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جامعة الإخوة منتوري قسنطينة 1
Frères Mentouri Constantine 1 University
Université Frères Mentouri Constantine 1

Faculty of Natural and Life Sciences كلية علوم الطبيعة والحياة

Department of Biochemistry and Cell and Molecular Biology

قسم الكيمياء الحيوية والبيولوجيا الخلوية والجزيئية

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Presented by: Allal yousra **the 30/06/2022**

Bouattit rayen

Evaluation board:

Supervisor: Mme T. Nardjes MOUAS (Prof - Mentouri Brothers University, Constantine 1).

Examiner 1: Pr. Djedouani Amel (ENS ,Constantine 3)

Examiner 2: Dr. Tabbi Aouatef (ENS, Constantine 3)

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Table of content

List of figures

List of tables

List of abbreviations

Introduction……01

THEORETICAL PART

Chapter one: Natural polyphenols…….03

I Natural polyphenols……....04
II Classification of polyphenols……..04
III Polyphenols as dietary supplements……05
IV Bioavailability of polyphenols……..05
V Diabetes and chronical disease related to gastro-intestinal system overview…….06
VI Natural polyohenols in prevention of chronical disease related to gastro-intestinal system and diabetes……07
VII Ceratonia siliqua L (carob)…….09
1. Origin and geographic distribution ……..…....09
2. Nutritional value…….……09
3. Current uses…….....09
a) Traditional use……...……...…...09
b) Food industry…….……..……..……09
c) Cosmetology…….……..……..…..09
4. Chemical composition ……..……...……..10
a. Primary metabolites…….……..……..…..10
b. Secondary metabolites…….……..……..…..11
VIII. Zingiber officinale (ginger)……12
1. Historical and popular uses…….…..……..12
2. Medicinal uses and some market preparation……..……..13

Chapter two: Dietary supplement and phytochemical…….16

I. Natural food supplement…….……..…17
II. Gummy form……....17

III. Natural products used in the dietary supplement industry…….……..…18
IV. Secondary metabolites responsible for the therapeutic effect and doses……....19
V. pharmacovigilance; regulation and control…….…....20

Chapter three: Extraction processes…….22

I. Enfleurage……...23
II. Decoction……....23
III. Maceration……..….24
IV. Percolation……..…….25
V. Infusion……..……..25

Experimental part

Chapter one: Materials and methods…….……..27

I. Materials…….……28
1. Plant material……..…..28
2. Formulation material……..…...28
II. Methods of study…….……29
1. Preparation of the plant samples……..…….……29
1.1. Carob (ceratonia siliqua) extraction……..…..29
1.2. Ginger (zingiber officinale) extraction…….…..30
2. The quality control…….…..….31

Chapter two: Results and discussion…….……33

I- Sweet ginger Gummies 1…….……..34
1. Taste tests…….…....34
2. Quality control…….....34
2.1.Organoleptic properties……..…..34
2.2.Physicochemical analysis……..……..35
2.3.Microbiological analysis……..……35
2.4.Stability test…….....36
II- Sweet ginger gummies 2……..…….37
1. Taste tests…….37
2. Quality control…….……...…….37
2.1.Organoleptic properties ……..…….37

2.2.Physicochemical analysis……..……..……38
2.3.Microbiologicalanalysis…….……....38
2.4.Stability test…….……....39
III- Sugar-free Ginger Gummies 1……..……...……40
1. Taste tests……..……...40
2. Quality control……..…..…..40
2.1.Organoleptic properties …….…….....40
2.2.Physicochemical analysis……41
2.3.Microbiologicalanalysis……..42
2.4.Stability test…….……...42
IV- Sugar-free Ginger Gummies 2……..…….……43
1. Taste tests…….……..……..…43
2. Quality control……..…..…43
2.1.Organoleptic properties ……..……43
2.2.Physicochemical analysis….……..……44
2.3.Microbiologicalanalysis.....45
2.4.Stability test.....45
V- Gummies with Carob acetic acid extract without added sugar …….....……46
1. Taste tests……..……..……46
2. Qualitycontrol…….……..…….…….46
2.1.Organoleptic properties …….……..……..…..…46
2.2.Physicochemical analysis……..…….....47
2.3.Microbiologicalanalysis…….……..…48
2.4.Stability test……..……..…….....48
VI- Gummies with aqueous extract of carob without added sugar ……..……....….49
1. Taste tests……...…….……49
2. Quality control……...…….……49
2.1.Organoleptic properties ……..……..…..49
2.2.Physicochemical analysis……..……..……50
2.3.Microbiologicalanalysis……..……...…..……51
2.4.Stability test…….……...……..…51

Chapter three: In silico study…….53

I- Structure activity relationship (SAR)…….……...……..……54

1. Ginger bioactive compound……..…54

2. Carob bioactive compounds…….…....56

II. Synergetic of active compounds…….…….……...…….60

1. Ginger active compounds synergy…….....…….…….60

2. Carob active compounds synergy…….....…….……...61

Conclusion and perspectives……...……63

Bibliographic references……..65

Abstract…….73

List of figures

Figure 1: Classification and chemical structure of major classes of dietary polyphenols.

Figure 2: Galactomannan

Figure 3: Saccharose

Figure 4: Ginger, ginger rhizome, and its major active components: 6-gingerol, 6-shogaol, and 6-paradol.

Figure 5:2D Structure of 6-gingerol (a), 6-shogaol (b), and 6-paradol (c**)**

Figure 6: Andrographolide structure

Figure 7: Some active compounds of curcuma domestica

Figure 8: Terpenes and terpenoids

Figure 9: Quinine, aconitine structure

Figure 10: Coumarinchemical structure description

Figure 11: Photos of the cold enfleurage device.

Figure12: The aqueous decoction of plant raw material**.**

Figure 13: Picture of maceration extraction

Figure 14: The pictorial representation of soxhlet extraction.

Figure 15: Bioactive compounds of ginger

Figure 16: The potential mechanism for the antioxidant action of 6-shogoal.

Figure 17: Gallicacid structure.

Figure 18: Graphical abstract of covalent enhancing activity of gallic acid.

Figure 19: Chemical structures of (**a**) D-pinitol and (**b**) locust bean gum (LBG).

Figure 20: Insulin-sensitizing mechanism of D-Pinitol.

Figure 21: Insulin-mimetic mechanism of D-pinitol.

Figure 22: HPLC chromatogram of standard**.**

List of tables

Table1: Biological and pharmacological activities of Ceratonia siliqua L.

Table2: Natural products used in the dietary supplement industry.

Table 3: Secondary metabolites responsible for the therapeutic effect

Table4: Formulation of Carob in gummy making with decoction and their taste tests.

Table5: Formulation of Carob in gummy making with infusion and their taste tests.

Table6: Formulation of Ginger gummy making with enfleurage, acetic acid extraction and taste tests.

Table7: Formulation of Ginger gummy making with enfleurage and their taste test

Table 8: Sweet ginger gummies 1 taste tests

Table 9: Sweet ginger gummies 1 organoleptic properties identification

Table 10: Sweet ginger gummies 1 organoleptic property analyses

Table 11: Sweet ginger gummies 1 physicochemical analysis identification

Table 12: Sweet ginger gummies 1 physicochemical analysis

Table 13: Sweet ginger gummies 1 microbiological analysis identification

Table 14: Sweet ginger gummies 1 microbiological analysis

Table 15: Sweet ginger gummies 1 stability test identification

Table 16: Sweet ginger gummies 1 stability test analyses

Table 17: Sweet ginger gummies 2 taste tests

Table 18: Sweet ginger gummies 2 organoleptic properties identification

Table 19: Sweet ginger gummies 2 organoleptic property analyses

Table 20: Sweet ginger gummies 2 physicochemical analysis identification

Table 21: Sweet ginger gummies 2 physicochemical analysis

Table 22: Sweet ginger gummies 2 microbiological analysis identification

Table 23: Sweet ginger gummies 2 microbiological analysis

Table 24: Sweet ginger gummies 2 stability test identification

Table 25: Sweet ginger gummies 2 stability test analyses

Table 26: Sugar-free Ginger Gummies 1 taste tests

Table 27: Sugar-free Ginger Gummies 1 organoleptic properties identification

Table 28: Sugar-free Ginger Gummies 1 organoleptic properties analyses

Table 29: Sugar-free Ginger Gummies 1 physicochemical analysis identification

Table 30: Sugar-free Ginger Gummies 1physicochemical analysis

Table 31: Sugar-free Ginger Gummies 1 microbiological analysis identification

Table 32: Sugar-free Ginger Gummies 1 microbiological analysis

Table 33: Sugar-free Ginger Gummies 1 stability test identification

Table 34: Sugar-free Ginger Gummies 1 stability test analyses

Table 35: Sugar-free Ginger Gummies 2 taste tests

Table 36: Sugar-free Ginger Gummies 2 organoleptic properties identification

Table 37: Sugar-free Ginger Gummies 2 organoleptic properties analyses

Table 38: Sugar-free Ginger Gummies 2 physicochemical analysis identification

Table 39: Sugar-free Ginger Gummies 2physicochemical analysis

Table 40: Sugar-free Ginger Gummies 2 microbiological analysis identification

Table 41: Sugar-free Ginger Gummies 2 microbiological analysis

Table 42: Sugar-free Ginger Gummies 2 stability test identification

Table 43: Sugar-free Ginger Gummies 2 stability test analyses

Table 44: Carob gummies with acetic acid extract taste tests

Table 45: Carob gummies with acetic acid extract organoleptic properties identification

Table 46: Carob gummies with acetic acid extract organoleptic properties analyses

Table 47: Carob gummies with acetic acid extract physiochemical analysis identification

Table 48: Carob gummies with acetic acid extract physiochemical analysis

Table 49: Carob gummies with acetic acid extract microbiological analysis identification

Table 50: Carob gummies with acetic acid extract microbiological analysis

Table 51: Carob gummies with acetic acid extract stability test identification

Table 52: Carob gummies with acetic acid extract stability test analyses

Table 53: Gummies with aqueous extract of carob taste tests

Table 54: Gummies with aqueous extract of carob organoleptic properties identification

Table 55: Gummies with aqueous extract of caroborganoleptic properties analyses

Table 56: Gummies with aqueous extract of carob physicochemical analysis identification

Table 57: Gummies with aqueous extract of carob physicochemical analysis

Table 58: Gummies with aqueous extract of carob microbiological analysis identification

Table 59: Gummies with aqueous extract of carob microbiological analysis

Table 60: Gummies with aqueous extract of carob stability test identification

Table 61: Gummies with aqueous extract of carob stability test analyses

Table62: In vitro hypoglycemic potentials of ginger and its bioactive constituents

Table63: The chemical components of carob and their biological evaluation

Table64: Phenolic acid composition (mg/g dry weight) of particular phenolicfractions of carob pods

List of abbreviations

ROS: Reactive oxygen species

LDL: Low-density lipoprotein

SOD: Superoxyde dismutase

GI: Gastro intestinal

IBD: Intestinal brush border

HO-1: Hemeoxygenase-1

GPx: Glutathione peroxidase

TEER: Transepithelial resistance

ZO-1: Zonula occludens-1

HPLC: High performance liquid chromatography

DAD: Diode array detection

MS: Mass spectrometry

COX: Cyclooxygenase

Ppm: Parts per million

HDL: High Density Lipoprotein

DSS: Dextran sodium sulfate

ALT:Alanine aminotransférase

AST:Aspartate aminotransférase

ALP: Alkaline phosphatase

TCM: Traditional Chinese Medicine

NSAIDS: Non-steroidal anti-inflammatory drugs

HRSV: Human Respiratory Syncytial Virus

IFN-β*:* Interferon *beta*

PI3K: Poshatidylinositol-3-kinese

Akt: Protein kinese B

NF-κB: Nuclear factor kappa B cells

TNF-α: Tumor necrosis factor α

VEGF: Vascular Endothelial Growth Factor

IL-8: Interleukin 8

DNA: Deoxyribose nucleic acid

PH: Potential hydrogène

WHO: World Health Organization

CNPM: The National Pharmacovigilance and Materiovigilance Center

SAR: Structure activity relationship

MDA: Malondialdehyde

Nrf2: Nuclear factor, erythroid 2-related factor 2

GSH/GSSG: Glutathione/glutathione disulfide

HO-1: Heme oxygenase-1

MT1: Metallothionein 1

AKR1B10: Aldo-keto reductase family 1 member B10

FTL: Ferritin light chain

GGTLA4: γ-glutamyltransferase-like activity 4

GCLM: Glutamate-cysteine ligase modifier

Keap1: Kelch-like ECH-associated protein 1

GSTP1: Glutathione S-transferase P1

NQO1: Quinone dehydrogenase 1

NADPH: Nicotinamide adenine dinucleotide phosphate

AGEs: Advanced glycation end products

MGO: Methylglyoxal

CML: Nε-carboxymethyl-lysine

3T3-L1: Cell line derived from (mouse) 3T3 cells

AMPK:AMP-activated protein kinase

GLP-1: Glucagon-like peptide 1

GLUT4: Glucose transporter type 4

HbA1c: Glycated hemoglobin A

TG: Tissue triglyceride

TC: Total cholesterol

DM2: Type 2 diabetes mellitus

LDL-C: Low-density lipoprotein cholesterol

GERD: Gastroesophageal reflux disease

MoAs: Mechanism of Action

LBG: Locust bean gum

NIDDM : Noninsulin-dependentdiabetes mellitus

T2DM : Type 2diabetesmellitus

EFSA: European Food Safety Authority

GA: Gallicacid

GM : Gut microbiome

NPs: Nanoparticles

Introduction:

The popularity of herbal medicines and dietary supplements is increasing all over the world. This is partly due to the many side effects attributed to synthetic drugs, helped by the perception that herbal products are “natural” and therefore inevitably “safe**”.(Končić, 2018)**as well public interest and consumer demand.

During the last few decades, the interests of the consumers have burgeoned in the natural products due to the raised awareness. Among various bioactive molecules, polyphenols are recognized as a food article is an outstanding source of variety of compounds with extraordinary diverse composition. Quite a significant amount of experimentation on its biological activity and promising application of these compounds has been executed. Polyphenols are the secondary metabolites of plant origin and are widely distributed. These compounds attained the prominent position due to their wide distribution in plant-based foods and significant evidence of negative correlation of their consumption with cancers, diabetes, and cardiovascular diseases.

Due to their structural diversity and possessing therapeutic activities with antioxydant and antidiabetic property, researchers have focused on phenolic compounds exploring their use as medicinal agents.

