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A comparative study of extract of two plants, *Zingiber Officinal* and *Cinnamon Verum*, study its effect on *Fusarium solani* isolated from potatoes

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الدكتور عبد المالك زعتر والأستاذ بوراس ياسين الذي شاركنا عناء البحث

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كما لا يفوتنا في هذا المقام أن نتقدم بعظيم الشكر والامتنان إلى كل من سند في مد العون لنا ونخص بالذكر:

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وإلى كل من ساهم وساعد في إنجاز هذا البحث أو أسدى نصيحة أو توجيها

جزاهم الله عنا كل الخير

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إلى جسر المحبة والعطاء والصدق والوفاء : **إخوتنا وأخواتنا**

إلى رفقاء الدرب الطويل والمشوار الدراسي الصعب "**أصدقائنا الأعزاء**

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نهدي ثمرة جهدنا هذا.

Abstract

The objective of this study is to test the antioxidant and anti-inflammatory potential and the effects of the aqueous extract of two plants, *Zingiber Officina*l and *Cinnammomum Verum*, on the mycelial growth of *Fusarium solani* isolated from potatoes.

The quantitative analysis ofthe polyphenols and flavonoids of the aqueous extracts show that the content ofphenolic compounds is 1,073 mg EAG/ml E and 3,133 for the *C.verum* and *Z.officinal* extracts respectively, since theflavonoid content was 0,761 mg EQ/ml E and 0,602 for c.verum and z.officinal extracts respectively.

The antioxidant activity of the extracts was estimated by the use of DPPH, FRAP and TAC . The results showed an inhibition of free radicals .

The anti-inflammatory activity of the extracts was estimated by the use of protein denaturation and anti-hemolysis test. our extracts are capable of anti-inflammatory activity with a very high percentage of inhibition of protein denaturation of the order of 61.53% in the aqueous extract c.verum and 56.66% in the aqueous extract z.officinal. While the inhibitory power of H2O2-induced hemolysis is noted in c.verum extracts with high activity (97.87%) at 0.08 mg/l and in z. officinal with (78.71%) at 0.08 mg/ml.

The antifungal activity was also tested against the species *Fusarium solani* where it showed good antifungal activity on *Fusarium solani* particularly at a concentration of 0.8 mg/ml with an inhibition of the order of 65% and 60% for z.officinal extract and c.verum extract respectively.

Key words:*Fusarium solani* , z.officinal, c.verum, antifungal activity,Antioxydant, anti-inflammatoire.

Resumé

L'objectif de cette étude est de tester le potentiel antioxydante , anti-inflammatoire et les effets de l'extrait aqueux de deux plantes, *Zingiber Officinal* et *Cinnammomum Verum*, sur la croissance mycélienne de *Fusarium solani* isolé de pomme de terre.

L'analyse quantitative des polyphénols et des flavonoïdes des extraits aqueux montre que la teneur en composés phénoliques est de 1,073 mg EAG/ml E et 3,133 pour les extraits *C.verum* et *Z.officinal* respectivement, alors que la teneur en flavonoïdes était de 0,761 mg EQ/ml E et 0,602 pour les extraits c.verum et z.officinal respectivement.

L'activité antioxydante des extraits a été estimée par l'utilisation de DPPH, FRAP et TAC . Les résultats ont montré une inhibition importante des radicaux .

L'activité anti-inflammatoire des extraits a été estimée par l'utilisation d'un test de dénaturation des protéines et d'anti-hémolyse. Nos extraits sont capables d'activité anti-inflammatoire avec un pourcentage très élevé d'inhibition de la dénaturation des protéines de l'ordre de 61,53% dans l'extrait aqueux c.verum et 56,66% dans l'extrait aqueux z.officinal. Alors que le pouvoir inhibiteur de l'hémolyse induite par H2O2 est noté dans les extraits de c.verum avec une activité élevée (97,87%) à 0,08 mg/l et dans z. officinal avec (78,71 %) à 0,08 mg/ml.

L'activité antifongique a également été testée contre l'espèce *Fusarium solani* où elle a montré une bonne activité antifongique sur *Fusarium solani* notamment à une concentration de 0,8 mg/ml avec une inhibition de l'ordre de 65% et 60% pour l'extrait de z.officinal et c.verum extrait respectivement.

Mots clés : *Fusarium solani* , *Z.officinal, C.verum*, activité antifongique. Antioxydante ,anti inflammatoire

ملخص

الهدف من هذه الدراسة هو اختبار القدرة المضادة للأكسدة والمضادة للالتهابات وتأثير المستخلص المائي لنبتتين، *Cinnammomum Verum* و*Zingiber Officinal*، على النمو الفطري للفطر *Fusarium solani* المعزول من البطاطس.

أظهر التحليل الكمي للبوليفينولوالفلافونويد للمستخلصات المائية أن محتوى المركبات الفينولية هو 1.073 مجم / EAG مل E و 3.133 لمستخلصات .C و 0.602 لمستخلصاتc.verumوz.officinalعلى التوالي.

تم تقدير النشاط المضاد للأكسدة للمستخلصات باستخدام DPPH و FRAP و .TAC أظهرت النتائج تثبيط الجذور الحرة.

تم تقدير النشاط المضاد للالتهابات للمستخلصات باستخدام اختبار تمسخ البروتين واختبار انحلال الدم. مقتطفاتنا قادرة على النشاط المضاد للالتهابات مع نسبة عالية جدا من تثبيط تمسخ البروتين بنسبة 61.53٪ في المستخلص المائيc.verumو 56.66٪ في المستخلص المائي .z.officinalبينما لوحظت القوة المثبطة لانحلال الدم الناجم عن H2O2 في مستخلصاتc.verumذات النشاط العالي (97.87 ٪) عند 0.08 مجم / لتر وفي .z أوفيسينال بنسبة (78.71٪) عند 0.08 مجم / مل.

