disorders. Further studies should be pursued to assess the cytotoxicity, which open the door for their clinical uses as a result of their efficacy.

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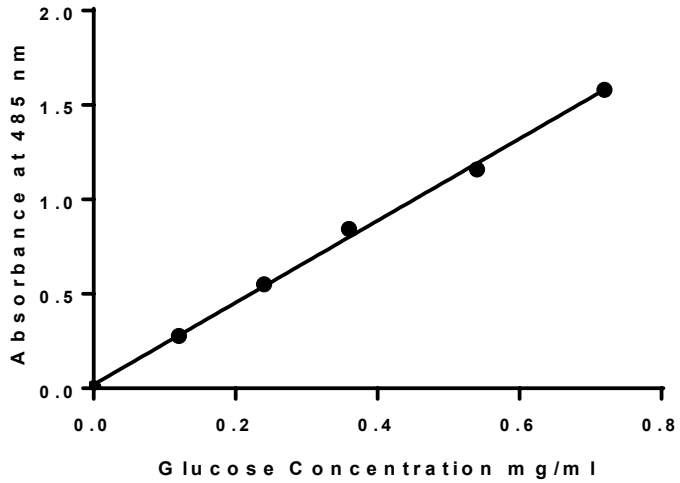
ANNEXES

Annexes

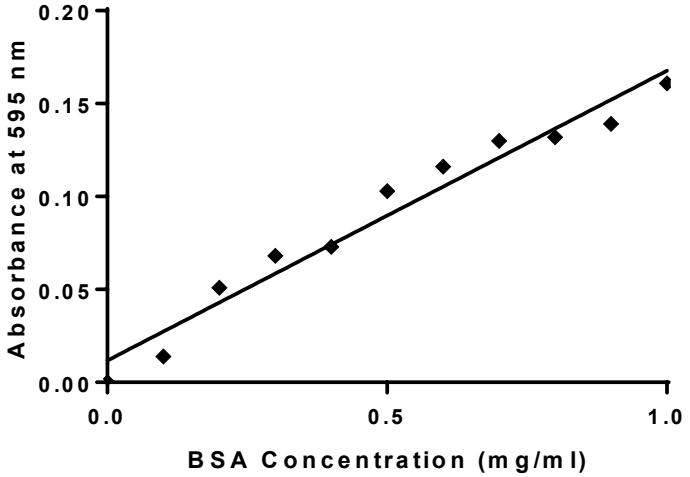
Annex 1: Glucose standard curve for total sugar estimation



Y = 2.17*X + 0.01967

0.998

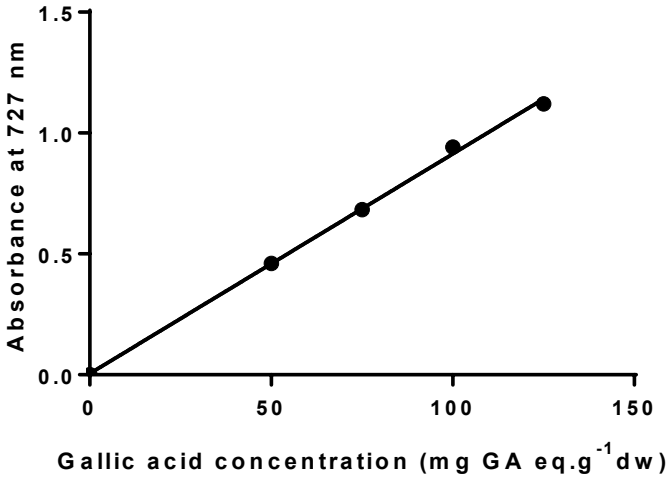
Annex 2: BSA standard curve for protein estimation