Gastrointestinal diseases are among the most common problems in tropical countries and commonly manifest as diarrhea, abdominal pain, abdominal distention, gastrointestinal bleeding, intestinal obstruction, mal absorption, or malnutrition. Infectious diarrheal diseases are an important cause of morbidity and mortality in childhood. **(Ashwin, 2020)** instead of chemical compounds with side effects, natural products have become a powerful tool to combat against health problems.By standardizing and evaluating the health of active plant-derived compounds, herbal drugs can help the emergence of a new era of the healthcare system to treat human diseases.**(Ouelebani and al. 2016)**

In the present study, Carob (Ceratonia siliqua) and ginger (Zingiber Officinale) a common medicinal plants mainly used in food and traditional folk medicine, were chosen to be tested in a new class of formulations namely **GUMMIES**rich in phytochemical constituents including phenolic compounds, dietary fibers… Due to their medicinal effect as natural antioxidant or antidiabetic; they became common and widely used in natural dietary supplement industry.

Thus, the general inquiry in the present research is how to produce locally and efficiently low cost natural dietary supplements with an effective therapeutic effect without toxicity.

For this purpose, the manuscript was divided into two parts:

The first part: Theoretical part which contains three chapters.

Chapter one: Natural polyphenols

Chapter two: Dietary supplements and phytochemical

Chapter three: Extraction processes

The second part: Experimental part

Chapter one: Materials and methods with extraction and formulation

Chapter two: Results and discussion of quality control.

Chapter three: In silico study (SAR, synergy)

And finally, a conclusion and future perspective.

Chapter one

Natural polyphenols

I. Natural polyphenols:

Polyphenols are natural compounds synthesized exclusively by plants with chemical features related to phenolic substances and eliciting strong antioxidants properties. **(Rajeev K Singla, 2019)**

Natural polyphenols have attracted great interests in medicine, food and cosmetics due to their versatile functions such as antioxidant, anticancer and antibacterial.**(Hui wang, 2020)**

Polyphenols are a group of water-soluble organic compounds, mainly of natural origin. The compounds having about 5-7 aromatic rings and more than 12 phenolic hydroxyl groups are classified as polyphenols. These are the antioxidants which protect the body from oxidative damage. In plants, they are the secondary metabolites produced as a defense mechanism against stress factors. Antioxidant property of polyphenols is suggested to provide protection against many diseases associated with reactive oxygen species (ROS), this group of wonder compounds is present in surplus in natural plants and food products. Intake of polyphenols through diet can scavenge ROS. **(Sharma, 2018)**

Polyphenols have become an emerging field of interest in nutrition in recent decades. A growing body of research indicates that polyphenol consumption may play a vital role in health through the regulation of metabolism, weight, chronic disease, and cell proliferation. Over 8,000 polyphenols have thus far been identified, though their short- and long-term health effects have not been fully characterized. **(Cory, 2018)**

II. Classification of Polyphenols:

Although polyphenols are chemically characterized as compounds with phenolic structural features, this group of natural products is highly diverse and contains several sub-groups of phenolic compounds. Fruits, vegetables, whole grains and other types of foods and beverages such as tea, chocolate and wine are rich sources of polyphenols. The diversity and wide distribution of polyphenols in plants have led to different ways of categorizing these naturally occurring compounds. Polyphenols have been classified by their source of origin, biological function, and chemical structure.**(tsao, 2010)**

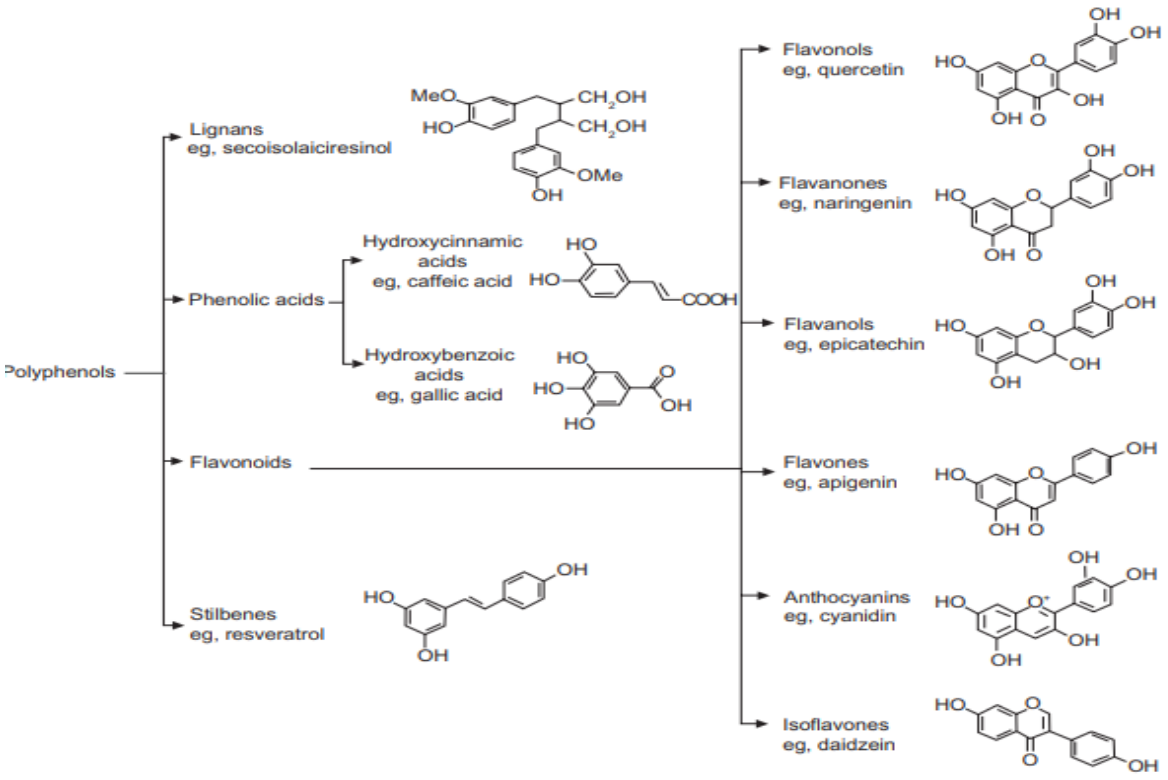


Fig1: Classification and chemical structure of major classes of dietary polyphenols. **(Martin, 2009)**

III. Polyphenols as dietary supplements

Dietary polyphenols, ie, phenolic acids and flavonoids, are a primary source of antioxidants for humans and are derived from plants including fruits, vegetables, spices, and herbs. Based on compelling evidence regarding the health effects of polyphenol-rich foods, new dietary supplements and polyphenol-rich foods are being developed for public use. Consumption of such products can increase dietary polyphenol intake and subsequently plasma concentrations beyond expected levels associated with dietary consumption and potentially confer additional health benefits.**(Martin, 2009)**

IV. Bioavailability of polyphenols:

It is important to realize that the polyphenols that are the most common in the human diet are not necessarily the most active within the body, either because they have a lower intrinsic activity or because they are poorly absorbed from the intestine, highly metabolized, or rapidly eliminated. In addition, the metabolites that are found in blood and target organs and that result from digestive or hepatic activity may differ from the native substances in terms of biological

activity. Extensive knowledge of the bioavailability of polyphenols is thus essential if their health effects are to be understood. Metabolism of polyphenols occurs via a common pathway. The aglycones can be absorbed from the small intestine. However, most polyphenols are present in food in the form of esters, glycosides, or polymers that cannot be absorbed in their native form. These substances must be hydrolyzed by intestinal enzymes or by the colonic microflora before they can be absorbed. When the flora is involved, the efficiency of absorption is often reduced because the flora also degrades the aglycones that it releases and produces various simple aromatic acids in the process. During the course of absorption, polyphenols are conjugated in the small intestine and later in the liver. This process mainly includes methylation, sulfation, and glucuronidation. This is a metabolic detoxication process common to many xenobiotics that restricts their potential toxic effects and facilitates their biliary and urinary elimination by increasing their hydrophilicity. The conjugation mechanisms are highly efficient, and aglycones are generally either absent in blood or present in low concentrations after consumption of nutritional doses. Circulating polyphenols are conjugated derivatives that are extensively bound to albumin. Polyphenols are able to penetrate tissues, particularly those in which they are metabolized, but their ability to accumulate within specific target tissues needs to be further investigated. Polyphenols and their derivatives are eliminated chiefly in urine and bile. Polyphenols are secreted via the biliary route into the duodenum, where they are subjected to the action of bacterial enzymes, especially -glucuronidase, in the distal segments of the intestine, after which they may be reabsorbed. This enterohepatic recycling may lead to a longer presence of polyphenols within the body.**(Manach, 2004)**

V. Diabetes and chronical disease related to gastro-intestinal system overview:

Diet and nutrition are known to play key roles in many chronic gastrointestinal diseases, regarding both pathogenesis and therapeutic possibilities. A strong correlation between symptomatology, disease activity and eating habits has been observed in many common diseases, both organic and functional, such as inflammatory bowel disease and irritable bowelsyndrome. New different dietary approaches have been evaluated in order improve patients' symptoms, modulating the type of sugars ingested, the daily amount of fats or the kind of metabolites produced in gut. Even if many clinical studies have been conducted to fully understand the impact of nutrition on the progression of disease, more studies are needed to test the most promising approaches for different diseases, in order to define useful guidelines for patients. **(Bengmark, 2005)**

Introduction the chronic metabolic disorder diabetes mellitus (DM) is a fast-growing global problem with huge social, health, and economicconsequences. DM is a major endocrine

metabolic disorder characterized by increased blood glucose, due to insulin production deficiency by pancreatic cells or by the ineffectiveness of the endogenous insulin. It is estimated that globally 285 millionpeople (approximately 6.4% of the adult population) are sufferingfrom this disease and the number is estimated to increase to 430million in the absence of better control or cure.Around 90% of all cases are associated with type 2 DM(noninsulin-dependent diabetes mellitus, NIDDM). Type 2 DM (T2DM) mainly associated with dysfunction of pancreatic cellas well as insulin resistance in skeletal muscle, liver and fat cellsleading to hyperglycemia and complications such as neuropathy,nephropathy, retinopathy and cardiomyopathy. Therefore,effective control over the elevated blood glucose (glycemic control) in diabetic patients is the main objective for reversing DM,preventing complications and improving quality of life.Beside glycemic control, continuous medical care along withpatient self-management is required for prevention of acute aswell as long-term complications.An increased prevalence of DM and its related complicationslead many researchers to for search hypoglycemic agents with better efficacy.Many new compounds from natural origindemonstrated the potential for treatment of DM and its complications.**(Kamble, 2013)**

VI. Natural polyphenols in prevention of chronical disease related to gastro-intestinal system and diabetes:

The natural polyphenols exemplifies a novel and relevant strategy in the treatment of human degenerative disorders. They play an important role by promoting the growth of Bifidobacterium sp. in the human GI tract this bacteria reduces the pH in the intestinal area and henceforth provides protection there. There are certain metabolic products of polyphenols that are demarked separately as they provide improvised actions against IBD patients. The herbal polyphenols gets hydrolysed through the intestinal brush border cells and thereby able to reduce the enzyme produced toxicity. This property shows a promising characteristic of the polyphenols that has the ability of modulating the human gut micro flora and enhancing certain friendly mechanisms within. On the line parallel to this, focus will also be on other human degenerative disorders that can be prevented by the action of the polyphenols.

Intestinal diseases have also been associated with oxidative stress. Specifically, oxidative stress has been shown to cause a defective barrier function leading to intestinal pathologies. Thus, Yang et al. investigated the protective effects of Red-osier dogwood (*Cornus stolonifera* Michx.) polyphenolic extracts against hydrogen peroxide-induced damage in Caco-2 intestinal epithelial cells. The results showed that Red-osier dogwood extract’s treatment increased cell viability and

decreased ROS through increased expression of antioxidant enzymes such as hemeoxygenase-1 (HO-1), superoxide dismutase (SOD), and glutathione peroxidase (GPx) in Caco-2 cells.

The expression of all these enzymes was probably due to the enhanced protein expression of the nuclear factor (erythroid-derived 2)-like 2 (Nrf-2), the most important transcription factor regulating antioxidant genes' expression. Red-osier dogwood extract was also shown to increase the transepithelial resistance (TEER) value through inhibition of disorganization of tight junction proteins such as zonula occludens-1 (ZO-1) and claudin-3.

Finally, Red-osier dogwood extract decreased in Caco-2 cells markers (e.g., interleukin 8) of inflammation which plays important role in intestinal diseases. In general, there is interdependence between oxidative stress and inflammation resulting in many chronic diseases. Anti-inflammatory activity was also shown to be possessed by polyphenolic extracts from mulberry species. In particular, Negro et al. isolated polyphenolic extracts from Italian mulberry local varieties belonging to *MorusAlba* and *Morus nigra* species. The *M.Alba* and *M. nigra* extracts contained five main anthocyanin compounds as identified by HPLC/DAD/MS analysis. The extracts from all the tested mulberry varieties exhibited in vitro strong free radical scavenging and inhibited cyclooxygenase (COX) activity (a marker of inflammation). It is not only the polyphenols that affect the gastrointestinal system, but also the gastrointestinal digestion may affect polyphenols activity.

For example, David et al. used a simulated in vitro digestion model to investigate gastrointestinal digestion's effects on the antioxidant capacity of Cornelian (*Cornus mas* L.) cherry fruit extract. The results showed that presence of three anthocyanins (i.e., cyanidin-3-*O*-galactoside, pelargonidin-3-*O*-glucoside, and pelargonidin-3-*O*-rutinoside) found in Cornelian cherry fruits, was not significantly affected by the gastric digestion. However, intestinal digestion decreased the anthocyanin content and antioxidant activity of the fruit extract indicating that its polyphenolic content stability during gastrointestinal digestion should be taken into consideration for estimating its bioavailability. **(Stagos, 2019)**

For diabetes; Natural products have become a powerful tool to combat against oxidative stress since phytochemicals provide the main source for antioxidants. These antioxidants help in improving insulin secretion and hepatic glycogen storage and reduce oxidative stress. **(Pasupuleti, 2020)**

Antioxydant may act as physical barriers to prevent ROS generation or access to important biological sites ; chemical traps/sinks that absorb energy and electrons,quenching ROS (carotenoids,anthocyanidins) ; catalytic systems that neutralise or divert ROS (antioxidant

enzymes SOD (superoxyde dismutase), catalase, gluthioneperoxydase) ; binding/inactivation of metal ions to prevent generation of ROS (ferritin, ceruloplasmin, catechins) ; and cain-breaking antioxydants which scavenge and destroy ROS (ascorbic acid, tocopherols, uric acid, glutathione, flavonoids**) (karadag et al.,2009).**

VII.Ceratonia siliqua L(carob):

1. Origin and Geographic Distribution:

Ceratonia siliqua L. belongs to legumes' family, it is widely cultivated in Mediterranean region, where it is considered as a natural component of biodiversity and a famous local product, used by local population since ancient times for alimentation as well as traditional remedy. **(Mouas et al.2021)**

2. Nutritional Value

Carob fruit is relatively caloric sice 100 g of carob flour give about 222 kcal/933 kJ **(Mouas et al.2021)**.