كما تم اختبار الفعالية المضادة للفطريات ضد نوع *Fusarium solani* حيث أظهر نشاط مضاد للفطريات جيد على *Fusarium solani* على وجه الخصوص بتركيز 0.8 مجم / مل مع تثبيط بنسبة 65٪ و 60٪ لمستخلصz.officinal و .c استخراج فيروم على التوالي.

الكلمات الأساسية: *Fusarium solani* : ، z.officinal، c.verum، نشاط مضاد للفطريات ، مضاد للأكسدة ، مضاد للالتهابات.

List of Abbreviations

Abs Absorbance

Alcl3 Trichlorure d’aluminium.

AA ascorbic acid

C verum cinamomom verum

DPPH 1,1-diphenyl 1-2-picrylhydrazyl

Ext Extract

FeCl3 Chlorure de fer

GAE Gallic acid equivalent

I (%) Percentage of inhibition.

IC50 Inhibitory concentration at 50%

mg EAG/gES mg gallic acid equivalent per g of dry extract

OMS organisation mondiale de la santé

Q quercétine

TAC Total antioxidant capacity

TFC Total flavonoids content

TPC Total phenolics content

µlMicrolitre

Z officinale *zingiber officinal*e

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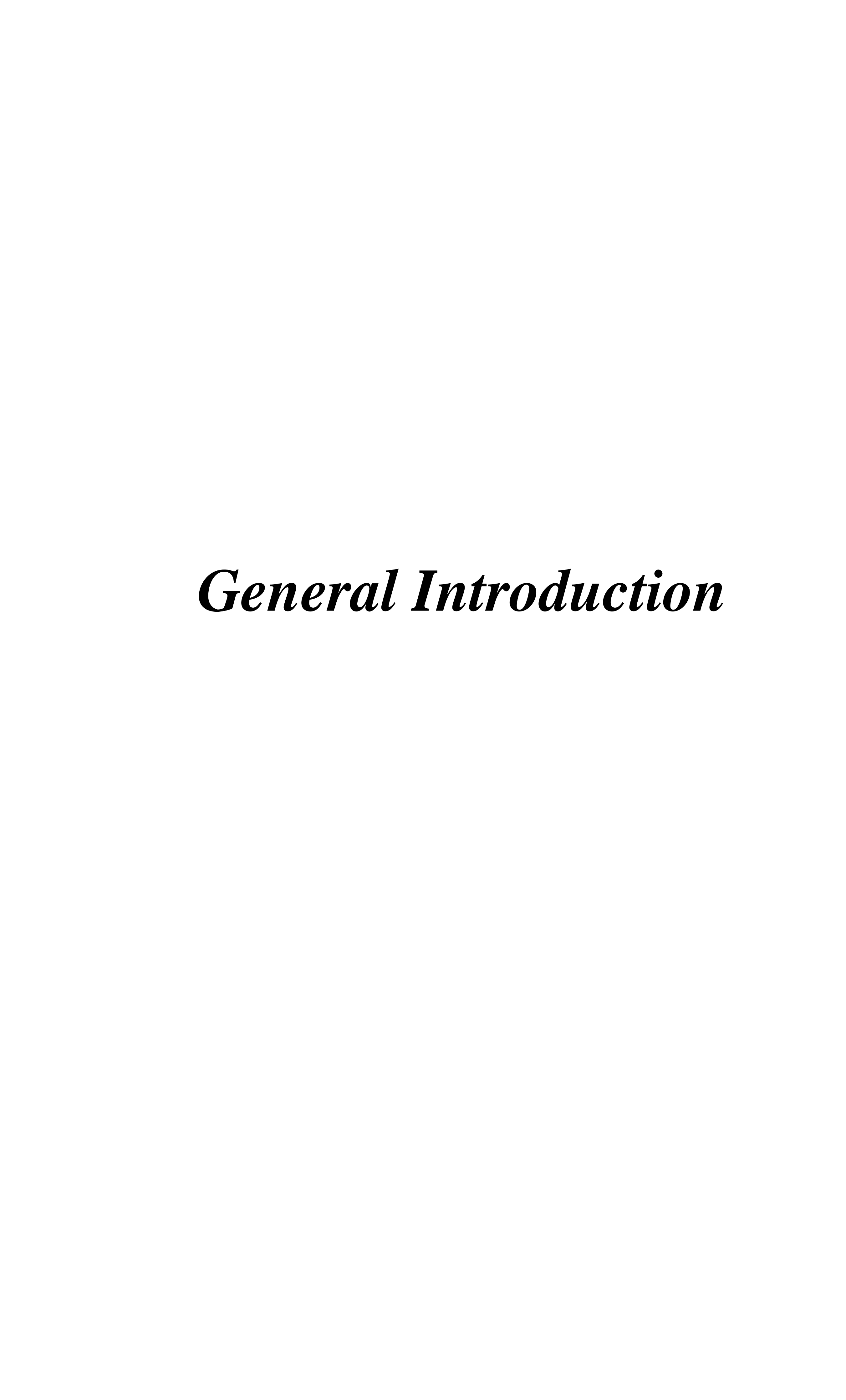


General Introduction

The potato (Solanum tuberosum L.) plays a key role in the food systemworld. It is the main non-grain foodstuff in the world, it comes in fourthposition after wheat, rice and maize which constitute the basis of human nutrition **(FAOSTAT,2015).**

Indeed, world potato production is estimated at 368.1 million tonnes,for a cultivated area of 19.4 million hectares, i.e. an average yield of 18.9 tonnes perhectare. This figure does not include plants (seeds) which represent 32.2 million tonnes**(FAOSTAT, 2015).**