0.9611

Y = 0.1559*X + 0.01177

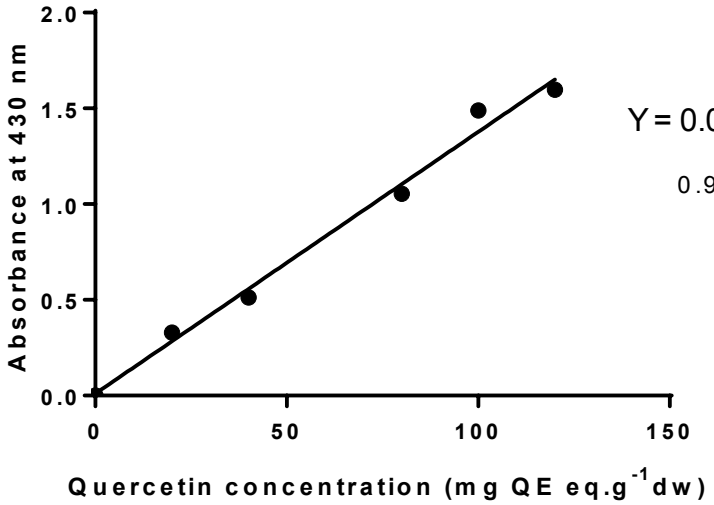
Annex 3: Gallic acid standard curve for total phenolic content



Y = 0.009087*X + 0.005108

0.9983

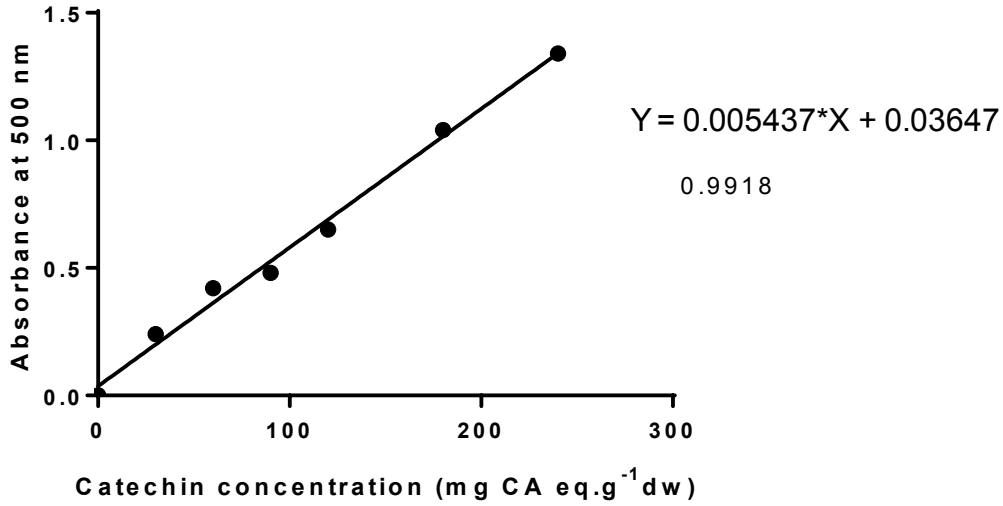
Annex 4: Quercetin standard curve for total flavonoid content



Y = 0.01367*X + 0.01032

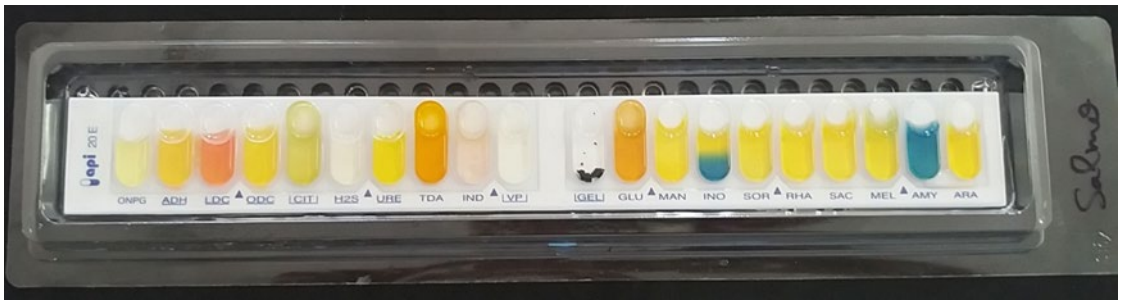
0.9897

Annex 5: Catechin standard curve for tannin content



Annex 6: API systems

- API20 E:

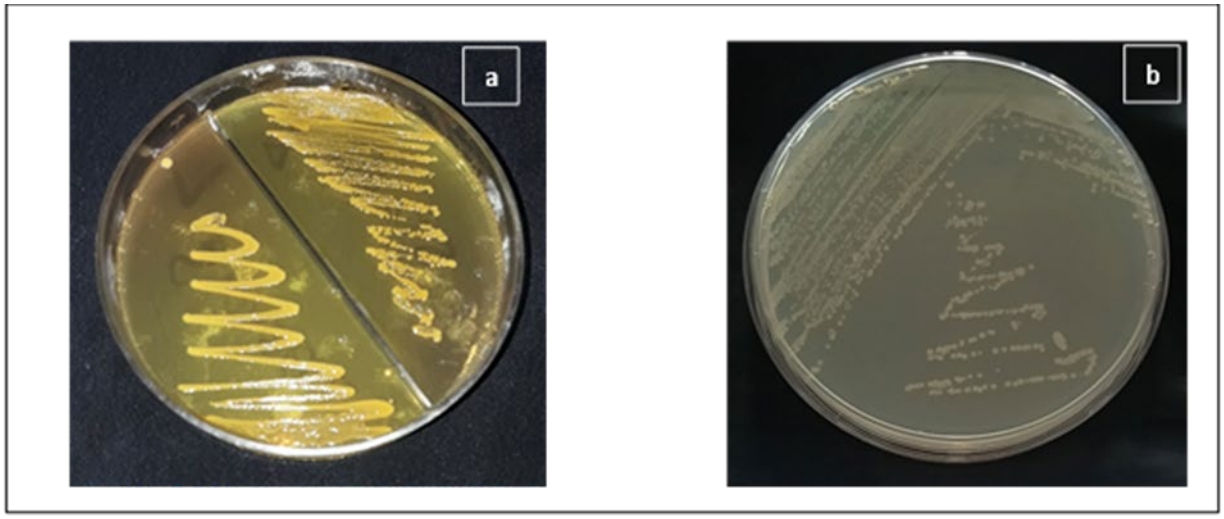


- API STAPH:

- API 50 CHL:

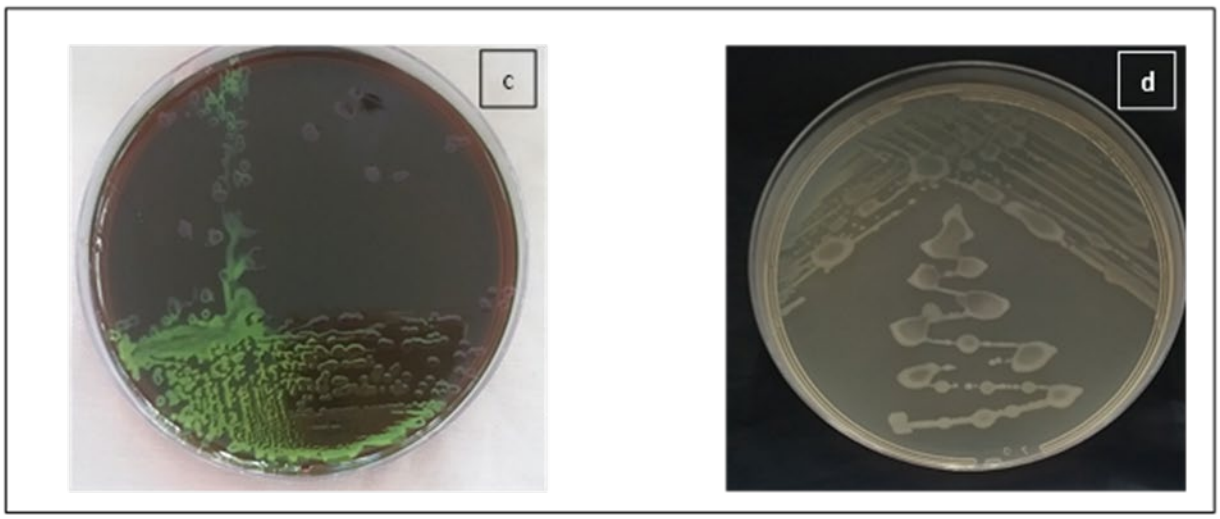

api 50 CH
0 1 2 3 4 5 6 7 8 9
api 50 CH
10 11 12 13 14 15 16 17 18 19
api 50 CH
20 21 22 23 26 27 28 29
api 50 CH
30 31 32 33 34 35 36 37 38 39
api 50 CH
40 41 42 43 44 45 46 47 48 49

Annex 7: *Staphylococcus aureus* pure culture



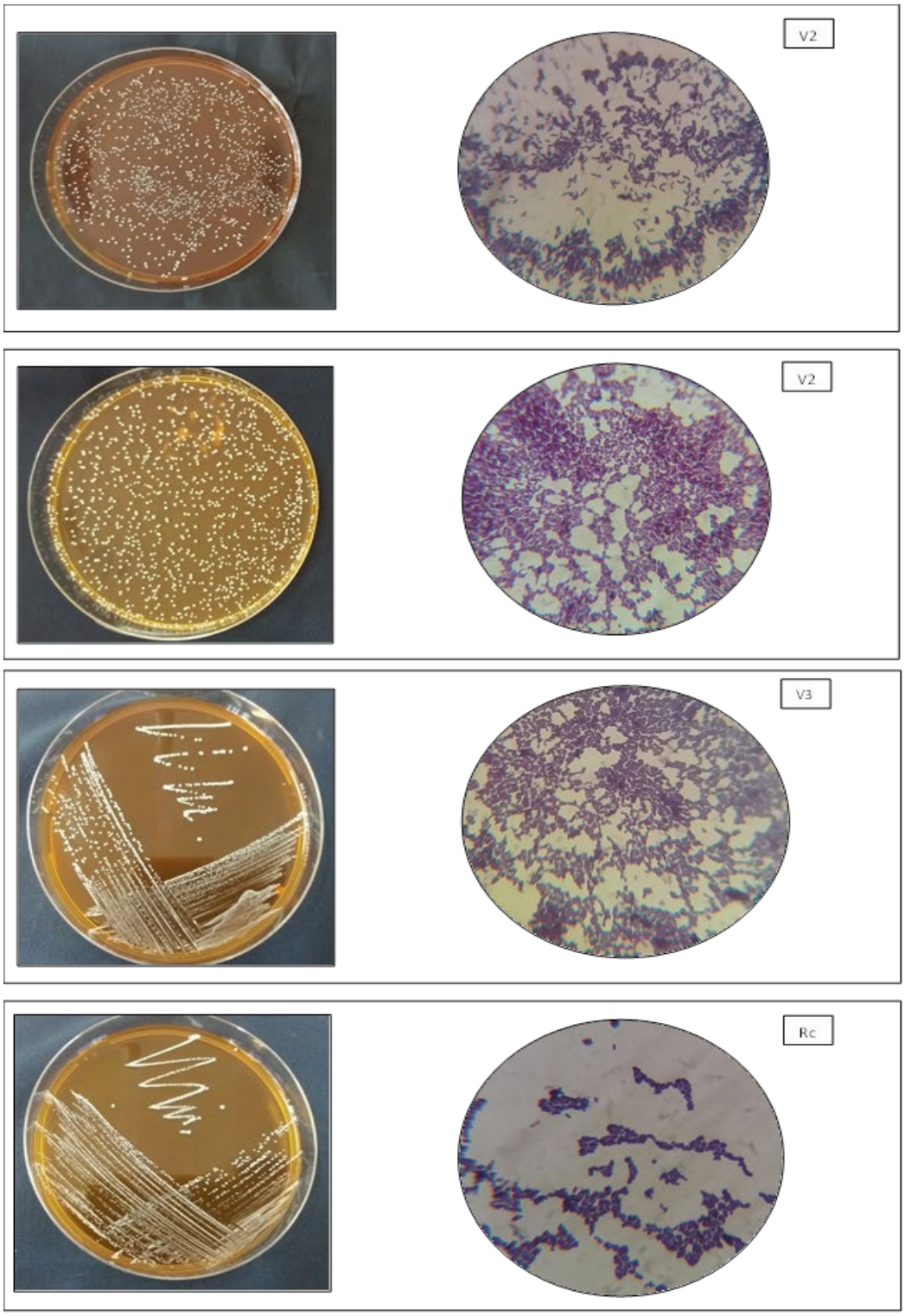
Staphylococcus aureus colonies in Petri dish on Chapman agar (a), on nutrient agar (b).