3. Current Uses

a) Traditional Use

Several ethnopharmacological surveys reported carob tree among most cited plants by herbalist and local informant for nutritional value and treating gastro-intestinal system diseases. **(Mouas et al.2021)**

b) Food Industry:

Carob pod pulp is used as cattle feed in addition to barley floor.

Carob flour obtained by grinding terrified dried pods after shelled is widely used in dietary food industry due to its high content in sugars, free gluten and phenols whichrecommended for oeucolic persons, it is also used in preparation of milk flour, drinks, citric acid, jams, sirup, honey as substitute of cacao in chocolate and biscuits.

Seeds tires is used as substitute of pectin, gelatin, stabilizer, fixer in several products such as cheese, sauces, mayonnaise; it is also used as thickening E410 in candy production.**(Mouas et al. 2021)**

c) Cosmetology

Due to its capacity to form viscous solutions at low concentrations, its thinking, emulsifier and stabilizing proprieties; it is used as natural adjuvant in soaps, creams, toothpaste...

4. Chemical Composition

a. Primary Metabolites

Carob tires

Major constituent of carob tires is a galactomannane (80–85%), afunctional polysaccharide present in carob seeds, in addition to 13% of lipids; 4% of proteins, 1–4% of celluloses and lignin and 1% of ashes and water content. **(Mouas et al,2021)**

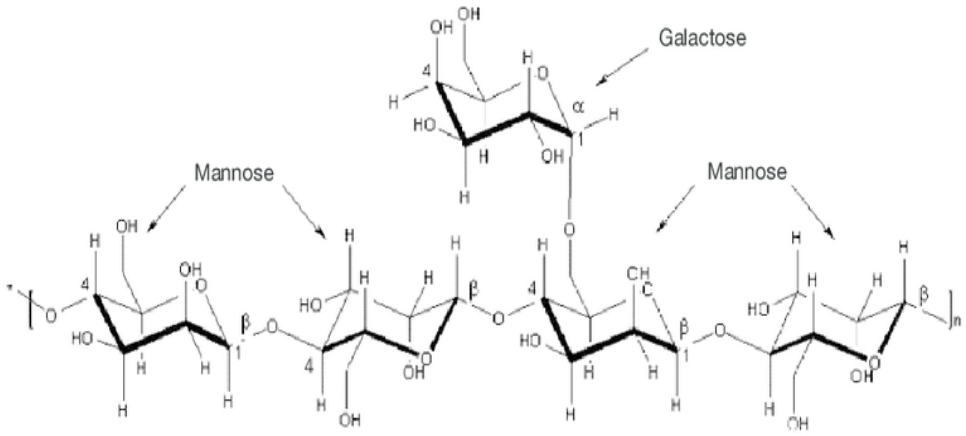


Fig 2: galactomannane**(Patrick,2009)**

Pod pulp

Pod pulp is rich in simple hydrocarbs (saccharose, fructose, glucose) and fibers and its content depends on cultivars, soils, seasons and climates.

Algerian cultivars reports 37,5 to 45,3% total sugars content, and according to Avallone (1997)**,** the average composition is 27–40% of Saccharose, 3–5% of Glucose, 3–8% of Fructose, 2–6% of Proteins, 0,4–0,6% of Lipids, 2–3% of ashes and 27–50% of Fibers. **(Mouas et al,2021)**

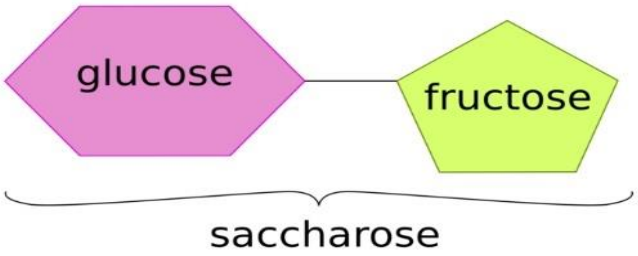


Fig 3: saccharose **(internet)**

b. SecondaryMetabolites:

Phenols

Carob is an interesting phenols source (16–20%), with a high molecular weight even in comparison with other plants, responsible of its antioxidant activity.

According to Owen (2003), flavones content in carob is about 0.132 g/kg), Würsch (1984) and Saura-Calixto (1988) reported a tannin content of 16–20% on dry weight in pods.

Kamal K. et al. *(*2013) reported that coumarins content in carob flour is about 4, 49 ppm while lignins are about 33.06 ppmprevious work on Ceratonia siliqua L.

Recently, the studied tree was reported to have multiple pharmacological activities, especially in the digestive tract, including antioxidant, antidiarrheal, antibacterial, anti-ulcer and anti-inflammatory actions. **(Rtibi et al, 2017)**

Another study indicates that carob has beneficial effects on blood cholesterol levels, blood sugar levels, liver and kidney functions.**(Attia et al, 2014)(Mouas et al,2021)**.

Table1: Biological and pharmacological activities of Ceratonia siliqua L. **(Tazir, 2020)**

Therapeuticactivities	Action	References
cholesterol-lowering effect	Consumption of carob fiber reduced LDL/HDL cholesterol levels and triglycerides	Zunft et *al*, 2003
Hypoglycemic effect	The immature carob prevents intestinal glucose absorption by inhibition of electrogenic glucose transport	Rtibi et *al*, 2017
Anti-inflammatory effects and Antiulcer	The aqueous extract of carob pods exhibits a protective effect against inflammation of the intestinal tract introduced by DSS (dextran sodium sulfate)	Rtibi et *al*, 2016
Effect of carob on liver function	The administration of carob fiber caused a significant decrease in ALT, AST, ALT and ALP for diabetics and a significant decrease in total protein, albumin, AST, ALT, and ALP for hypercholesterolemia.	Attia et *al*, 2014
Effect of carob on kidney functions	The administration of carob fiber in rats induced a significant decrease in urea, uric acid and creatinine for diabetics and a decrease in urea and creatinine for hypercholesterolemia.	
The antifungal effect	The methanolic leaf extract caused complete inhibition of mycelial growth at 25 mg/ml. This inhibition exceeds 59% from the dose of 3.125 mg/ml.	Fadel et *al*, 2011

The antiproliferative effect	Carob leaf extract inhibits tumor cell proliferation. Carob extract showed a much higher antiproliferative effect in neuroblastoma and on lines human breast cancer cells.	Corsi et *al*, 2002 Roseiro et *al*, 2013

VIII. Zingiber officinale

1. Historical and Popular Uses:

Ginger is used worldwide as a cooking spice, condiment and herbal remedy.

It has a pH of 5.6 to 5.9, similar to that of figs, fennel, leeks, parsnips and lettuce

TheChinese have used ginger for at least 2500 years as a digestive aid and antinausea remedy and totreat bleeding disorders and rheumatism; it was also used to treat baldness, toothache, snakebite,and respiratory conditions1.

In Traditional Chinese Medicine (TCM), ginger is considered apungent, dry, warming, yang herb to be used for ailments triggered by cold, damp weather.

Ginger is used extensively in Ayurveda, the traditional medicine of India, to block excessiveclotting (i.e. heart disease), reduce cholesterol and fight arthritis.

In Malaysia and Indonesia,ginger soup is given to new mother for 30 days after their delivery to help warm them and tohelp them sweat out impurities.

In Arabian medicine, ginger is considered an aphrodisiac.

Some Africans believe that eating ginger regularly will help repel mosquitoes.

Ginger migrated westward to Europe by Greek and Roman times.

The Greeks wrappedginger in bread and ate it after meals as a digestive aid. Subsequently, ginger was incorporateddirectly into bread and confections such as gingerbread.

Ginger was so valued by the Spanishthat they established ginger plantations in Jamaica in the 1600's.

Nowadays, ginger is extensively cultivated from Asia to Africa and the Caribbean and isused worldwide as a nausea remedy, as an anti-spasmodic and to promote warming in case ofchills.

Ginger is also extensively consumed as a flavoring agent; it is estimated that in India,the average daily consumption is 8 -10 grams of fresh ginger root. **(Kathi J, 1999)**

Chemical analysis of ginger shows that it contains over 400 different compounds. The major constituents in ginger rhizomes are carbohydrates (50–70%), lipids (3–8%), terpenes, and phenolic compounds.

Terpene components of ginger include zingiberene, β-bisabolene, α-farnesene, β-sesquiphellandrene, and α- curcumene, while phenolic compounds include gingerol,paradols, and shogaol

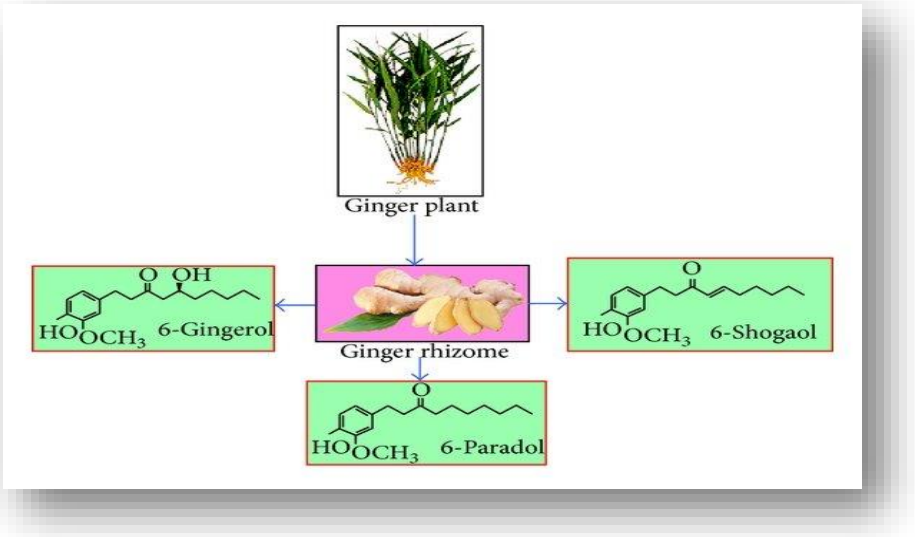


Fig 4: Ginger, ginger rhizome, and its major active components:6-gingerol, 6-shogaol, and 6-paradol.**(Sahdeo,2015)**

These gingerols (23–25%) and shogaol (18–25%) are found in higher quantity than others. Besides these, amino acids, raw fiber, ash, protein, phytosterols, vitamins (e.g., nicotinic acid and vitamin A), and minerals are also present. **(Sahdeo,2015)**

2. Medicinal Uses and Some Market Preparations:

Arthritis: It reduces inflammatory eicosanoids without the side effects of other anti-inflammatory drugs and NSAIDS.

Heart and circulatory problems: Ginger offer substantial protection from stroke and heart attack because of its ability to help prevent blood clotting.

Its antioxidant constituents strengthened the cardiac muscle and also lower serum cholesterol levels by interfering with cholesterol biosynthesis.

Fever reducer: It can assist in lowering a fever. Its antibacterial/antiviral effects help to reduce the incidence of colds altogether.

Digestive problems: It is commonly used for indigestion because it absorbs and neutralizes toxins in the stomach. It also improves the production and secretion of bile from the liver and gallbladder. Bile aids in the digestion of fats, which helps to lower cholesterol levels.

It is also used as Antioxidant, Antitoxic, Eicosanoid balance, Enzyme activity, Probiotic support, Serotonergic, Systemic stimulant

Some of the market preparations containing Ginger (Shunth) are pachnol, Hajmola, Hingoli, garam masala, chana masala, dristi eye drops, chaat masala, divyachurna, aloo bhujia, pav bhaji masala, shahi paneer masala, etc...**(Yogeshwar Sharma, 2017)**

- **Antiviral effect:**

Fresh rhizome of *Z. officinale* has been proven with an antiviral effect against Human Respiratory Syncytial Virus (HRSV) infection via decreasing HRSV-induced plaque formation in respiratory mucosal cell lines.

Therefore, high concentration of *Z. officinale* could stimulate mucosal cells to secrete IFN-β which responsible in counteracting viral infections by reducing viral attachment and internalization.

The lyophilized juice extract of *Z. officinale* is considered as containing antiviral effect against Hepatitis C viral infection. **(Kankanam,2020)**

- **Anti-inflammatory Effect:**

Z. officinale is highly effect in inflammations associated with alimentary channel such as colitis.

The plant responsible with poshatidylinositol-3-kinese (PI3K), protein kinese B (Akt) and the nuclear factor kappa light chain enhancer of activated B cells (NF-κB), as well as 6-shogaol responsible in protective effects against tumor necrosis factor α (TNF-α) induced intestinal dysfunction in human intestinal cell models. **(Kankanam,2020)**

- **Anti-cancer Effect**

Z. officinale exhibits anti-inflammatory and anti-tumorigenic effects due to its bio active molecules such as 6-gingerole, 6-shogaol, 6-paradol and zerumbone, as a result prevention or control from colorectal, gastric ovarian, liver, breast and prostate cancers is possible.

Z. officinale activates enzymes such as glutathione peroxidase, glutathione s transferase and glutathione reductase and suppress colon carcinogenesis.

gingerol is effect in liver cancers by arresting cell cycle and induction of apoptosis. Growth inhibition of human epidermoid carcinoma cells via reactive oxygen species (ROS) induced apoptosis is exhibited by gingerol with considerable amount of toxicity.

Active compounds of *Z. officinale* effect in controlling ovarian cancers via inhibition of NF-κB activation and diminished the secretion of VEGF and IL-8. **(Kankanam,2020)**

- **Antioxidant Activity**

Z. officinale is effective in Parkinson's disease because zingerone, an active ingredient in ginger scavenged peroxide and hydroxyl ions as well as suppress lipid peroxidation.

Ginger consists with Reno protective effect in renal failures because of anti-inflammatory properties by attenuating serum C-reactive protein levels and antioxidant effects by reducing lipid peroxidase marker, malondialdehyde levels and increasing renal superoxide dismutase activity. **(Kankanam,2020)**

Through proper digestion and absorptions, as well as maintaining proper circulations ginger supports elevation of waste productions while physiological functions.

Chapter two:

Dietary supplements andPhytochemicals

I. Natural Food supplements:

Food supplements are concentrated sources of nutrients or other substances that can have nutritional or physiological effects, where the purpose is to supplement the normal diet. Food supplements are marketed in "dose" forms, such as pills, tablets, capsules, and liquids in measured doses. Supplements can be used to correct nutritional deficiencies or to maintain adequate intake of certain nutrients. However, in some cases excessive intake of vitamins and minerals might be harmful or because unwanted side effects; therefore, indications for their maximum levels are necessary to ensure their safe use in food supplements.**(EFSA, 2015b)**

II. Gummyform:

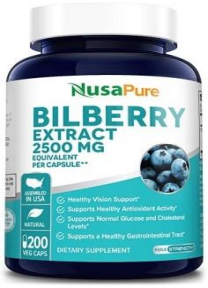
The increased interest in functional materials of natural origin has resulted in a higher market demand for preservative-free, "clean label", or natural ingredients-based products. The gummy bear food supplements are more acceptable to consumers and have fewer limitations compared to other dosage forms. **(Čižauskaitė, 2019)**

By looking at previous research and references, it was proven that this is possible, so we can produce natural ingredients-based gummy.