In Algeria, potato cultivation occupies 28% of the market gardening area. in 2013. It is a staple food for the Algerian consumer. So the consumption of this food increased from 35 kg in 1990 to 56 kg in 2005 to reach 102 kg inhabitant/year in 2012 **(FAO, 2014).**

The agricultural production of potatoes in the El-Wad region has witnessed a continuous boom in recent years, which made it one of the richest agricultural regions at the national level, and it participates in more than a third of the national production (45%). Which gives it the first place among the states producing this crop (CAW, 2020)

In cultivation, the potato is attacked by several pests and diseases such asaphids, viral diseases and especially fungal diseases. She can get aset of fungal diseases that affect all or part of the plant during the growth phasevegetation or during storage of tubers as well as fungal diseasesmainly alternaria and downy mildew (**Roetschi, 2001**), and one of the most common fungal diseases is dry rot. Dry rot This disease is considered one of the most important fungal diseases that affect potato tubers during storage and causes a rate of up to 60%. This fungus can also infect the parts used as seeds and causes losses of up to 15%. Fusarium sp. The onset of dry rot symptoms appear as depressed areas on the outer surface of the tuber With the development of the infection, the outer shell of the tuberculosis wrinkles, and tissue death occurs below this area Symptoms on the inner parts of the tuberculosis are in the form of an ulcerated area of dark black or brown color. These parts often dry out and separate from the rest of the tubercle with a clear line The causative fungus enters the tuberculosis and causes rot in the middle of the tuber. This affected area appears in the form of a cavity. These cavities are often covered with mycelium and the fungus spores are of different colors, which are either yellow or purple. Diagnosis of the disease is a complex matter, as it is similar to several diseases such as bacterial soft rot caused by bacteria, especially if the tubers are stored under high humidity conditions. Fusarium dry rot is caused by several species of the genus Fusarium, the most common of which is the fungus Fusarium sambucinum. However, there are other species such as *F.solani* Mushroom spores can stay in the soil for long periods, and these spores are abundantly present on potato tubers, especially during spring Potato tubers that are used as seeds and that are kept at 2-2.5 °C is the same temperature suitable for fungus dormancy, and

as a result, disease occurs during the storage period. When tubers infected with the fungus are used in cultivation, this is one of the most important factors that help spread the disease and increase its severity.

Uses of pesticides against general potato diseasescauses harmful effects on cultivation, production and the farmer **(INRA, 2005).**

In this study, is to test in vitro antioxidant ,anti inflammatory and the effect of ***Z Officinale*** and ***Cinnamomom verum***plant extracts on the mycelial growth of **Fusarium** isolated from potato.

So, what is this excerpt? What is its effect on the fungus mentioned?

In order to answer this problem, the effect of this plant extract was studied according to thenext artboard; the study is divided into two main parts:

I. The bibliographic part; It consists of three chapters:

1.Presentation of the plants studied

2. General information on mushrooms

II. The practical part; It consists of two chapters:

1. Materials and methods.

2. Results and discussion

Literature review

Chapter I: General about the Potato

I.1 Definition

The potato (Solanum tuberosum L.) is the most important non-cereal forfood for the world's population **(Kumar *and al.*, 2014**). It is one of the main cropsthat contribute to global food needs **(Amara and Mourad, 2013).** She is consideredas the most important tuber **(Amiri *and al.,* 2013).**

I.2.Presentation and origin of the potato

The potato***(Solanum tuberosum L.*)** is a tuberous, herbaceous and perennial plantbelonging to the Solanaceae family **(Moule, 1972).** It originates from Latin America,more precisely the Andes and Peru. It is grown as a seasonal plant**(Nyabyenda, 2005).**

I.3. Classification

According to (Boumlik, 1995), the systematic position of the potato is as follows:

Table 01:the systematic position of the potato

Reign	vegetal
Phylum	Angiosperms
Class	Dicotyledons
Subclass	Gamopetals
Order	Polemonial
Family	Solanaceae
Genus	Solanum
Species	Solanum tuberosum L.

I.4. Botanical description

The potato consists of two distinct parts**.**

I.4.1. Aerial part

- Aerial stems, 2 to 10 in number and sometimes more **(Soltner, 2005).**
- The leaves are alternate, arranged on the stem following a spiral phyllotaxy with a spiralgenerator most often turning in the left direction **(Rousselle *and al.,* 1996).**
- Flowers can occur on aerial stems and always be located at the tip of a stem.They are white or darker in color **(Rousselle *and al.,* 1996).**
- The fruits are spherical or ovoid berries 1 to 3cm in diameter. Fruit production isgenerally rare or nil **(Rousselle *and al.,* 1996).**

I.4.2. Underground part

- Roots are adventitious, fasciculate, which are born at the level of the buried nodes of the stems
- leaves at stolon nodes **(Rousselle *and al.,* 1996).**
- Potato stolons are lateral stems that grow horizontally atfrom the buds of the underground part of the stems **(Sawyer, 1987).**
- The tubers are modified stems and they represent the main storage organ of the plantpotato **(Rousselle *and al.,* 1996).**

I.4.3. Characteristics of the tuber

A.External structure

There are two ends: The heel (or hilum) attached to the mother plant by the stolon and the crown(apical end opposite the heel) where most of the eyes are concentrated **(Figure 01).**eyes are arranged spirally on the surface of the tuber, each eye has several buds whichgive rise to germs **(Sawyer, 1987).**