Annex 8: *Escherichia Coli* pure culture



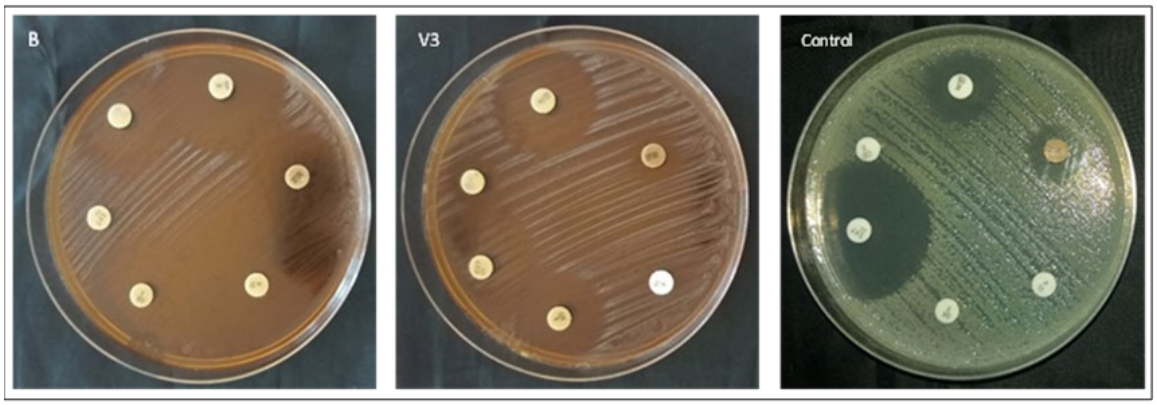
Escherichia Coli colonies in Petri dish on EMB agar (c), on nutrient agar (d).

Annex 9: *Lactobacillus sp* pure culture



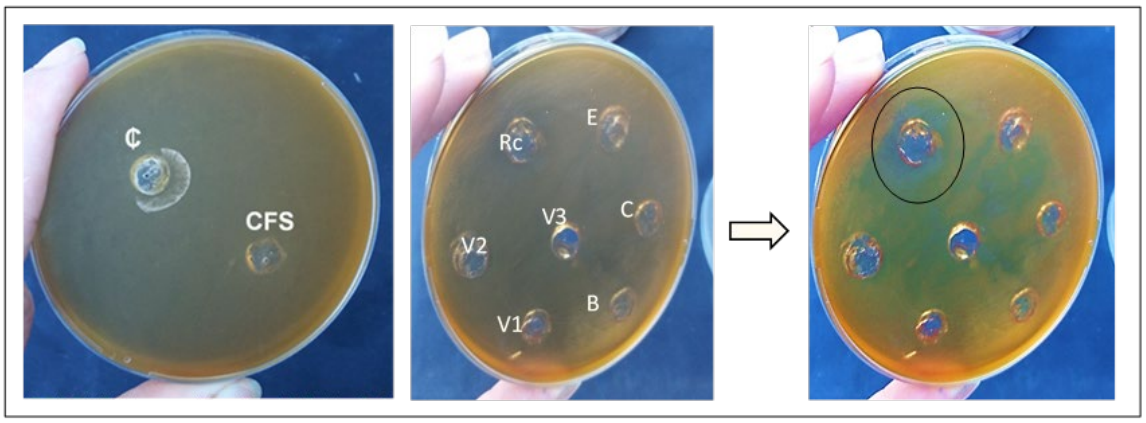
Colony morphology and gram strain of pure isolated *Lactobacillus sp.*