It has been reported that a gummy bear's base usually consists of the jellifying agent (pectins, modified starch, gelatin etc.) and sugars, where water-soluble ingredients can be dissolved and the insoluble ones are suspended in the viscous matrix. Therefore, the application range of gummies in the pharmaceutical and food industry as a novel drug delivery system, which is more acceptable to children and some adults due to the confectionary appearance and taste, is wide. Some studies have determined that the composition of gummy bears, especially the concentration and origin of gelling agent and sugars, has a significant impact on the rheological properties of the product. An increasing amount of gelatin in a food matrix has been shown to increase the thickness of the product associated with a reduction in the perception of flavor. According to L. DeMars and R.G. Ziegler, gelled products are easily made on a gelatin base though opportunities still exist for improving and modifying their texture since various possible textural changes have never been adequately defined and quality evaluation assays of gummy bears have been suggested and performed, it is relevant to produce a superior gummy bear base from the health perspective composed of natural ingredients, which could be further used to incorporate various active ingredients and additives. **(Čižauskaitė, 2019)**

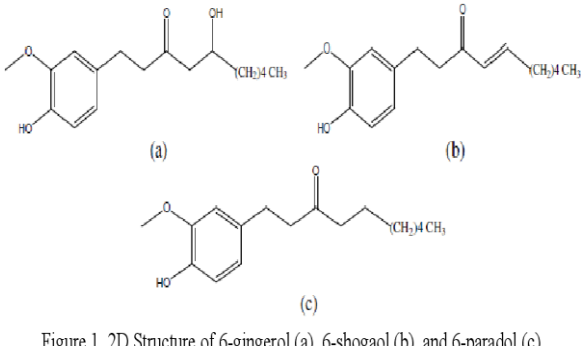
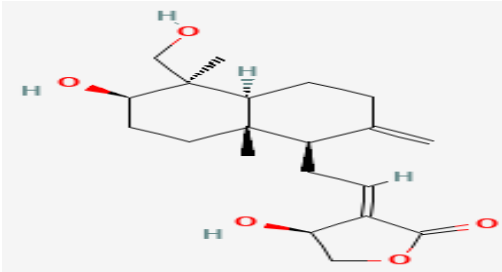
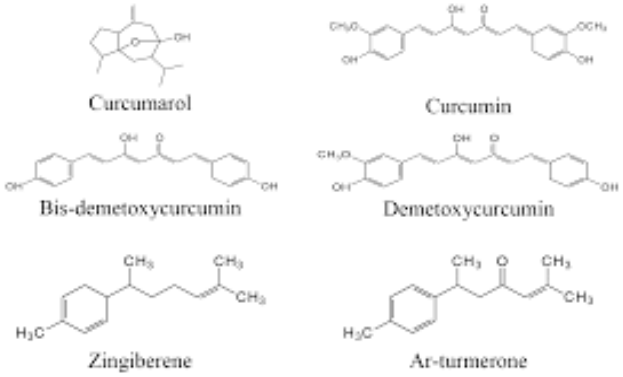
III. Natural products used in the dietary supplements industry:

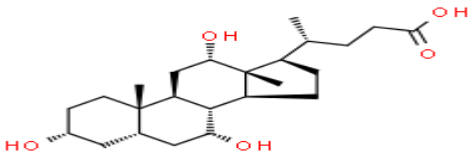
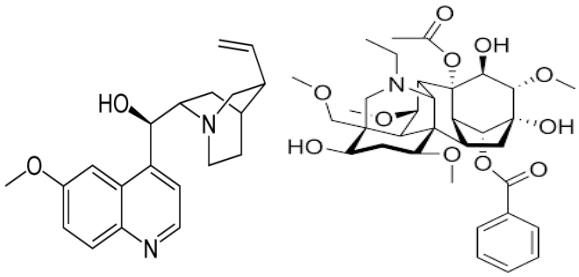
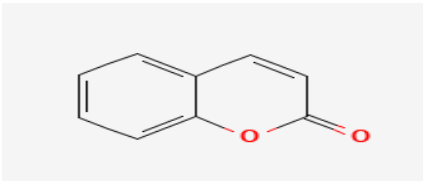
Table2: Natural products used in the dietary supplement industry.

Natural product	Therapeutic effect	Food supplement mark	Dose
Garlic	Reduce cardiovascular disease risk, anti-tumor, anti-microbial, benefits hyperglycemia		1000 mg per soft gel.
zingiber officinale (ginger)	digestive support		550 mg.
Turmeric	Provides antioxidant benefits		550 mg per serving.
Carob	Weight loss; reduce blood sugar and insulin levels, and lower cholesterol levels.		120 ml.
Bilberry	Healthy vision support, antioxidant activity, supports normal glucose and cholesterol levels also healthy gastrointestinal tract.		2500 mg per capsule.

IV. Secondary metabolites responsible for the therapeutic effect:

Table 3: Secondary metabolites responsible for the therapeutic effect

Secondary metabolites	Therapeutic potential	Structure
Active isolated from zingiber officinale (paradol, gingerol)	Increase appetite; treat the digestive tract disorder such as nausea and vomit, to treat common cold, cough, diarrhea, malaria, fever and arthritis.	 Figure 1. 2D Structure of 6-gingerol (a), 6-shogaol (b), and 6-paradol (c) **Fig 5:**2D Structure of 6-gingerol (a), 6-shogaol (b), and 6-paradol (c**) (saptarini, 2013)**
Andrographolide isolated from A.panicu-lata ness	Treat high blood pressure, fever, malaria, diabetes, gastrointestinal disorders, inflammation, dysentery, and cancer.	 **Fig6:**Andrographolide structure **(pubchemncbi, 2022)**
Curcumarol, curcumin, bis-demetoxycurcumin, demetoxycurcumin, zingiberene, Ar-turmerone (from curcumadomestica).	Treat diabetes, leprosy, gastrointestinal disorders, tonic, laxative, rheumatic, antiseptic, hepatic disorders, and cancer.	 **Fig7:** Some active compounds of curcuma domestica **(sholikhah, 2016)**

Terpenoids	Inhibit cancer cell proliferation and metastasis.	 **Fig 8:** Terpenes and terpenoids **(chemsrc, 2022)**
Alkaloids (quinine, aconitine)	Antimalarial activity, treatment of rheumatism, neuralgia, sciatica, purgative, antitussive, and sedatives in snake bite, fever, and insanity.	 **Fig 9:** Quinine, aconitine structure
Coumarin	Anticoagulant (inhibit the action of vitamin k), antioxidant (protect the cellular DNA from oxidative damage), antibacterial, anti-inflammatory, antitumor, antiviral.	 **Fig 10:** Coumarin chemical structure description **(pubchem ncbi,2022)**

V. Pharmacovigilance; regulation and control:

The World Health Organization (WHO) defines pharmacovigilance as the science and activities relating to the detection, evaluation, understanding and prevention of adverse effects or any other problem related to marketed medicines. It encompasses in particular risk management and the prevention of medication errors, the dissemination of information on medication, action in favor of the rational use of medication and preparedness for crisis situations **(WHO, I.S.D.B, 2005).**

In Algeria,the National Pharmacovigilance and Materiovigilance Center (CNPM) is concerned by this guard, it was created by Executive Decree No. 98-192 of 8 Safar 1419 corresponding to June 3, 1998 on the creation, organization and operation of a CNPM.

According to the decree, the mission of the center is:

Monitoring of adverse reactions due to the use of medicinal products placed on the market and of incidents or risks of incidents resulting from the use of medical devices.

The realization of any study or work concerning the safety of use of drugs and medical devices during various administrations and uses to perform prophylactic diagnostic and therapeutic acts.**(cnpm.org.dz)**

However food supplements are not subject to regulation in our country they fall under the agri-food regulatory frameworks; they are considered as food.

As mentioned in the guide:

Regulations concerning medical devices, reagents, medicinal plants, cosmetic products and food supplements are not mentioned in this guide. **(cnpm.org.dz).**

However,a toxic file related to the following consumption of natural products as: Dietary compounds, cosmetics, and phytoproducts are provided on the CNPM web site, to detect and alert on any sort of toxicity regarding this class of natural products, these cards can be fill in and sent by the pharmacy, Doctors or patients themselves in case of observed intoxication.

Chapter three:

Extraction processes

Classic methods still the most used extraction process in artisanal and industrial scale, for their safety for environment and sensitive bioactive compounds, large yield, economy, and above all their practical use, it involves:

I. Enfleurage

Enfleurage is one of the oldest processes. It is based on the affinity of perfumes for fats. In this extraction system, there are two methods depending on the resistance of the plant to heat: cold enfleurage and hot enfleurage.

Cold enfleurage can treat the most delicate flowers (such as jasmine or tuberose). Practically, the flower petals are manually and delicately placed one by one on glass plates coated with a thin layer of odorless grease. Then, these plates are superimposed on wooden frames. The volatile substances diffuse and are absorbed by the fat layer. After a few days, the fat is saturated with plant essence. **(Abuakar, 2020)**

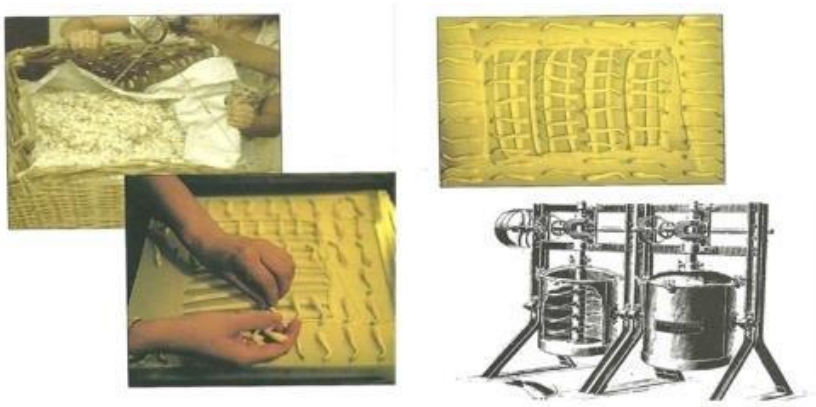


Fig 11: Photos of the cold enfleurage device. **(Chenni,El abed, 2017)**

The flowers are periodically renewed 10 to 15 times until the fat is saturated (2 kg of flowers for 1 kg of fat). Once the fragrant fat has been collected, it is melted in a water bath, decanted and filtered. After cooling, a floral ointment is obtained which faithfully restores the smell of the flower and which is then exhausted with alcohol. Hot enfleurage consists of infusing the least fragile flowers (such as rose de Mai, cassia, violet or orange blossom) in odorless fats or oils previously heated in a bain-marie.**(Abuakar, 2020)**

II. Decoction:

In this process, the crude drug is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. Thisprocedure is suitable for extracting water-soluble, heat-stable constituents.This process is typically used in preparation of Ayurvedic extracts called“quath” or “kawath”. The starting ratio of crude drug to water is fixed, e.g.1:4 or 1:16; the

volume is then brought down to one-fourth its original volumeby boiling during the extraction procedure. Then, the concentrated extracts filtered and used as such or processed further.

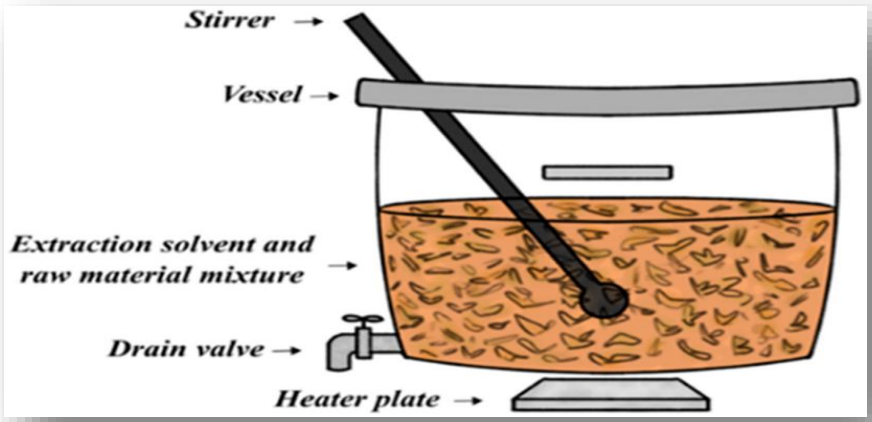


Fig 12: The aqueous decoction of plant raw material. **(Miralrio, 2020)**

III. Maceration

This is an extraction procedure in which coarsely powdered drug material, either leaves or stem bark or root bark, is placed inside a container; the menstruum is poured on top until completely covered the drug material. The container is then closed and kept for at least three days. The content is stirred periodically, and if placed inside bottle it should be shaken time to time to ensure complete extraction. At the end of extraction, the micelle is separated from marc by filtration or decantation. Subsequently, the micelle is then separated from the menstruum by evaporation in an oven or on top of water bath. This method is convenient and very suitable for thermosensitive plant material.**(Abdullahi R,2020)**

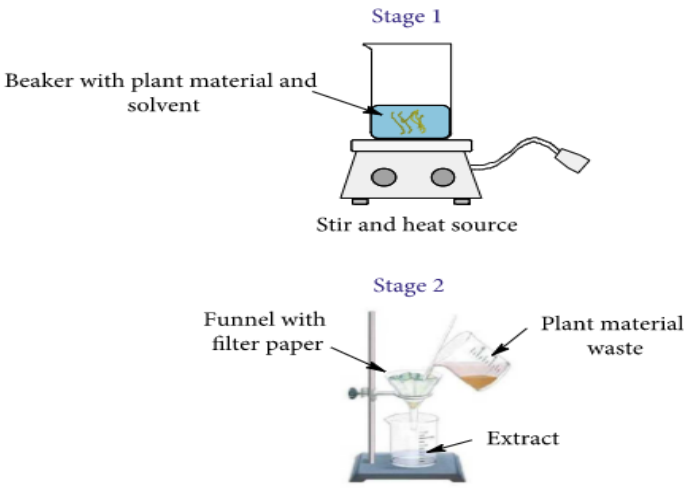


Fig13: Picture of maceration extraction.**(Luna, 2020)**

IV. Percolation

The apparatus used in this process is called percolator. It is a narrow-cone-shaped glass vessel with opening at both ends. A dried, grinded, and finely powdered plant material is moistened with the solvent of extraction in a clean container. More quantity of solvent is added, and the mixture is kept for a period of 4h. Subsequently, the content is then transferred into percolator with the lower end closed and allow to stand for a period of 24h.The solvent of extraction is then poured from the top until the drug material is completely saturated. The lower part of the percolator is then opened, and the liquid allowed dripping slowly. Some quantity of solvent was added continuously, and the extraction taken place by gravitational force, pushing the solvent through the drug material downward. The addition of solvent stopped when the volume of solvent added reached 75% of the intended quantity of the entire preparations. The extract is separated by filtration followed by decantation. The marc is then expressed and final amount of solvent added to get required volume.**(Abdullahi R, 2020)**

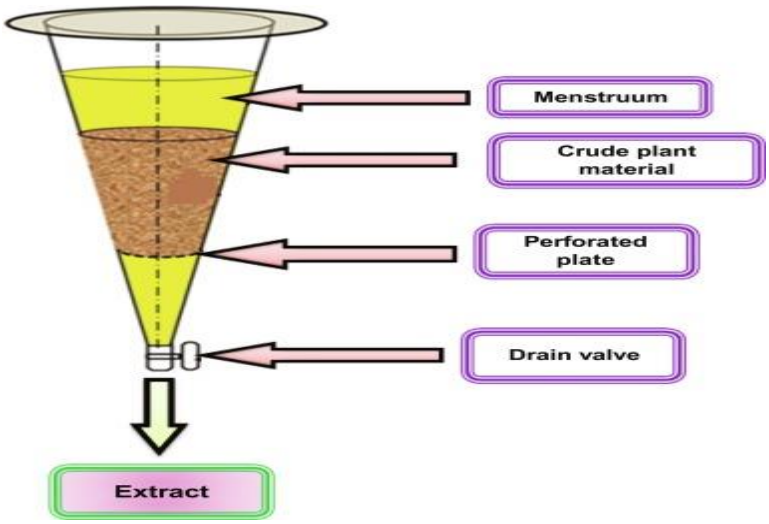


Fig14: The pictorial representation of soxhlet extraction. **(Alara, 2021)**

V. Infusion:

This is an extraction process such as maceration. The drug material is grinded into fine powder, and then placed inside a clean container. The extraction solvent hot or cold is then poured on top of the drug material, soaked, and kept for a short period of time this method is suitable for extraction bioactive constituents that are readily soluble. In addition, it is an appropriate method for preparation of fresh extract before use. The solvent to sample ratio is usually 4:1 or 16:1 depending on the intended use.