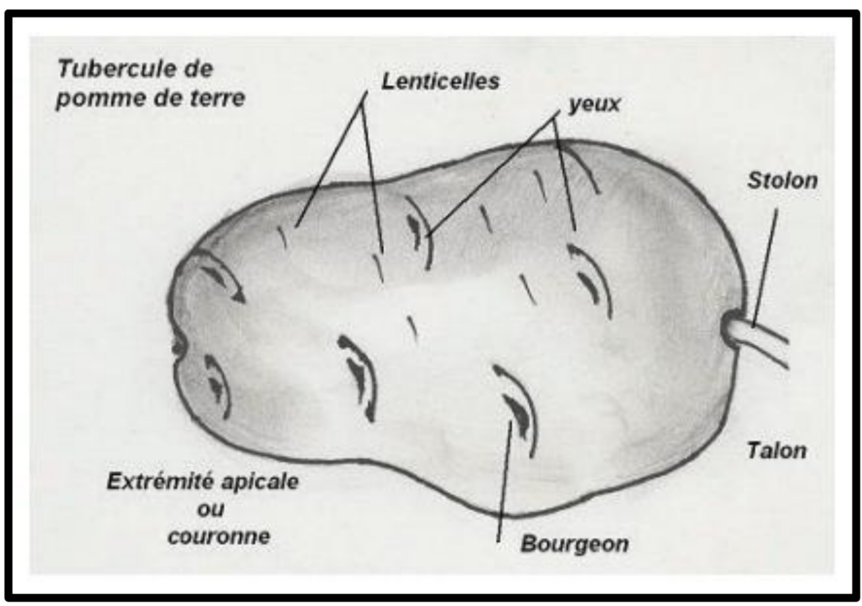


Figure01: External structure of the potato tuber (Rolot and Vanderhofstadt, 2014)

B. Internal structure

On the longitudinal section of a mature tuber **(Figure 02),** we observe:

- The periderm becomes firm and nearly impermeable to chemicals, gases and liquids.
- The lenticels provide communication between the outside and the inside of the tuber.
- The "flesh" of the tuber located below the skin **(Rousselle *and al.,* 1996).**

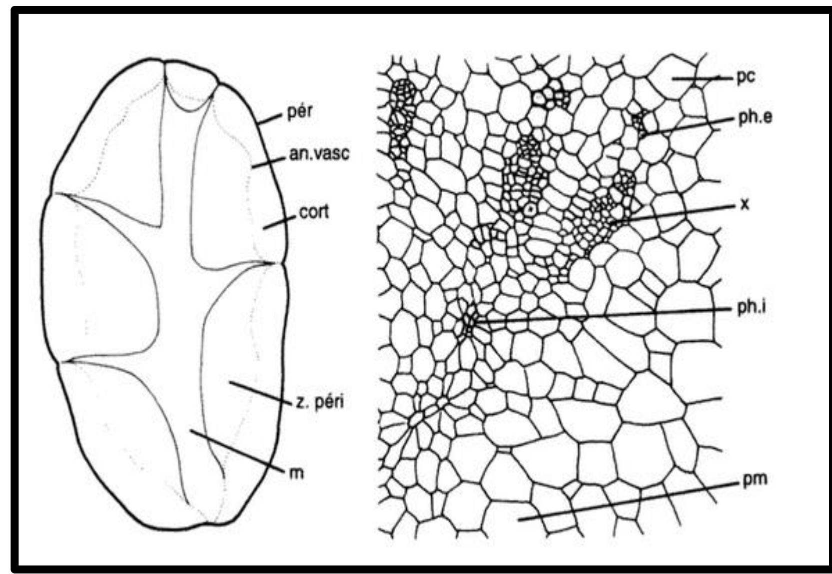


Figure 02: Longitudinal section of a tuber (Rousselle *and al.,* 1996).

I.4.2 Chemical composition of the tuber

The tuber consists mainly of water (about 80% of the weight). The rest (20%) is formedby dry matter: amino acids, proteins, starch, sugars (sucrose), vitamins (C, B1), salts minerals (K, P,), fatty and organic acids (citric, ascorbic) **(FAO, 2008).**

I.5. Potato physiology and propagation

It can reproduce in two ways:

I.5.1. Sexual reproduction

The fruit is a spherical or ovoid berry 1 to 3 centimeters in diameter, it usually contains several dozen seeds. The potato is very little propagated by seed in practice agricultural, however the seed is the tool for varietal creation **(Rousselle *and al*., 1996).**

I.5.2. Vegetative reproduction

This takes place in three stages:

A. Dormancy

It is a period of rest that also exists in seeds. The duration of dormancy isdepending on the variety and the storage conditions, and especially the temperature, it can be veryshort or very long **(Polese, 2006).**

B. Germination

The germ begins its growth if there is no dormancy induced by the environmental conditions.

It is generally the main bud of the eye located at the top of the crown which enters thefirst in growth. It gives rise to a germ which exerts apical dominance over theother buds, and delays their germination **(Mazoyer, 2002).**

C. Tuberization

Tuberization corresponds to the elongation of the stolons, the differentiation at the end of thesedaughter tubers and their increase in size **(Mazoyer, 2002).**

I.6. Cultivation technique

A. Soil preparation: The soil must be prepared to a depth of at least 25-30 cm **(Bamouh, 1999).**

B. Fertilization: The potato is demanding in nitrogen, phosphorus and potash (3.2 kg of nitrogen,1.6 kg of phosphoric acid and 5.5 to 6 kg of potash per ton of tuber) **(Mazoyer, 2002).**

C. Plant material: The choice of tuber is made according to the variety **(Bamouh, 1999).**

D. Planting: It can only take place after the total lifting of dormancy **(Bamouh, 1999).**

E. Irrigation: The water needs of the potato are estimated between 400 and 600 mm depending onthe climatic conditions, the type of soil and the length of the **cycle (Bamouh, 1999).**