Annex 10: Antibiogram of *Lactobacillus*



Antibiogram of some isolated *Lactobacillus sp* and the used control was E coli strain.

Annex 11: Antimicrobial activity of *Lactobacillus*



Antimicrobial activity of CFS of *Lactobacillus* isolates against *Salmonella typhimurium* : each *Lactobacilli* strain had a control plate where ₵: the bacterial suspension and CFS: cell free supernatant.

Annex 12: ^{13}C-NMR Data for Aldoses[2]

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
D-Hexopyranoses						
α - Gal	93.2	69.4	70.2	70.3	71.4	62.2
β - Gal	97.3	72.9	73.8	69.7	76.0	62.0
α - Glc	92.9	72.5	73.8	70.6	72.3	61.6
β - Glc	96.7	75.1	76.7	70.6	76.8	61.7
α - Man	95.0	71.7	71.3	68.0	73.4	62.1
β - Man	94.6	72.3	74.1	67.8	77.2	62.1
D- Pentopyranoses						
α - Ara	97.6	72.9	73.5	69.6	67.2	
β - Ara	93.4	69.5	69.5	69.5	63.4	
α - Xyl	93.1	72.5	73.9	70.4	61.9	
β - Xyl	97.5	75.1	76.8	70.2	66.1	
D-Hexofuranoses						
α - Gal	95.8	77.1	75.1	81.6		63.3
β - Gal	101.8	82.2	76.6	82.8	71.5	63.6
β - Glc	103.8	81.8		82.1		

[2] (Bock and Pedersen 1983)

Abstract

This work gives scientific evidence to the traditional medicinal use of *Crataegus azarolus* fruits and wild *Tuberaria lignosa* leaves, highlighting their interest as sources of bioactive compounds that could be used as therapeutic agents. We isolated and characterized polysaccharides from *Crataegus azarolus* fruits (AZWSP). This latter yielded a 2,19 % fraction, composed of 85.58% total carbohydrates, 4.26% of protein content also contained 5.34% of polyphenols. The preliminary structure features were characterized using FT-IR, Maldi-TOF SM and the ^{1}H and ^{13}C NMR analysis. Several mechanisms for antioxidant capacity were examined, including determining relative free radical-scavenging (IC_{50} = 99 ± 2 µg.ml^{-1}), hydroxyl radical scavenging (IC_{50} = 106 ± 3 µg.ml^{-1}) and anti-lipid peroxidation activities (IC_{50} = 127 ± 17 µg.ml^{-1}), as well as reducing power (EC_{50} =115 µg.ml^{-1}). Furthermore, the pulp polysaccharide remained 83% undigested in the artificial digestion process and stimulated the growth o of various isolated *lactobacillus* species (having probiotic potential), showing a considerable prebiotic ability.

Given *Tuberaria lignosa* traditional use, the phytochemical screening was investigated the total phenolic content was higher in methanolic fraction (130,96± 3,28 mg GA eq. g^{-1}E) and the same for total flavonoid (12,68±1,9 QE. g^{-1}E) and tannins (23,73± 2,49 mg CE. g^{-1}E). Ellagitanins and flavonoids were the main phenolic compounds found, HHDP-glucose, punicalin and punicalagin the most abundant compounds. Antioxidant activity assayed was always higher in methanolic fraction compared with aqueous extract (using CAT, FRAP and DPPH assays). Likewise, the preliminary antibacterial study using the disc diffusion method revealed an interesting profile of antimicrobial action against positive and negative Gram bacteria; the MIC varied between 0,78 to 3,125 mg.ml^{-1}. The antifungal activity of *Tuberaria lignosa* was reported as well using direct contact assay.