Experimental Part

Chapter one:

Materials and methods

For this study, a vegetable and a spice: Carob and Ginger respectively, very prized by costumers were chosen to be formulated as pilots according to previous *in vitro* investigations and market survey on functional and dietary foods made by our research team **(teniou t, 2021)**, which revealed their high therapeutic potential, low cost and availability. Its medicinal effect with the structure activity relationship and synergy interactions were also investigated.

I. Materials:

1. Plant material:

The plants used in the present study are:

Ginger (zingiber officinale):

Ginger has a very long history of use in various forms of traditional and alternative medicinePeople typically use it in cooking or herbal tea, and some take ginger supplements for their possible health benefits.

Ginger can be used fresh, dried, powdered, or juice

Carob (Ceratonia siliqua):

Carob products consumed by humans come from the dried, sometimes roasted, pod, which has two main parts: the pulp accounts for 90% and the seeds 10% by weight. Carob pulp is sold either as flour or "chunks".

On the way to making gummy, we can use carob in its four forms: carob flour, kibbled carob, carob seeds or whole carob.

2. Formulation material:

All adjuvant used to make the gummy are natural with health benefits without toxic effect at low cost:

Adjuvant

- Vegetable Butter.
- Natural vinegar.
- Vegetable gelatin.
- Stevia sugar.
- Distilled water.

Standard laboratory equipment

II. Methods of study:

As mentioned above, the extraction protocols from which we chose decoction and infusion for carob (ceratonia siliqua) powder, and enfleurage to ginger (zingiber officinale).

1. Preparation of the plants samples:

For carob, it was used as powder after drying and grinding it or just crack it, for gingerit was cut fresh or grinding after drying to use the powder also as a juice after grinding it fresh and filtering.

1.1.Carob (ceratonia siliqua) extraction:

- **Decoction :**

Table4: Formulation of Carob in gummy making with decoction and their taste tests.

Gummy making	Quantity of ingredients
First attempt	400ml water 40g carob powder 50g gelatin
Second try	200ml water 30g carob powder 20g gelatin

- **Infusion:**

Table5: Formulation of Carob in gummy making with infusion and their taste tests.

Gummy making	Quantity of ingredients
First attempt	100ml vinegar 15g carob 15g gelatin
Second try	100ml vinegar 12g carob 10g gelatin

1.2.Ginger (zingiber officinale) extraction:

- **Enfleurage:**

With acetic acid as preservative:

Table6: Formulation of Ginger gummy making with enfleurage,acetic acid extractionand taste tests.

	Quantity of ingredients	
Without sugar	First try	Water =100 ml Ginger = 35g Vinegar = 50 ml Butter = 20 g Gelatin = 5 teaspoons +25 teaspoons water
	Second try	Ginger juice =100g Butter =33 g Vinegar = 1 spoon Gelatin = 5 teaspoons +25 teaspoons water
With sugar	First try	Sugar = 5 g Water =100 ml Ginger =35 g Butter =33 g Vinegar = 1 spoon Gelatin =5 teaspoons +25 teaspoons water

Without acetic acid:

Table7: Formulation of Ginger gummy making with enfleurage andtheir taste test.

	Quantity of ingredients	
Without sugar	First try	Water =100 ml Ginger = 45 g Butter = 40 g Gelatin = 5 teaspoons+25 teaspoons water
	Second try	Ginger juice =100g Butter =33 g Gelatin = 5 teaspoons +25 teaspoons water
With sugar	First try	Sugar = 10 g Ginger = 50g Butter =50 g Gelatin =5 teaspoons +25 teaspoons water
	Secondtry	Sugar = 5 g Water =100 ml Ginger =35 g Butter =17 g Gelatin =5 teaspoons+25 teaspoons water

2. **The quality control:**

It’s important for ensuring consumer safety and efficacy, this study were to assess the quality of our product byanalyzing; physicochemical, Microbiological, organoleptic and taste properties.

(1)

(2) (3)

(4)

(1) Weight the amount of ginger, carob powder, sugar, gelatin and butter

(2) Carob extract after boiling and filtering

(3) Ginger mixture after melting it with butter and sugar

(4) Put ginger and carob mixture in silicone mall and remove it after it colds and became thick.

Chapter two:

Results and discussion

The quality control of the finished products was carried out by analysing:

- ✓ The organoleptic properties,
- ✓ The physicochemical analysis,
- ✓ Microbiological quality and stability study by accelerated aging test,

According to the Algerian official newspaper, Algerian or international standards.

I- Sweet ginger Gummies 1

1. Taste tests

Table 8: Sweet ginger gummies 1 taste tests

	Opinion
Taster1	Buttery flavor with spicy aroma
Taster2	Sour with pungent melting in the mouth
Taster3	Delicious lightly sweet and spicy

2. Quality control

2.1.Organoleptic properties

Identification

Table 9: Sweet ginger gummies 1 organoleptic properties identification

Product: Gummies with 1 sweet ginger.	**Date of Manufacture:**
Nature of the Product: Gelatin.	Product Category: Other Confectionery Products (Caramels, candies, nougats,halkouma.)
Type of packaging: Food.	

Analyses

Table 10: Sweet ginger gummies 1 organoleptic property analyses

Analyses performed	**Results**	**Methods**
Aspect	Gelatinous	Sensory
Color	Light green	Sensory
Odor	Spicy, characteristic of ginger.	Sensory
Overall conclusion:	Conforms to the product data sheet.	

2.2. Physicochemical analysis

Identification

Table 11: Sweet ginger gummies 1 physicochemical analysis identification

Product: Gummies with 1 sweet ginger.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category: Other Confectionery Products (Caramels, candies, nougats,halkouma.)
Type of packaging: Food.	

Analyses

Table 12: Sweet ginger gummies 1 physicochemicalanalysis

Analyses performed	**Results**	**Methods**
Solubility	Very good	Cold mixing
Ph	5.43	Ph meter
Brix° Soluble Dry Extract	19%	Refractometer
Overall conclusion:	**Conforms to the product data sheet.**	

2.3. Microbiological analysis

Table 13: Sweet ginger gummies 1 microbiological analysis identification

Product: Gummies with 1 sweet ginger.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category: Other Confectionery Products (Caramels, candies, nougats,halkouma.)
Type of packaging: Food.	

Analyses

Table 14: Sweet ginger gummies 1 microbiological analysis

Analyses performed	**Sample**					**Reference**	**Standards**
	1	**2**	**3**	**4**	**5**		
Aerobic Germs at 30°c*10	4.0	3.9	4.3	3.8	4.1	*NA ISO 4833*	$r<10^5<10^6$
Total coliforms	00	00	00	00	00	NA ISO 4831	$r<2<10^2$
Moisissures	03	00	02	00	00	[ARRÊTÉ 02/06/2015] J.O. N°48 2015	$r<10<10^2$
Salmonella/25g	Abs	Abs	Abs	Abs	Abs	NA ISO 6579	Abs
OVERALL CONCLUSION:	Satisfactory result according to the interdepartmental decree of 04 October 2016 establishing the microbiological criteria for foodstuffs (JORADPN°39 of 02 July 2017).						

2.4. Stability test

Table 15: Sweet ginger gummies 1 stability test identification

Product: Gummies with 1 sweet ginger.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category:
Type of packaging: Food.	

Analyses

Table 16: Sweet ginger gummies 1 stability test identification analyses

Analyses performed	**1control unit at 20-25°c**	**2 units at 30°c for 21 days**	**References**
Physicochemical characters: -Appearance	No apparent bulging, flaking or leakage defects were found	No apparent bulging, flaking or leakage defects were found	
-pH	5.22	5.43	NFV 08-402
Microbiological Characteristics: -Microbial Flora Count /15*10-4 mm^2	240,35	195	
-R factor	/	0.811	
General conclusion	The product is stable according to the interdepartmental order of 04 October 2016 establishing the microbiological criteria for food (JORADPN°39 of 02 July 2017).		

II- Gummies with Sweet Ginger 2

1. Taste tests

Table 17: Sweet ginger gummies 2 taste tests

	Opinion
Taster1	Slightly peppery and sharp
Taster2	It has spicy aroma much like garlic
Taster3	Chewy and lightly sweet

2. Quality control

2.1.Organoleptic properties

Identification

Table 18: Sweet ginger gummies 2 organoleptic properties identification

Product: Gummies with 2 sweet ginger	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category: Other Confectionery Products (Caramels, candies, nougats,halkouma.)
Type of packaging: Food.	

Analyses

Table 19: Sweet ginger gummies 2 organoleptic property analyses

Analyses performed	**Results**	**Methods**
Aspect	Gelatinous	Sensory
Color	Light green	Sensory
Odor	Spicy, characteristic of ginger.	Sensory
Overall conclusion:	Conforms to the product data sheet.	

2.2.Physicochemical analysis

Identification

Table 20: Sweet ginger gummies 2 physicochemical analysis identification

Product: Gummies with 2 sweet ginger	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category: Other Confectionery Products (Caramels, candies, nougats,halkouma.)
Type of packaging: Food.	

Analyses

Table 21: Sweet ginger gummies 2 physicochemical analysis

Analyses performed	**Results**	**Methods**
Solubility	Very good	Cold mixing
Ph	4.46	Ph meter
Brix° Soluble Dry Extract	19%	Refractometer
Overall conclusion:	Conforms to the product data sheet.	

2.3.Microbiological analysis

Table 22: Sweet ginger gummies 2 microbiological analysis identification.

Product: Gummies with 2 sweet ginger	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category: Other Confectionery Products (Caramels, candies, nougats,halkouma.)
Type of packaging: Food.	

Analyses

Table 23: Sweet ginger gummies 2 microbiological analysis

Analyses performed	**Sample**					**Reference**	**Standards**
	1	**2**	**3**	**4**	**5**		
Aerobic Germs at 30°c*10	2.9	3.2	3.5	3.1	3.4	*NA ISO 4833*	$r<10^5<10^6$
Total coliforms	00	00	00	00	00	NA ISO 4831	$r<2<10^2$
Moisissures	01	00	00	00	00	[ARRÊTÉ 02/06/2015] J.O. N°48 2015	$r<10<10^2$
Salmonella/25g	Abs	Abs	Abs	Abs	Abs	NA ISO 6579	Abs
OVERALL CONCLUSION:	Satisfactory result according to the interdepartmental decree of 04 October 2016 establishing the microbiological criteria for foodstuffs (JORADPN°39 of 02 July 2017).						

2.4.Stability test

Table 24: Sweet ginger gummies 2 stability test identification.

Product: Gummies with 2 sweet ginger	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category:
Type of packaging: Food.	

Analyses

Table 25: Sweet ginger gummies 2 stability test identification analyses.

Analyses performed	**1control unit at 20-25°c**	**2 units at 30°c for 21 days**	**References**
Physicochemical characters: -Appearance	No apparent bulging, flaking or leakage defects were found	No apparent bulging, flaking or leakage defects were found	
-pH	4.3	4.49	NFV 08-402
Microbiological Characteristics: -Microbial Flora Count /15*10-4 mm^2	305,5	200,1	
-R factor	/	0,655	
General conclusion	The product is stable according to the interdepartmental order of 04 October 2016 establishing the microbiological criteria for food (JORADPN°39 of 02 July 2017).		

III- Sugar-free Ginger Gummies 1

1. Taste tests

Table 26: Sugar-free Ginger Gummies 1 taste tests

	Opinion
Taster1	So bitter with acrid flavor
Taster2	Strongly flavored but has soft texture
Taster3	Lightly sour and moist

2. Quality control

2.1.Organoleptic properties

Identification

Table 27: Sugar-free Ginger Gummies 1organoleptic properties identification

Product: Gummies with ginger 1 without sugar.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category: Other Confectionery Products (Caramels, candies, nougats,halkouma.)
Type of packaging: Food.	

Analyses

Table 28: Sugar-free Ginger Gummies 1organoleptic properties analyses

Analyses performed	**Results**	**Methods**
Aspect Color Odor	Gelatinous Light green Spicy, characteristic of ginger.	Sensory Sensory Sensory
Overall conclusion:	Conforms to the product data sheet.	

2.2.Physicochemical analysis

Identification

Table 29: Sugar-free Ginger Gummies 1 physicochemical analysis identification

Product: Gummies with ginger 1 without sugar.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category: Other Confectionery Products (Caramels, candies, nougats,halkouma.)
Type of packaging: Food.	

Analyses

Table 30: Sugar-free Ginger Gummies 1physicochemical analysis

Analyses performed	**Results**	**Methods**
Solubility Ph Brix° Soluble Dry Extract	Average 6.25 12%	Cold mixing Ph meter Refractometer
Overall conclusion:	Conforms to the product data sheet.	