F. Harvest: Maturation is indicated by yellowing of lower leaves, dryingstems and the firmness of the tuber skin **(Bamouh, 1999).**

G. Storage: To ensure proper storage, only uninjured tubers are stored.conservative. The ideal storage conditions are temperature (2 to 4°C for seed potatoes, 4 to 8°C for ware potatoes) and relative humidity (90 to 95%l: Le choix de tubercule s'effectuéselon la variété**(Bamouh, 1999).**

H. Plantation: Elle ne peut avoir lieu qu'après la levée totale de la dormance **(Bamouh, 1999).**

I. Irrigation: The water needs of the potato are estimated between 400 and 600 mm depending onthe climatic conditions, the type of soil and the length of the cycle **(Bamouh, 1999).**

J. Harvest: Maturation is indicated by yellowing of the lower leaves, dryingstems and the firmness of the tuber skin **(Bamouh, 1999).**

K. Storage: To ensure proper storage, only uninjured tubers are stored.keep. The ideal storage conditions are temperature (2 to 4°C for seed potatoes, 4 to 8°C for ware potatoes) and relative humidity (90 to 95%while avoiding the accumulation of CO2 by ventilation) **(Bamouh, 1999).**

The potato affected by diseases. If these meet favorable conditions,they can cause very significant damage in a short time **(AL-Salihy et al., 2014)**

6. Main fungal diseases of potato

Table 02: The main fungal diseases of potatoes

Diseases	Organs inffected	Description of the damage	Means of control
Mildew caused by Phytophthora infestants (Bryant and al., 2006).	On sheets Figure 03: Detachbrown (Rolot andVanderhofstadt, 2014). On stems Figure 04: Lesionblackish (Rolot and Vanderhofstadt, 2014).	Visible in 3 to 7 days and it can be completely destroyed in less than a week (Boulet, 2013). -Small black spots (Fig. 04) which turn into brown or black lesions, lesion colors are black (Fig. 05). Infected stems are weakened and can lead to the death of certain parts of the plant (Gaucher, 1998). - Superficial and irregular discoloration (Fig.06), necrotic lesions penetrate from the surface into the tuberculous tissue. Secondary infections can cause soft rot (Gaucher, 1998).	- Avoid excess nitrogen, eliminate diseased plants and weeds that constitute a hearth of contamination, use of healthy seeds, application of organocupric products and synthetic organic products (mancozeb) (Bamouh, 1999).

	On the tubers Figure 05: Lesiondry, brown necrotic (Rolot andVanderhofstadt, 2014).		
black Rhizoctonia causedbyRhizoctonia solani	On vegetation On tubers Figure 06:Small rounded black spots on the tuber (Rolot and Vanderhofstadt, 2014).Vanderhofstadt, 2014).	Intervening at the time of emergence on young potato shoots (Grosch et al., 2005). Lack or delay in emergence, dry and well-defined necroses on the underground part of the stems or stolons. The formation of small rounded brownish or black spots (Fig.07) (Gaucher, 1998).	- Usehealthy seeds,spincultural,treatthe seedswith somefungicides(Bamouh, 1999).

Alternariose caused by *Alternaria solani*	On sheets Figure 07: Brown spots on the leaves (Agrios, 2005). On tubers Figure 08: Spotsdark brown to black(John, 2002).	Small occasional brown spots on older leaves (Fig.08). These lesions are round measuring 3 to 10 mm in diameter and are composed of concentric rings of dead tissue, sometimes presence of the yellow halo (Gaucher, 1998). -Dark brown to black spots (Fig.0 9), circular to elliptical and depressed, these spots penetrate in the pulpit to a depth of 1 to 2 mm (Jean, 2002).	- Burn the top of the cultures of the family of nightshades so reduce the primary inoculum, practice a crop rotation and fungicides after a rain (Bamouh, 1999).
Dartrose caused by Colletotrichum coccodes	Stolons, root On tubers	- Wilting, chlorosis and drying from the top of the plant to the base of the stems, The formation of greyish spots, punctuated with black dots (Fig. 10) (Marot et al., 2008).	Long rotation periods (Kerr, 2014).

	Figure 09: Spots atgrayishcolor, and black spots (Kerr, 2014).		
Silver spot caused by *Helminthosporium solani*	On tubers Figure 10: Silver scab on tuber (Rolot and Vanderhofstadt, 2014).	-Circular spots of silvery aspect with irregular contour (Fig. 11) on the surface of the tuber at the level of these spots, there are tiny black punctuations, these spots spread out with age and take on a silvery reflection (Marot et al., 2008).	Application of fungicides before planting or at harvest time, storage in chambers cold (Kerr, 2014).
Fusariose	On tuber	Fusarium wilts are recognizable by a surface of the tuber wrinkled in more or less concentric wrinkles around the point of infection (Fig. 12) and on which	The use of healthy tubers, chemical treatment with thiabendazole (Rousselle et al., 1996).

	Figure 11: Fusarium symptoms on the tuber (Rolot and Vanderhofstadt, 2014).	fruiting pads, the pionnotes, form (Rousselle et al., 1996).	
Gangrene	On tuber Figure 12: Rotdry (Polese, 2006).	Decayed rot called a "boost" symptom (Rousselle et al., 1996). - Irregular circular depressions of 0.5 to 3cm in diameter (Fig. 13) (Gaucher, 1998).	-Store the tubers in good conditions. -Chemical treatment before storage (Polese, 2006).
Verticilliose caused by Verticillium		Yellow coloration followed by wilting (Fig. 14) (Rousselle et al., 1996).	Burn plant debris before cultivation, treated against nematodes (Bamouh, 1999).