The results obtained during the research work indicate that the yellow Hawthorn polysaccharide and *Tuberaria lignosa* plant extract are endowed with great properties namely prebiotics and antimicrobial; that can alter the gut microbiota and improve the host health, and therefore can be exploited as pharmaceuticals.

Keywords: *Crataegus azarolus*; prebiotics; probiotics; *Tuberaria lignosa*; Antimicrobial activities; Antioxidant activities.

Résumé

Ce travail apporte des preuves scientifiques aux utilisations médicinales traditionnelles des fruits de *Crataegus azarolus* et des feuilles de *Tuberaria lignosa*, soulignant leur intérêt comme sources de composés bioactifs qui pourraient être utilisés comme agents thérapeutiques. Nous avons isolé et caractérisé les polysaccharides des fruits de *Crataegus azarolus* (AZWSP). Ce dernier a donné une fraction de 2,19 %, composée de 85,58 % de glucides totaux, 4,26 % de protéines et 5,34 % de polyphénols. Les propriétés préliminaires de la structure ont été caractérisées par FT-IR, Maldi-TOF SM et l'analyse ^{1}H et ^{13}C NMR. Plusieurs mécanismes de la capacité antioxydante ont été examinés, y compris la détermination des activités relatives de piégeage des radicaux libres (IC_{50} = 99 ± 2 µg.ml^{-1}), de piégeage des radicaux hydroxyles (IC_{50} = 106 ± 3 µg.ml^{-1}) et d'anti-peroxydation des lipides (IC_{50} = 127 ± 17 µg.ml^{-1}), ainsi que le pouvoir réducteur (EC_{50} =115 µg.ml^{-1}). De plus, le polysaccharide de la pulpe est de 83% non digéré dans le processus de digestion artificiel et a stimulé la croissance de diverses espèces de *Lactobacillus* isolées (ayant un potentiel probiotique), montrant de ce fait une capacité prébiotique considérable. Étant donné l'utilisation traditionnelle de *Tuberaria lignosa*, le screening phytochimique a été étudié. La teneur en phénols totaux était plus élevée dans la fraction méthanolique (130,96± 3,28 mg GA eq. g^{-1}E) et il en était de même pour les flavonoïdes totaux (12,68±1,9 QE. g^{-1}E) et les tanins (23,73± 2,49 mg CE. g^{-1}E). Les ellagitanins et les flavonoïdes étaient les principaux composés phénoliques trouvés, le HHDP-glucose, la punicaline et la punicalagine étant les composés les plus abondants. L'activité antioxydante testée était toujours plus élevée dans la fraction méthanolique par rapport à l'extrait aqueux (en utilisant les tests CAT, FRAP et DPPH). De même, l'étude antibactérienne préliminaire utilisant la méthode de diffusion des disques a révélé un profil intéressant d'action antimicrobienne contre les bactéries Gram négatif et positif; la CMI variait entre 0,78 et 3,125 mg.ml^{-1}. L'activité antifongique du *Tuberaria lignosa* a également été rapportée en utilisant le test de contact direct. Les résultats *in vitro* obtenus au cours de cette étude indiquent que le polysaccharide d'azarole et l'extrait de la plante *Tuberaria lignosa* sont dotés de grandes propriétés à savoir prébiotiques et antimicrobiennes; qui peuvent modifier le microbiote intestinal et améliorer la santé de l'hôte, et par conséquent peuvent être exploitées comme produits pharmaceutiques.

Mots clés : *Crataegus azarolus* ; prébiotiques ; probiotiques ; *Tuberaria lignosa* ; Activités antimicrobiennes ; Activités antioxydantes.