2.3.Microbiological analysis

Table 31: Sugar-free Ginger Gummies 1 microbiological analysis identification

Product: Gummies with ginger 1 without sugar.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category: Other Confectionery Products (Caramels, candies, nougats,halkouma.)
Type of packaging: Food.	

Analyses

Table 32: Sugar-free Ginger Gummies 1 microbiological analysis

Analyses performed	**Sample**					**Reference**	**Standards**
	1	**2**	**3**	**4**	**5**		
Aerobic Germs at 30°c*10	4.2	3.9	4.1	4.5	3.7	*NA ISO 4833*	$r<10^5<10^6$
Total coliforms	00	00	00	00	00	NA ISO 4831	$r<2<10^2$
Moisissures	00	01	00	00	03	[ARRÊTÉ 02/06/2015] J.O. N°48 2015	$r<10<10^2$
Salmonella/25g	Abs	Abs	Abs	Abs	Abs	NA ISO 6579	Abs
OVERALL CONCLUSION:	Satisfactory result according to the interdepartmental decree of 04 October 2016 establishing the microbiological criteria for foodstuffs (JORADPN°39 of 02 July 2017).						

2.4.Stability test

Table 33: Sugar-free Ginger Gummies 1 stability test identification

Product: Gummies with ginger 1 without sugar.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category:
Type of packaging: Food.	

Analyses

Table 34: Sugar-free Ginger Gummies 1 stability test analyses

Analyses performed	**1control unit at 20-25°c**	**2 units at 30°c for 21 days**	**References**
Physicochemical characters: -Appearance -pH	No apparent bulging, flaking or leakage defects were found 6.1	No apparent bulging, flaking or leakage defects were found 6.25	NFV 08-402
Microbiological Characteristics: -Microbial Flora Count /15*10-4 mm^2 -R factor	320,3 /	246,7 0,77	
General conclusion	The product is stable according to the interdepartmental order of 04 October 2016 establishing the microbiological criteria for food (JORADPN°39 of 02 July 2017).		

IV- Sugar-free Ginger Gummies 2

1. Taste tests

Table 35: Sugar-free Ginger Gummies 2 taste tests

	Opinion
Taster1	Clammy texture with butter flavor
Taster2	Pungent and spicy but have pudding texture
Taster3	Slight bitter gentle on the tongue

2. Quality control

2.1.Organoleptic properties

Identification

Table 36: Sugar-free Ginger Gummies 2 organoleptic properties identification

Product: Gummies with ginger 2 without sugar.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category: Other Confectionery Products (Caramels, candies, nougats,halkouma.)
Type of packaging: Food.	

Analyses

Table 37: Sugar-free Ginger Gummies 2organoleptic properties analyses

Analyses performed	**Results**	**Methods**
Aspect Color Odor	Gelatinous Light green Spicy, characteristic of ginger.	Sensory Sensory Sensory
Overall conclusion:	Conforms to the product data sheet.	

2.2.Physicochemical analysis

Identification

Table 38: Sugar-free Ginger Gummies 2 physicochemical analysis identification

Product: Gummies with ginger 2 without sugar.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category: Other Confectionery Products (Caramels, candies, nougats,halkouma.)
Type of packaging: Food.	

Analyses

Table 39: Sugar-free Ginger Gummies 2physicochemical analysis

Analyses performed	**Results**	**Methods**
Solubility Ph Brix° Soluble Dry Extract	Average 4.23 12%	Cold mixing Ph meter Refractometer
Overall conclusion:	Conforms to the product data sheet.	

2.3.Microbiological analysis

Table 40: Sugar-free Ginger Gummies 2 microbiological analysis identification

Product: Gummies with ginger 2 without sugar.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category: Other Confectionery Products (Caramels, candies, nougats,halkouma.)
Type of packaging: Food.	

Analyses

Table 41: Sugar-free Ginger Gummies 2 microbiological analysis

Analyses performed	**Sample**					**Reference**	**Standards**
	1	**2**	**3**	**4**	**5**		
Aerobic Germs at 30°c*10	2.0	2.2	2.4	2.1	2.3	*NA ISO 4833*	$r<10^5<10^6$
Total coliforms	00	00	00	00	00	NA ISO 4831	$r<2<10^2$
Moisissures	00	00	00	00	00	[ARRÊTÉ 02/06/2015] J.O. N°48 2015	$r<10<10^2$
Salmonella/25g	Abs	Abs	Abs	Abs	Abs	NA ISO 6579	Abs
OVERALL CONCLUSION:	Satisfactory result according to the interdepartmental decree of 04 October 2016 establishing the microbiological criteria for foodstuffs (JORADPN°39 of 02 July 2017).						

2.4.Stability test

Table 42: Sugar-free Ginger Gummies 2 stability test identification

Product: Gummies with ginger 1 without sugar.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category:
Type of packaging: Food.	

Analyses

Table 43: Sugar-free Ginger Gummies 2 stability test analyses

Analyses performed	**1control unit at 20-25°c**	**2 units at 30°c for 21 days**	**References**
Physicochemical characters: -Appearance	No apparent bulging, flaking or leakage defects were found	No apparent bulging, flaking or leakage defects were found	
-pH	4.15	4.23	NFV 08-402
Microbiological Characteristics: -Microbial Flora Count /15*10-4 mm^2	190,8	153,2	
-R factor	/	0,802	
General conclusion	The product is stable according to the interdepartmental order of 04 October 2016 establishing the microbiological criteria for food (JORADPN°39 of 02 July 2017).		

V- Gummies with Carob acetic acid extract without added sugar

1. Taste tests

Table 44: Carob gummies with acetic acid extract taste tests

	Opinion
Taster 1	Very slightly sweet much like raw chocolate
Taster 2	Sour with chewy texture
Taster 3	Very inviting sweet flavor and mellows fast

2. Quality control

2.1.Organoleptic properties

Identification

Table 45: Carob gummies with acetic acid extract organoleptic properties identification

Product: Gummies with acetic acid extract of carob without added sugar.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category: Other Confectionery Products (Caramels, candies, nougats,halkouma.)
Type of packaging: Food.	

Analyses

Table 46: Carob gummies with acetic acid extract organoleptic properties analyses

Analyses performed	**Results**	**Methods**
Aspect Color Odor	Gelatinous Dark brown Caramelized and pungent, characteristic of carob and acetic acid.	Sensory Sensory Sensory
Overall conclusion:	Conforms to the product data sheet.	

2.2.Physicochemical analysis

Identification

Table 47: Carob gummies with acetic acid extract physiochemical analysis identification

Product: Gummies with acetic acid extract of carob without added sugar.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category: Other Confectionery Products (Caramels, candies, nougats,halkouma.)
Type of packaging: Food.	

Analyses

Table 48: Carob gummies with acetic acid extractphysiochemical analysis

Analyses performed	**Results**	**Methods**
Solubility Ph Brix° Soluble Dry Extract	Good 3.69 18%	Hot infusion. Ph meter Refractometer
Overall conclusion:	Conforms to the product data sheet.	

2.3.Microbiological analysis

Table 49: Carob gummies with acetic acid extract microbiological analysis identification

Product: Gummies with acetic acid extract of carob without added sugar.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category: Other Confectionery Products (Caramels, candies, nougats,halkouma.)
Type of packaging: Food.	

Analyses

Table 50: Carob gummies with acetic acid extract microbiological analysis

Analyses performed	**Sample**					**Reference**	**Standards**
	1	**2**	**3**	**4**	**5**		
Aerobic Germs at 30°c*10	1.0	1.5	1.2	1.0	1.1	*NA ISO 4833*	$r<10^5<10^6$
Total coliforms	00	00	00	00	00	NA ISO 4831	$r<2<10^2$
Moisissures	00	00	00	00	00	[ARRÊTÉ 02/06/2015] J.O. N°48 2015	$r<10<10^2$
Salmonella/25g	Abs	Abs	Abs	Abs	Abs	NA ISO 6579	Abs
OVERALL CONCLUSION:	Satisfactory result according to the interdepartmental decree of 04 October 2016 establishing the microbiological criteria for foodstuffs (JORADPN°39 of 02 July 2017).						

2.4.Stability test

Table 51: Carob gummies with acetic acid extract stability test identification

Product: Gummies with acetic acid extract of carob without added sugar.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category:
Type of packaging: Food.	

Analyses

Table 52: Carob gummies with acetic acid extract stability test analyses

Analyses performed	**1control unit at 20-25°c**	**2 units at 30°c for 21 days**	**References**
Physicochemical characters: -Appearance	No apparent bulging, flaking or leakage defects were found	No apparent bulging, flaking or leakage defects were found	
-pH	3.5	3.69	NFV 08-402
Microbiological Characteristics: -Microbial Flora Count /15*10-4 mm^2	69,7	45,9	
-R factor	/	0,658	
General conclusion	The product is stable according to the interdepartmental order of 04 October 2016 establishing the microbiological criteria for food (JORADPN°39 of 02 July 2017).		

VI- Gummies with aqueous extract of carob without added sugar

1. Taste tests

Table 53: Gummies with aqueous extract of carob taste tests

	Opinion
Taster 1	It tend to be little earthy
Taster 2	Have a slight sweetness that is reminiscent of caramel
Taster 3	Unique taste like cacao good to be sweet

2. Quality control

2.1. Organoleptic properties

Identification

Table 54: Gummies with aqueous extract of carob organoleptic properties identification

Product: Gummies with aqueous extract of Carob without added sugar.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category: Other Confectionery Products (Caramels, candies, nougats,halkouma.)
Type of packaging: Food.	

Analyses

Table 55: Gummies with aqueous extract of carob organoleptic properties analyses

Analyses performed	**Results**	**Methods**
Aspect Color Odor	Gelatinous Dark brown Caramelized, characteristic of Carob.	Sensory Sensory Sensory
Overall conclusion:	Conforms to the product data sheet.	

2.2. Physicochemical analysis

Identification

Table 56: Gummies with aqueous extract of carob physicochemical analysis identification

Product: Gummies with aqueous extract of Carob without added sugar.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category: Other Confectionery Products (Caramels, candies, nougats,halkouma.)
Type of packaging: Food.	

Analyses

Table 57: Gummies with aqueous extract of carob physicochemical analysis

Analyses performed	**Results**	**Methods**
Solubility Ph Brix° Soluble Dry Extract	Very good 4.39 22%	Hot decoction Ph meter Refractometer
Overall conclusion:	Conforms to the product data sheet.	

2.3. Microbiological analysis

Table 58: Gummies with aqueous extract of carob microbiological analysis identification

Product: Gummies with aqueous extract of Carob without added sugar.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category: Other Confectionery Products (Caramels, candies, nougats,halkouma.)
Type of packaging: Food.	

Analyses

Table 59: Gummies with aqueous extract of carob microbiological analysis

Analyses performed	**Sample**					**Reference**	**Standards**
	1	**2**	**3**	**4**	**5**		
Aerobic Germs at 30°c*10	2.5	2.7	2.9	3.0	3.2	*NA ISO 4833*	$r<10^5<10^6$
Total coliforms	00	00	00	00	00	NA ISO 4831	$r<2<10^2$
Moisissures	00	00	00	00	00	[ARRÊTÉ 02/06/2015] J.O. N°48 2015	$r<10<10^2$
Salmonella/25g	Abs	Abs	Abs	Abs	Abs	NA ISO 6579	Abs
OVERALL CONCLUSION:	Satisfactory result according to the interdepartmental decree of 04 October 2016 establishing the microbiological criteria for foodstuffs (JORADPN°39 of 02 July 2017).						

2.4. Stability test

Table 60: Gummies with aqueous extract of carob stability test identification

Product: Gummies with aqueous extract of Carob without added sugar.	Date of Manufacture:
Nature of the Product: Gelatin.	Product Category:
Type of packaging: Food.	

Analyses

Table 61: Gummies with aqueous extract of carob stability test analyses

Analyses performed	**1control unit at 20-25°c**	**2 units at 30°c for 21 days**	**References**
Physicochemical characters: -Appearance -pH	No apparent bulging, flaking or leakage defects were found 4.26	No apparent bulging, flaking or leakage defects were found 4.39	NFV 08-402
Microbiological Characteristics: -Microbial Flora Count /15*10-4 mm^2 -R factor	103,8 /	90,4 0,871	
General conclusion	The product is stable according to the interdepartmental order of 04 October 2016 establishing the microbiological criteria for food (JORADPN°39 of 02 July 2017).		

- **Discussion**

Formulation:

The obtained gummy form is practical and very suitable for adults as well as children, it contains an efficient non-toxic dose of therapeutic natural agents, different recipes were tested in order to study the quality control and stability of the final products

Quality contrôle and stability :

- All obtained forms were tested according to national standards, which gave satisfactory results and are suitable to be produced and commercialized in local market, which doesn't need sanitary insurance according to the CNPM.
- We also observed that preservatives and sugar are not needed in case of natural fruit pulp and ginger.
- And are stable at least for 15 days at room temperature < 20°.

Otherwise, tested formulations could be more optimized for better organoleptic proprieties as taste, texture…

Chapter three:

In silico study

I- Structure activity relationship (SAR):

1. Ginger bioactive compounds:

Ginger is abundant in active constituents, such as phenolic and terpene compounds. The phenolic compounds in ginger are mainly gingerols, shogaols, and paradols. In fresh ginger, gingerols are the major polyphenols, such as 6-gingerol, 8-gingerol, and 10-gingerol. With heat treatment or long-time storage, gingerols can be transformed into corresponding shogaols. Afterhydrogenation, shogaols can betransformedintoparadols**. (Mao, 2019)**

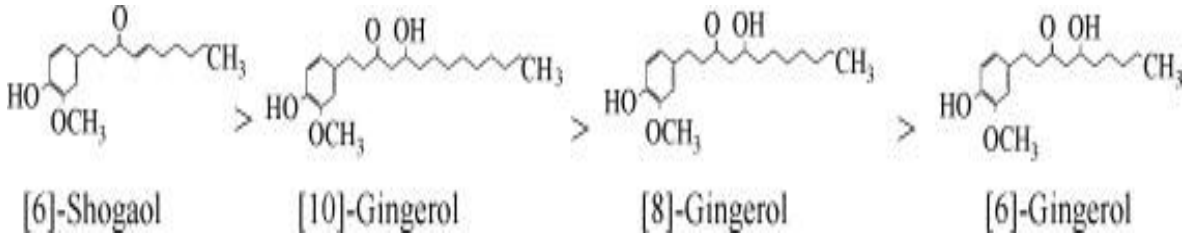


Fig15: Bioactive compounds of ginger **(Dugasani, 2010)**

Antioxidant activity:

Ginger and its bioactive compounds (such as 6-shogaol) exhibited antioxidant activity via the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway. In human colon cancer cells, 6-shogaol increased intracellular glutathione/glutathione disulfide (GSH/GSSG) and upregulated Nrf2 target gene expression, such as with heme oxygenase-1 (*HO-1)*, metallothionein 1 (*MT1*), aldo-keto reductase family 1 member B10 (*AKR1B10*), ferritin light chain (*FTL*), and γ-glutamyltransferase-like activity 4 (*GGTLA4*). Besides, 6-shogaol also enhanced the expression of genes involved in glutathione synthesis, such as the glutamate-cysteine ligase catalytic subunit (*GCLC*) and the glutamate-cysteine ligase modifier subunit (*GCLM*). Further analysis revealed that 6-shogaol and its metabolite activated Nrf2 via the alkylation of cysteine residues of Kelch-like ECH-associated protein 1 (Keap1). Moreover, ginger phenylpropanoids improved Nrf2 activity and enhanced the levels of glutathione S-transferase P1 (GSTP1) as well as the downstream effector of the Nrf2 antioxidant response element in foreskin fibroblast cells. In a human mesenchymal stem cell model, ginger oleoresin was investigated for its effects on injuries that were induced by ionizing radiation. The treatment of oleoresin could decrease the level of ROS by translocating Nrf2 to the cell nucleus and activating the gene expression of *HO-1* and *NQO1* (nicotinamide adenine dinucleotide phosphate (NADPH) quinone dehydrogenase 1), in addition, ginger extract could reduce the production of ROS in human fibrosarcoma cells

with H_2O_2-induced oxidative stress. In stressed rat heart homogenates, ginger extract decreased the content of malondialdehyde (MDA), which was related to lipid peroxidation.