	Figure 13: Yellow color and wilting of tuber and leaves(Agrios, 2005)		

Chapter II: Presentation of the plants studied

II 1.*Zingiber officinal*

II.1.1. Habitat, geographical distribution

Ginger or rhizome of *Zingiber officinaleRoscoe* the family of *Zingiberaceae*(**Gigone, 2012),** grows in tropical regions, particularly in Southeast Asia. Maximum variation of cultivated ginger is found in India and neighboring countries from Southeast Asia **(B.Sasikumar, Saji, *and al.* 1999).** From there the ginger then went quickly usable thanks to its trade from all over Southeast Asia, to West Africa and the Caribbean. This oriental spice probably crossed the first time the Mediterranean Sea thanks to the Phoenicians to reach Europe during the Roman Empire from the 1st century **(Gigon 2012).** Ginger used as a spice for over 200 years (**Stoliva, 2007),** and many other spices have been used as medicine**.** Ginger is an important ingredient in phytotherapy **(Thomson, 2002).**

II.1.2. Botanical description

Specified herbaceous tropical perennial to 3m tall. The underground part used is the rhizome (**Gigon 2012).** Its rhizome is we them and fragrant, pale beige skin, flesh pale yellow juicy and fragrant, it becomes more and more fibrous with age, covered with leaves scaly and provided at its lower part with cylindrical roots. Its leaves are persistent bisériées, long, narrow, lanceolate, pointed and 20cm long. There is two kinds of stems: sterile tall stems used for chlorophyll assimilation and shorter stems (approximately 20cm) bearing irregular spike flowers. the inflorescence is in progress very tight axillary spikes, stem covered with scales. She has fragrant flowers white yellow, with red streaks on the lips **(Figure14).**Flowering takes place between August and November. Its fruits are trivalve capsules containing black seeds **(Faivre Cl *and al* ., 2006).**

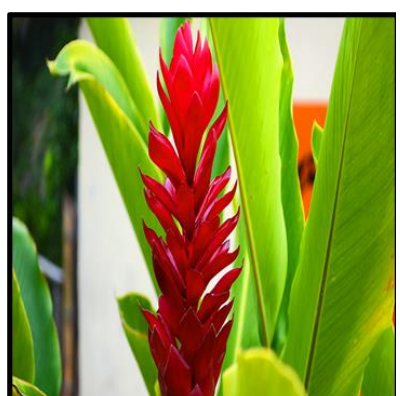


Figure 14: Leaves, flowers and fresh rhizome of ginger (Gigon, 2012).

In the market, ginger comes in two forms, white (peeled) and black (unpeeled) Its popular name in the Maghreb is “Skendjbir” a deformation of “Zandjabil” which is the Arabicized form

of "Singabera" its name "Pali" (ancient language of India). The Greek physician Dioscorides believed that ginger was imported from Arabia (**Baba aissa ,2000).**

Ginger is one of the best-known and most popular spices. It's a plant that greatly exhausts the soil. It is mainly grown in India, the main producing country. (More than 50%), but also in Sri Lanka, China, Japan, Jamaica, Nigeria, South America, Australia, … The commercial product is prepared from these rhizomes dug up when the upper parts of the plant wither. In the country's producers, the rhizomes are eaten fresh. **(Richard ,1992).**

II.1.3.Botanical classification

Ginger (*Zingiber officinale*) is a plant species native to India that is classified as follows:

Table 03: Botanical classification of ginger**(Faivre and al . 2006; Gigon, 2012)**

Nom commun	Ginger, white spice, ginger, jenjanb
Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Angiosperms
Class	Liliopsida
Subclass	*Zingiberidae*
Order:	*Zingiberales*
Family	*Zingiberaceae*
Subfamily	*Zingiberoideae*
Genus	*Zingiber*
Species	*Zingiber officinaleRoscoe*

II.1.4.The composition of ginger

Chemical analysis of ginger indicates that it contains over 400 different compounds **(Grzanna, 2005)**, which are carbohydrates (50-70%), terpenes, phenolic compounds, lipids (3-8%) **(S. Prasad, 2015),** two types of fatty acids: oleic and linolic (10%), starches (60%), proteins, vitamins and minerals **(Kim H.S, 2015),** an oleoresin complex and an enzyme, zingibain**(Gigone, 2012),** The oleoresin of the compounds responsible for the very marked flavor contains ginger.Some belong to the vanilloid family and are known as of 3-, 6-, 8-, 10 and 12-gingerols. these compounds on a side chain of length variable, respectively of 7, 10, 12, 14 or 16 carbons (**Gigon, 2012, Ok and Jeong, 2012),** they are accompanied by gingediols and paradols, Zingerone and shogaol are products of the degradation of gingerol under the action of heat **(Gigone, 2012).**The characteristic odor and flavor of ginger are due to volatile oils mainly in gingerols and shogaols **(S. Prasad, 2015)** .

II.2.*Cinnamomum verum*

II.2.1 Definition

The word *cinnamon* would come from the Latin canna meaning reed or pipe, because of the shape under including on reception from the Far East.

Known since antiquity, *cinnamon* is an aromatic spice that comes from the bark internal *cinnamon*.

Cinnamon is native to Ceylon or currently Sri Lanka and the Malabar coasts from India. We currently find "the *cinnamon* gardens" mainly on the part southern part of the island: in the lowlands around Galle, Matara and Colombo. The Sri Lanka is the leading exporter of real *cinnamon* in quantity and quality.