ملخص

يقدم هذا العمل أدلة علمية على الاستخدامات الطبية التقليدية لثمار *Crataegus azarolus* و أوراق *Tuberaria lignosa* البرية ، مما يسلط الضوء على اهتمامها كمصدر للمركبات النشطة بيولوجيا التي يمكن استخدامها كعوامل علاجية. قمنا بعزل وتمييز السكريات المتعددة من ثمار *Crataegus azarolus* (AZWSP). هذا الأخير أسفر عن مردود يقدر ب 2,19 ٪ ، يتكون من 85.58 ٪ من إجمالي الكربوهيدرات ، و 4.26 ٪ من البروتين يحتوي أيضا على 5.34 ٪ من متعددات الفينول. تم تمييز خصائص الهيكل الأولية باستخدام FT-IR و Maldi-TOF SM وتحليل الرنين المغناطيسي النووي ^{1}H و ^{13}C. تم فحص العديد من الآليات لتحليل قدرتها كمضاد أكسدة، بما في ذلك تحديد كسح الجذور الحرة النسبية (ميكروغرام / مل $IC_{50} = 99 \pm 2$) ، وإزالة جذور الهيدروكسيل (ميكروغرام / مل $IC_{50} = 106 \pm 3$) وأنشطة بيروكسيد مضادة للدهون (ميكروغرام / مل IC_{50} =، 127 ± 17 وكذلك قدرة الاسترجاع (ميكروغرام / مل $EC_{50} = 115$).

علاوة على ذلك، ظل متعدد السكريات غير مهضوم بنسبة 83٪ في عملية الهضم الاصطناعي وحفز نمو مختلف انواع بكتيريا حمض اللبن المعزولة (التي لديها إمكانات بروبيوتيك)، مما يدل على القدرة الكبيرة للسكر المعقد كبريبايوتك.

بالنظر إلى الاستخدام التقليدي ل *Tuberaria lignosa* ، تم التحقيق في الفحص الكيميائي النباتي كان إجمالي المحتوى الفينولي أعلى في المستخلص الميثانولي ($g^{-1}E$ 130,96 ±3,28mg GA eq.) ونفس الشيء بالنسبة لإجمالي الفلافونويدات ($g^{-1}E$ 12 ,68±1 ,9 QE.) والتانين ($g^{-1}E$ 23,73 ± 2,49 mg CE .). الإيلاجيتانين والفلافونويد كانت المركبات الفينولية الرئيسية التي تم العثور عليها ، فكانت HHDP-glucose و punicalin و punicalagin المركبات الأكثر وفرة .

كان فحص النشاط المضاد للأكسدة دائما أعلى في المستخلص الميثانولي مقارنة بالمستخلص المائي (باستخدام اختبارات CAT وFRAP وDPPH). وبالمثل، كشفت الدراسة الأولية المضادة للبكتيريا باستخدام طريقة الانشار القرصي عن نتائج مثيرة للاهتمام لنشاط المضاد للميكروبات ضد البكتيريا Gram négatif وpositif. تراوحت MIC بين 0,78 إلى 3,125 ملغم / مل. تم الإبلاغ عن النشاط المضاد للفطريات من *Tuberaria lignosa* أيضا باستخدام فحص الاتصال المباشر.

تشير النتائج التي تم الحصول عليها في المختبر خلال هذا العمل البحثي إلى أن متعدد السكر المستخلص من الزعرور الأصفر ومستخلص نبات *Tuberaria lignosa* يتمتعان بخصائص رائعة و هي البريبايوتيكس و مضادات الميكروبات. الذي يمكن ان يعدل ميكروبات الأمعاء ويحسن صحة المضيف.و بالتالي يمكن استغلاله كادوية .

الكلمات المفتاحية: زعرور; البريبايوتكس; البروبيوتيك; *Tuberaria lignosa* ; لأنشطة المضادة للميكروبات; أنشطة مضادة للأكسدة.