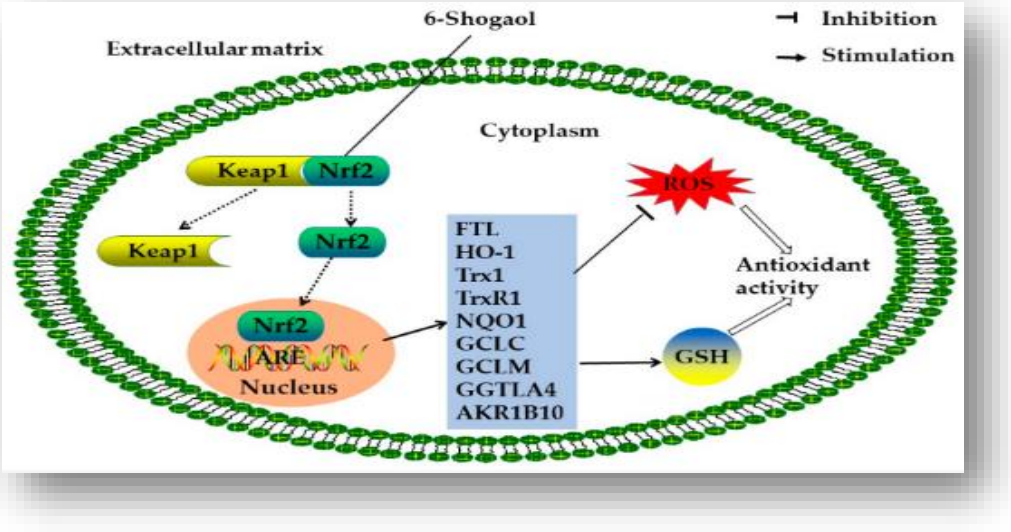


Fig16: The potential mechanism for the antioxidant action of 6-shogoal. **(Mao, 2019)**

Antidiabetic activity:

Diabetes mellitus is known as a severe metabolic disorder caused by insulin deficiency and/or insulin resistance, resulting in an abnormal increase in blood glucose. Prolonged hyperglycemia could accelerate protein glycation and the formation of advanced glycation end products (AGEs). Many research works have evaluated the antidiabetic effect of ginger and its major active constituents.

An in vitro experiment resulted in both 6-shogaol and 6-gingerol preventing the progression of diabetic complications, and they inhibited the production of AGEs by trapping methylglyoxal (MGO), the precursor of AGEs. Additionally, 6-gingerol reduced the levels of plasma glucose and insulin in mice with high-fat diet-induced obesity. Nε-carboxymethyl-lysine (CML), a marker of AGEs, was decreased by 6-gingerol through Nrf2 activation. In 3T3-L1 adipocytes and C2C12 myotubes, 6-paradol and 6-shogaol promoted glucose utilization by increasing AMPK phosphorylation. In addition, in a mouse model fed a high-fat diet, 6-paradol significantly reduced the level of blood glucose. In another study, 6-gingerol facilitated glucose-stimulated insulin secretion and ameliorated glucose tolerance in type 2 diabetic mice by increasing glucagon-like peptide 1 (GLP-1). Besides, 6-gingerol treatment activated glycogen synthase 1 and increased cell membrane presentation of glucose transporter type 4 (GLUT4),which increased glycogen storage in skeletal muscles. Furthermore, the consumption of ginger could reduce the levels of fasting plasma glucose, glycated hemoglobin A

($HbA1_C$),insulin, TG, and TC in patients with type 2 diabetes mellitus (DM2). Moreover, ginger extract treatment improved insulin sensitivity in rats with metabolic syndrome, which might have been relevant to the energy metabolism improvement induced by 6-gingerol. In addition, ginger extract alleviated retinal microvascular changes in rats that had diabetes induced by streptozotocin. Ginger extract could reduce the levels of NF-κB, TNF-α, and vascular endothelial growth factor in the retinal tissue. In a randomized, double-blind, and placebo-controlled trial, the ingestion of ginger decreased the levels of insulin, low-density lipoprotein cholesterol (LDL-C), and TG; decreased the homeostasis model assessment index; and increased the quantitative insulin sensitivity check index in patients with DM2.

The studies have demonstrated that ginger and its bioactive compounds could protect against diabetes mellitus and its complications, probably by decreasing the level of insulin, but increasing the sensitivity of insulin. **(Mao, 2019)**

Table62: In vitro hypoglycemic potentials of ginger and its bioactive constituents. **(Wang, 2020)**

In vitro study	Result/outcome	References
[6]-Gingerol on 3 T3-L1 cells	Enhanced differentiation of 3T3-L1 preadipocytes and insulin-sensitive glucose uptake	Sekiya et al. [42]
[6]-Shogaol or [6]-gingerol on 3 T3-L1 cells	Significant inhibition of TNF-α-mediated adiponectin expression in 3T3-L1 adipocytes. [6]-Shogaol acted as a peroxisome proliferator-activated receptor (PPAR)γ agonist, while [6]-gingerol acted by suppressing TNF-α-induced JNKs signaling	Isa et al. [43]
Ethyl acetate extract of ginger on L6 myotube cell surface	Stimulated glucose uptake and GLUT4 expression in L6 myotube cell surface, reduced lipid content in 3T3 adipocyte, and inhibited protein glycation. Inhibited α-amylase (IC_{50} = 980.2 μg/mL) and α-glucosidase (IC_{50} = 180.1 μg/mL)	Rani et al., [44]
Aqueous extract of ginger at 5, 10, 20, 40 g/L incubated with (PBS), glucose + BSA for 5 weeks	Dose-dependent, antidiabetic activity through inhibition of glucose diffusion and reduced glycation	Sattar et al., [45]

2. Carob bioactive compounds:

Carob fruit is a complex mixture of primary and secondary metabolites, with the presence of sugars and fibers being characteristic for these fruits, followed by a great diversity of polyphenols. Numerous minerals and amino acids are also present in carob fruits.

Numerous studies have revealed several physiological responses to carob fruit and its products that may be relevant to the promotion of human health and the prevention or treatment of some chronic diseases. Below we categorize the health benefits of the carob fruit. **(Goulas, 2016)**

Table63: The chemical components of carob and their biological evaluation**. (Goulas, 2016)**

Group of Chemical Constituents/Individual Substances	Biological Evaluation of Constituents/Disease	Carob Parts/Fraction
LBG/galactomannan	Gastrointestinal effects	Seed endosperm
D-Pinitol	Anti-diabetic activity	Carob pulp

Gallic acid:

Gallic acid (GA) is a naturally occurring polyphenol compound present in fruits, vegetables, and herbal medicines. GA has antioxidant, anticancer, anti-inflammatory, and antimicrobial properties. GA and its derivatives have multiple industrial uses, such as food supplements or additives. Additionally, recent studies have shown that GA and its derivatives not only enhance gut microbiome (GM) activities, but also modulate immune responses. **(Yang, 2020)**

Enhance antioxydant properties :

- Gallic acid binds to γ-AlOOH nanoparticles (NPs) electrostatically and covalently
- Pure and modified with gallic acid (GA) alumina NPs are non-cytotoxic
- Pure γ-AlOOH nanoparticles showed antioxidant activity, GA enhanced it
- Modification of NPs with GA makes them membrane-protective of oxidative hemolysis
- Prepared samples are colloidally stable hydrosols without additives. **(Martacov, 2019)**

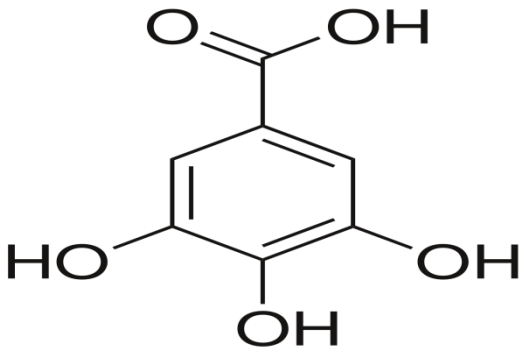


Fig 17 : Gallic acid structure. **(Internet)**

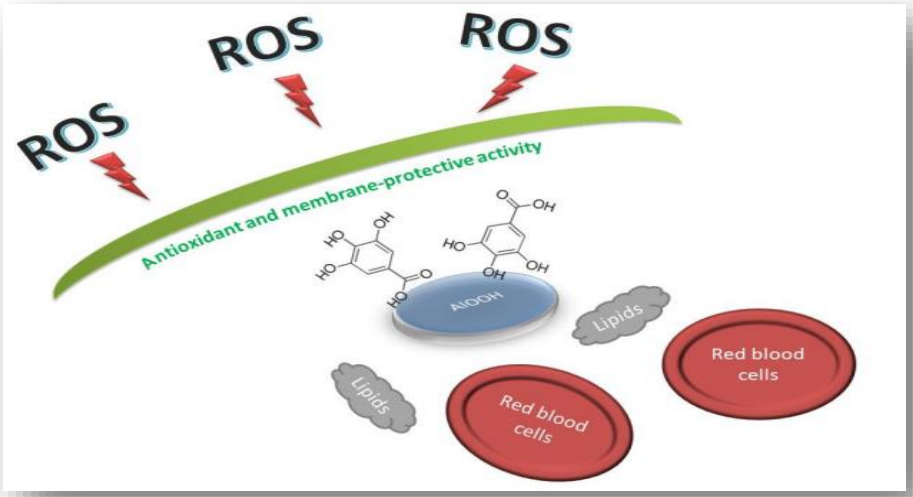


Fig 18: Graphical abstract of covalent enhancing activity of gallic acid. **(Martacov, 2019)**

D-Pinitol as Insulin Regulator:

In carob bean, the major cyclitol is D-pinitol (3-*O*-methyl-D-chiro-inositol) and its content showed great diversity (1.0–8.5 g 100 g^{-1}·d.m.) **(Goulas, 2016)**

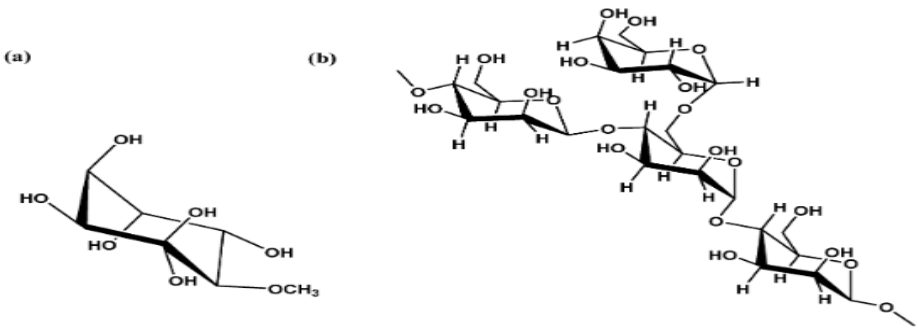


Fig 19: Chemical structures of (**a**) D-pinitol and (**b**) locust bean gum (LBG). **(Goulas, 2016)**

D-Pinitol has two mechanisms of action as an insulin regulator: insulin sensitizing and insulin mimetic.

K. Srivastava et al. present the insulin-sensitizing effect of D-Pinitol in their review article about this natural product, and a simplified illustration of this effect is shown in figure

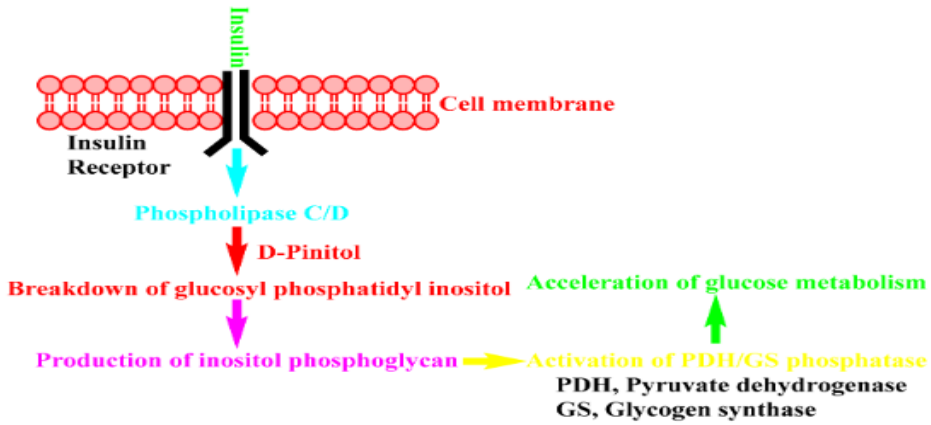


Fig20: Insulin-sensitizing mechanism of D-Pinitol. **(Azab, 2022)**

T. Antonowski et al. present the insulin-like (insulin-mimetic) activity of D-Pinitol. This publication, and others, demonstrates the simplified mechanism shown in figure 21:

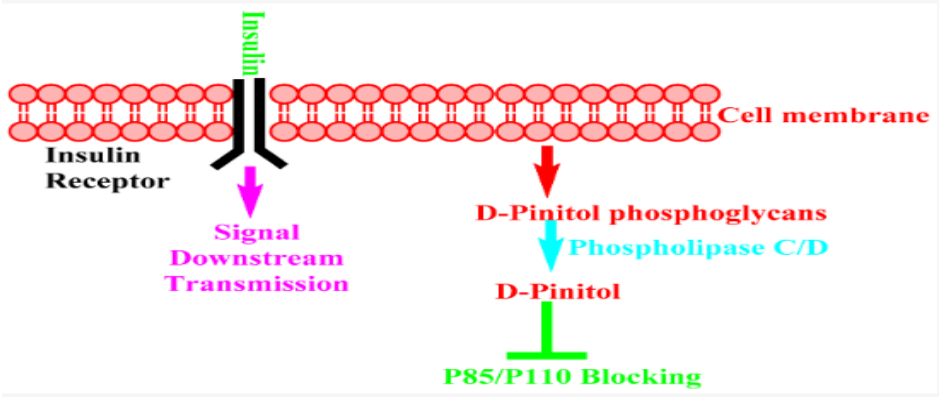


Fig21: Insulin-mimetic mechanism of D-pinitol. **(azab, 2022)**

Galactomannan:

Fenugreek is composed of a wide variety of bioactive compounds of which fiber, primarily the water-soluble fiber galactomannan, is suggested to be the effective component in observed reductions of heartburn and GERD symptoms. DiSilvestro found that the fenugreek fiber group (2000 mg, twice per day, standardized to contain 85% galactomannan), and the ranitidine group (75 mg, twice per day) both yielded reduced heartburn severity and incidence in subjects (n = 45)

The proposed mechanism for galactomannan's effect on heartburn and GERD involves the soluble fiber forming a raft when hydrated, acting as a barrier to ameliorate the rise of acid into the esophagus, and thus serving as an effective adjunct in the relief of GERD symptoms.