The Ceylon *cinnamon* is a tree about ten meters high**(Figure 15) (VERNON F. and RICHARD H., 1976)** and can reach 20m **(LEUNG ALBERT Y., 1980),** The first harvest is possible after five years, then it is done every two years in the rainy season when the bark is full of sap **(VERNON and RICHARD, 1976).** The bark is detached from young shoots or branches, by incision, cut into strips 30 cm long, which are left to dry then scraped to separate the cork. It comes in the form of pipes nested inside each other **(RICHARD and LOO, 1992).**

On the spice market, *cinnamon* can be presented in whole tubes (scraped epidermis inner bark), broken pipes, pieces, shavings or powder.

Figure15 :Cinamomom Verum tree and tubes(VERNON F. and RICHARD H., 1976)

II.2.2 Botanical classification

Table 04 : Botanical classification of Cinamomom verum **(Quézel et santa ,1962 et 1963).**

Kingdom	Plantae
Sub kingdom	Tracheophytes
Super division	Angiosperms
Class	Mgnoliids
Order	Laurales
Family	Lauraceae
Genus	Cinnamomum
Species	C. verum

II.2.3Chemical compositions

Knowledge of the composition, is of significant interest: given the strong demand from industrialized countries for *cinnamon*. The bark contains tannins, resins, mucilage, gum, sugars, calcium oxalate, little coumarin **(LEUNG ALBERT, 1980),** starch, polycyclic diterpenes,proanthocyanidolic oligomers and volatile oils.

II.3. Biological properties of phenolic compounds

II.3.1. Antioxidant activity

Antioxidants are substances capable of neutralizing or reducing damage by free radicals in the body and help preserve the cell level of non-cytotoxic concentrations of ROS. Our body reacts

Therefore, constantly to this permanent production of free radicals and we distinguish at cell level two unequally powerful lines of defense to detoxify the cell **(Zeb, 2020).** They exhibit abilities such as scavenging free radicals, reduction of enzymatic activity (pro-oxidant), chelation of pro-oxidant metals, inhibition of lipid peroxidation and quenching of singlet oxygen **(Omba*and al.*,2015).**

II.3.2.Anti-inflammatory activity

The anti-inflammatory effect of flavonoids comes from its ability to inhibit enzymatic, blocking the activities of enzymes such as cyclooxygenase and 5-lipoxygenase, which exerted functions in the transmission of arachidonic acid and therefore participate in the inflammatory response **(Jucá*and al*., 2020).**

II.3.3.Antifungal activity

Many flavonoids exhibit antifungal activities, the largest number belongs to flavanones and flavans. A prenylated flavanone (5, 7,4'-trihydroxy-8-methyl-6-(3-methyl-[2-butenyl])-(2S)-flavanone) **(Ali *and al.,* 2017)** Two flavanones naringenin (NAR) and pinocembrin inhibit the growth of strains of C.albicans**(Soberón*and al.,* 2020)** and also coumarin and its derivatives show activity antifungal against Candida albicans **(Jia *and al.,* 2019).**

Chapter III: General information about fungi

III.1. Definition

Fungi are eukaryotic organisms that do not constitute a monophyletic entity but on the contrary form a very heterogeneous group whose common essential characteristic is heterotrophic nutrition by absorption, which can take the form of saprophytism, parasitism or symbiosis. **(Nasraoui, 2006).**

a) **Symbiotic fungi:** These are mycorrhizal fungi, which result in beneficial interactions with plant roots **(Vander *and al* ., 1998).**
b) **Phytopathogenic fungi:** They have established antagonistic interactions with plants **(Vander, 2003).**
c) **Saprophytic (free-living) fungi:** They participate in the processes of decomposition of organic matter, immobilization of mineral elements and allowing neutral interactions with the plant. **(Klein et Paschke, 2004).**

III.2. Identification systems

III.2.1. Morphological identification

Identifying the many fungal species likely to colonize plants is a very important step. Indeed, not all species have the same physiological characteristics or the same requirements, identification can give valuable information on the origin of contamination and allow appropriate treatment. This identification was for a long time exclusively based on the observation of the cultural and morphological characteristics of the species. Recent advances in molecular biology have made it possible to offer tools to help with identification. (**Abdel Massih, 2007).** However, the complexity of the fungal kingdom means that, at present, these tools cannot completely replace morphological examination, which remains the basis of identification. **(Tabuc, 2007).**

III.2.2.Génétique identification

The identification of fungal genera is based on morphological criteria:

- **macroscopic appearance of the mycelium** (appearance, color, relief, size and smell of the colonies, as well as the fruiting structures).
- **microscopic appearance of reproductive structures** (thallus, spores, appearance of spores, modes of formation of conidia, mode of grouping of conidia, mode of implantation of conidiogenous cells, presence of protective structures resulting from asexual or sexual reproduction, presence of chlamydospores).

Many studies have aimed to develop identification tools based on the study of nucleic acids (DNA and RNA) which will no longer necessarily pass a morphological examination. **(Jin*and al* ., 2004).**

The most interesting methods are based on the amplification by PCR (Polymerase Chain Reaction) of certain specific regions such as the gene encoding the 28S ribosomal subunit (D1-D2 region) and of the ITS1 and ITS2 regions **.**

The molecular identification of fungal species is, at present, mainly applied in medical mycology to differentiate the species of interest. Indeed, invasive fungal infections are increasingly pathogenic as the primary cause of morbidity and mortality, particularly in immunocompromised patients **(Aguire, *and al* ., 2004).** This method is also used to differentiate and identify the molds responsible for food spoilage, mainly Penicillium species **(Boysen *and al* ., 2000; Hageskal*and al* ., 2006),** on the other hand for Fusarium, the existing molecular methods frequently give inconclusive results and the classic morphological examination still seems to be an essential method for the identification of the species belonging to this fungal genus **(Healy *and al* ., 2005).** If at present the molecular identification tools do not seem able to replace the classic morphological identification, it is likely that in the years to come, these methods will represent particularly useful tools for fungal detection and identification. in food. (**Tabuc, 2007).**