An animal study by Pandian et al. attributed galactomannan's superior antiulcerogenic ability to its observed reduction in gastric acid output but did not indicate a barrier mechanism. Based on this evidence, fenugreek and its constituent, galactomannan, hold promise for the management of upper GI symptoms or conditions.

As galactomannan is a component of other gums such as guar gum, locust bean gum, and partially hydrolyzed guar gum, these could be investigated for similar effects on heartburn reduction. In addition to galactomannan, soluble fiber as a whole is an evolving area of interest for upper GI symptom management. A prospective, open-label study (n = 30) observed an inverse relationship between increased fiber intake, specifically psyllium fiber (15 g per day), and occasional heartburn, esophageal sphincter resting pressure, and heartburn frequency in non-erosive GERD patients with previous low dietary fiber intake, defined as less than 20 g per day. This trial lacked a placebo and was short in duration (10 days). **(Schulz, 2022)**

II. Synergetic interactions:

1. Ginger active compounds synergy:

According to HPLC chemical profile of chosen plants in addition to establish medicinal indications in some references **(teniou,slimani, 2021)**, the following information's could be extracted:

- In regards of majority compounds present in both plants and their interactions with each other's, a possible synergetic effect could be observed in ginger betweencompounds with a close time of retention, because of similar chemical affinity when fractioning the crud.

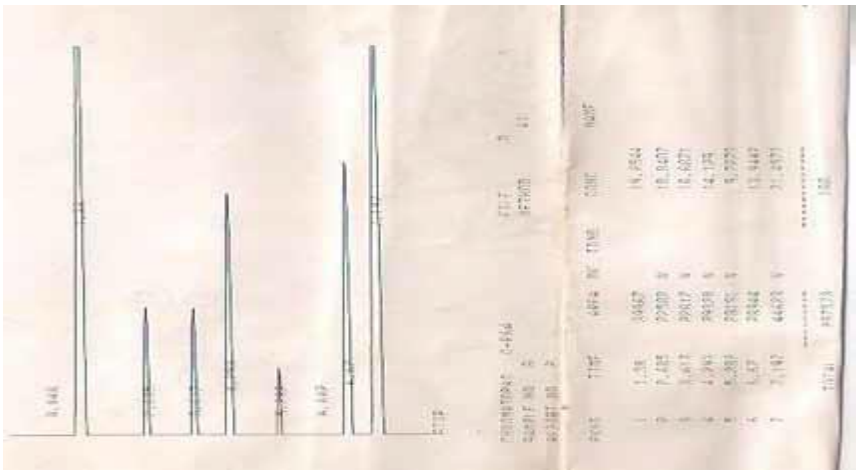


Fig22: HPLC chromatogram of ginger standard**. (Hasan, 2012)**

- This indicates possible short interactions especially on the OH sites in addition to cell medium (ph) influence.

2. **Carob active compounds synergy:**

This synergy may confer an additional therapeutic effect, more solubility and less toxicity for a better absorption in certain cells membrane and digestive tract medium helped with ph, and carried by high molecular sugars and fibersas the majority of polyphenols in plants exist as glycosides with different sugar units and acylated sugars at different positions of the polyphenol skeletons.**(tsao, 2010) (singla, 2019)**

Table64: Phenolic acid composition (mg/g dry weight) of particular phenolicfractions of carob pods***. (Ayaz, 2007)**

Phenolic fractions				
Compounds	Free	Esters§	Glycosides¶	Total†
Gallic acid	1,249.5 ± 206.9	1,550.5 ± 119.8	468.3 ± 40.3	3,268.4
Syringic acid	3.6 ± 0.7	n.d.	4.4 ± 0.6	8.0
Sinapic acid	1.8 ± 0.1	2.0 ± 0.2	0.7 ± 0.2	4.5
$\Sigma_{benzoics}$	1,253.1	1,551	472.7	3,276.8
$\Sigma_{cinnamic}$	1.8	2.0	0.7	4.5
$\Sigma_{benzoics}$ (%)	99.9	99.9	99.9	99.9
$\Sigma_{cinnamic}$ (%)	0.14	0.13	0.15	0.14
Total‡	1,254.9	1,552.5	473.4	3,280.9

* Values, means of three independent extractions and determinations (n = 3).
† Total is sum of each phenolic acid of four phenolic fractions.
‡ Total is sum of individual phenolic acids identified in each phenolic fraction.
§ MSPEs, methanol soluble phenolic esters.
¶ MSPGs,methanol soluble phenolic glycosides.

Bioavailability is also largely influenced by the structure of polyphenols. We have just begun to understand the reason why some flavonol glycosides are better absorbed than their aglycones, but very little is known on the influence of other structural parameters. **(scalbert, 2000)**

Polyphenols exist in foods and beverages in various chemical forms that determine their gut absorption. Chemical structures will also influence the conjugation reactions with methyl, sulfate or glucuronide groups and the nature and amounts of metabolites formed by the gut microflora absorbed at the colon level.

Understanding the structural factors that influence absorption and metabolism is essential to determine the polyphenols that are better absorbed and that lead to the formation of known active

metabolites. **Gut absorption**. Flavonoid glycosides. Certain classes of polyphenols, such as flavonols, isoflavones, flavones and anthocyanins, are usually glycosylated. The linked sugar is often glucose or rhamnose but can also be galactose, arabinose, xylose, glucuronic acid or other sugars (Harborne 1994). The number of sugars is most commonly one but can be two or three, and there are several possible positions of substitution on the polyphenol. The sugars can be further substituted, for example, with a malonic acid group.

The glycosylation influences chemical, physical and biological properties of the polyphenol. For example, partition coefficients measure the relative affinity of a compound for aqueous and organic phases and are important in determining whether a compound will passively diffuse across a biological membrane and how they might partition in a cell.

In these studies, most polyphenol glycosides are first deglycosylated and then converted to glucuronides or sulfates with or without methylation. **(scalbert, 2000)**

Conclusion and Perspectives

Conclusion

In Algeria, natural food market occupies an important place in cropping systems and in the diet of the population. Our target in the present study is to test a very prized technic of extraction in perfume industry the "**Enflorage**", to extract efficiently and safely sensitive bioactive compounds already known for their therapeutically effects in the prevention and maintenance of gastrointestinal disorders in several pharmacopeia, in order to formulate them into a new class of dietary products namely "**Gummies"**, the control of quality in addition to their chemical and biological interactions SAR, to establish synergetic effect of total crud in comparison of pure molecules effect.

- This research delivers an excellent extraction yield and solubility using Effleurage method in comparison with decoction and infusion ones.
- A good and characteristic organoleptic tests result for all formulations.
- A conform and satisfactory physic-chemical and microbiological quality.
- A high stability in time of gummy form.
- No toxic analyses are required in the case of these products according to previous clinical studies.
- Chemical fractioning by HPLC and SAR investigations, suppose a very interesting synergetic effect between active compounds present in crud, which make it more efficient and polyvalent than pure molecules.

  This insurance quality makes formulated Gummies good products for direct commercialization in pharmacies as well as in Super Market.

  As future research perspectives, followed actions are proposed:
- Rise up a start-up project, to shape this idea, with local market ability to commercialize those new natural products for Algerian costumer.
- To inspire new synthetic matrix and systems for improving pure bioactive compounds efficacy, safety, solubility and stability according to studied SAR and synergic effect.

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Année universitaire : 2021-2022

Présenté par : Allal yousra / Bouattit rayen

Formulation de polyphénols naturels comme Gummies dans la prévention et la régulation des maladies chroniques liées au système gastro-intestinal

Mémoire pour l'obtention du diplôme de Master en biochimie

Le marché des aliments naturels occupe une place importante dans les systèmes de culture et dans l'alimentation des populations.

La caroube (Ceratonia siliqua) et le gingembre (Zingiber Officinale) sont des plantes méditerranéennes typiques, principalement utilisées dans l'alimentation et la médecine populaire traditionnelle, ont été choisies pour étudier leurs effets protecteurs sur le diabète induit et les problèmes gastro-intestinaux.

Dans la présente recherche nous visons à formuler une nouvelle classe de produit diététique à savoir les gummies riches en composés bioactifs connus pour leur effet thérapeutique préalablement extrait par enfleurage, suivi d'un contrôle qualité en plus des interactions chimiques et biologiques RSA (relation structure activité).

Notre étude est finie par un excellent rendement d'extraction et une excellente solubilité par enfleurage ;

- Bonnes caractéristiques organoleptiques pour toutes les formulations.

- Qualité physico-chimique et microbiologique satisfaisante.

- Grande stabilité dans le temps de la forme gommeuse.

- Pas d'analyses toxiques selon les études cliniques précédentes.

- Effet synergique intéressant entre les composés actifs qui le rend plus efficace que les molécules pures.

selon cette qualité d'assurance, les gommes formulées conviennent aussi bien aux adultes qu'aux enfants, ainsi qu'à la commercialisation directe dans les pharmacies et les supermarchés.

Mots-clefs : Aliment fonctionnel, Ceratonia siliqua, Zingiber Officinale, produit diététique, gummies, enfleurage, contrôle de qualité.

Laboratoires de recherche :

Laboratoire

Encadreur : Dr. T. Nardjes MOUAS (prof- Université Frères Mentouri, Constantine 1).

Examinateur 1 : Pr. Djedouani Amel (ENS, Constantine 3)

Examinateur 2: Dr. Tabbi Aouatef (ENS, Constantine 3)

المقدم من : علال يسرى / بوعطيط ريان

السنةالدراسية: 2022-2021

تركيبة البوليفينول الطبيعية مثل حلوى الصمغ في الوقاية من الأمراض المزمنة المتعلقة بالجهاز المعدي المعوي وتنظيمها

أطروحة للحصول على درجة الماجستير في الكيمياء الحيوية

يحتل سوق الغذاء الطبيعي مكانة مهمة في أنظمة المحاصيل وفي النظام الغذائي للسكان.

يعتبر الخروب (Ceratonia siliqua) والزنجبيل (Zingiber Officinale) من نباتات البحر الأبيض المتوسط النموذجية ، وهما يستخدمان بشكل رئيسي في الغذاء والطب الشعبي التقليدي ، وقد تم اختيارهما للتحقيق في آثارهما الوقائية على مرض السكري المحرض ومشاكل الجهاز الهضمي.

نهدف في البحث الحالي إلى صياغة فئة جديدة من المنتجات الغذائية وهي الصمغ الغني بالمركبات النشطة بيولوجيًا المعروفة بتأثيرها العلاجي الذي تم استخراجه سابقًا عن طريق enfleurage ، يليه مراقبة الجودة بالإضافة إلى التفاعلات الكيميائية والبيولوجية SAR علاقة نشاط التركيب.

انتهت دراستنا بإنتاجية استخلاص ممتازة وقابلية للذوبان باستخدام enfleurage ؛

-خصائص حسية جيدة لجميع المستحضرات.

-جودة فيزيكو كيميائية وميكروبيولوجية مرضية.

-ثبات عالي على شكل صمغ مع مرور الوقت.

-لا توجد تحاليل سامة حسب الدراسات السريرية السابقة.

-تأثير تآزري مثير للاهتمام بين المركبات النشطة مما يجعلها أكثر كفاءة من الجزيئات النقية.

وفقًا لجودة التأمين هذه ، تعد العلكة المصنعة مناسبة جدًا للبالغين وكذلك الأطفال أيضًا للتسويق المباشر في الصيدليات والأسواق الكبرى.

الكلمات المفتاحية: الغذاء الوظيفي ، Ceratonia siliqua، Zingiber Officinale ، المنتجات الغذائية ، الصمغ ، enfleurage ، مراقبة الجودة.

مختبرات البحوث:

مختبر

المدرب: د. ت. نرجس مواس (أستاذة جامعية- جامعة الاخوة مونتوري ، قسنطينة 1) .

الممتحن 1:جدواني امال (استاذة جامعية ,ENS قسنطينة 3)

الممتحن 2:طابي عواطف (دكتورة ,ENS قسنطينة 3)

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Presented by: Allal yousra / Bouattit rayen

Natural polyphenols formulation as Gummies in prevention and regulation of chronic diseases related to gastro intestinal system

Thesis for obtaining the Master's degree in biochemistry

Natural food market occupies an important place in cropping systems and in population diet.

Carob (Ceratonia siliqua) and ginger (Zingiber Officinale) are typical Mediterranean plant, mainly used in food and traditional folk medicine, were chosen to investigate their protective effects on induced diabetes and gastrointestinal problems.

In the present research we aim to formulate new class of dietary product namely gummies rich in bioactive compounds known for their therapeutic effect previously extracted by enfleurage, followed by quality control in addition to chemical and biological interactions SAR (structure activity relationship).

Our study ended up with excellent extraction yield and solubility using enfleurage;

- Good organoleptic characteristics for all formulations.
- Satisfactory phisico-chemical and microbiological quality.
- High stability over time of gummy form.
- No toxic analyses according to previous clinical studies.
- Interesting synergic effect between active compounds which make it more efficient than pure molecules.

According to this insurance quality the formulated gummies are very suitable for adults as well as children's also for direct commercialization in pharmacies and super markets.

Key words: Functional food, Ceratonia siliqua, Zingiber Officinale, dietary product, gummies, enfleurage, quality control.

Research laboratories:
Laboratory

Supervisor: Mme. T. Nardjes MOUAS (Prof - Mentouri Brothers University, Constantine 1).

Examiner 1: Pr. Djedouani Amel (ENS, Constantine 3)

Examiner 2: Dr. Tabbi Aouatef (ENS, Constantine 3)