III.3.The main genera of phytopathogenic soil fungi

III.3.1.The genus Fusarium

This genus includes imperfect fungi belonging to the class of Deuteromycetes. The perfect or teleomorphic forms of some species of the Fusarium are known, and belong to the class of Ascomycetes (order of Hyphocreales, family Nectriaceae, genera Gibberella, Calonectria and Nectria). The genus includes nearly 40 species that are often widely distributed **(Nelson *and al* ., 1983).** Economically, the genus Fusarium is very important because it includes many phytopathogenic species, likely to induce diseases(fusarium) in many plants. He will concede among the mushrooms most aggressive telluric soils, causing wilting and rotting on many many cultivated plant species **(Benhamou *and al* ., 1997).**

In addition, many saprophytic species are able to develop into as secondary pathogens on senescent plant tissues. The species of genus Fusarium can thus attack cereals (corn, wheat, barley, oats), vegetables, ornamental plants and lots of fruit trees. The majority of *Fusarium solani* ecies are likely to produce mycotoxins and are thus implicated in poisoning in farm animals. **(Tabuc, 2007).**

The main species of Fusarium, given their frequency in different substrates, in particular cereals, of their toxigenic potential and their pathogenicity, are: F. culmorum, F. graminearum, F. oxysporum and F. verticilloides (F. moniliforme). **(Tabuc, 2007).** Fusarium culmorum, Fusarium graminearum are the pathogens of root rot disease that occur manifests itself on durum and soft wheat as well as on barley. This disease appears particularly in semi arid areas and during years with low rainfall. **(Ezzahiri, 2008).** In pea this type of disease can also be caused by F.oxysporumf.sp. *solani*. **(Haglund and Kraft, 2001).**

Vascular wilt is the disease that causes the greatest losses for many cultivated plants, such as vegetables, and tropical crops it is also caused by F. oxysporum**(Agrios, 2005).**

SECOND PART : EXPERIMENTAL

Chapter I :

Materials and Methods

I.1. The objective of the work

The purpose of this study is to highlight the biocidal effect of plant extract of mugwort (Z Officinale) and (Cinnamomon verum) ,against the fungus *Fusarium solani* and their antioxidant ,anti inflammatory activities .

Pour there achieve experimental work was carried out at the level of the agronomy laboratory of the faculty nature and life sciences from El ChahidHammaLakhder El-Oued University.

I.2Materiels

Table 05 : materials used

Precisionscale		Ultrasoniccleane r	
Microscope		Laboratoryoven	
Centerfuge		Spectrophotomet re	

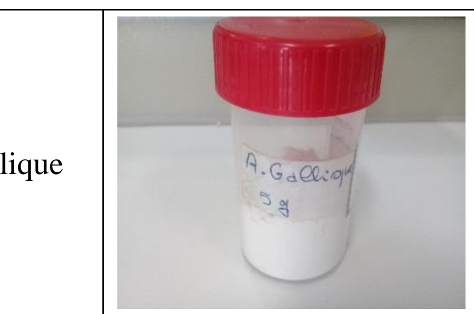
Analytical balance		Hotplatestirrer	
Ultrasoniccleaner		Spectrophotometer	
Water baths		Glass funnel	
Glass measuringcylinder		Fume hood	
Bechers		micropipette	

Erlenmeeyerflasks		supply wash bottle standard spout	
Plastic test tube		Spatula in laboratory	

I.3Using produit

Ferricchloride		Sulphuricacid	
Aceticacid		Wagner reagent	
ClorideHydrogen HCl		Chloroform	

Reducing compound		Sodium carbonate anhydrous	
Chloride		Methanol	
Antibiotique		Gentheraxine	
Albumine humaine		Acide ascorbique	
TCA solution		Potassium fericianure	
Chloride AlCl3		Folinciocalteu	

Acid gallique		Quercetin	

I.4. Plantmaterials

In this study, (*Zingiber officinale*) and *(Cinnamon verum*) were obtained from the market. Mechanical grinder powdered these herbs until a fine powder was obtained.

The powders of *Zingiber officinale* and *Cinnamon verum* stored at room temperature in airtight containers protected from bright light until the beginning of the experiment.

1.5. Aqueous extract preparation

The aqueous extract was prepared by adding 50 ml of distilled water to 5 g dry powder of plantat 50°C during 2 hours. After 24 h of maceration at room temperature, the mixture was filtered by Whatman paperthen evaporated by using rotary evaporator.**(Derouiche et al., 2019)**

Figure 16 : aqueous extract preparations .

1.6. Phytochemical analysis

The phytochemical analysis were carried out on the aqueous extracts prepared from the plant by qualitative characterization method according to(**Hamid EL-Haoud*et al.,* 2018)**

1.6.1Phenols

Introduce 5 ml of extract in a test tube and drops few of natural 5% ferric chloride solution. A dark green color indicates the presence of phenolic compounds

1.6.2. Flavonoids

In a test tube, introduce 5ml of extract, 5ml of diluted ammoniac and 1ml of H_2SO_4. The appearance of a yellow color indicates the presence of flavonoids.

1.6.3.Alkaloids

1 ml of aqueous extract were treated with a few drops of hydrochloric acid then 1–3 drops of Wagner reagent were added. The appearance of brown precipitate reveals the presence of alkaloids in the sample.

1.6.4.Tannins

In a test tube, introduce 5 ml of extract and add 1 ml of a 2% aqueous solution of ferric chloride (FeCl3). The presence of tannins was indicated by a greenish or bluish-blackish coloration.

1.6.5.Terpenoids

The formation of a reddish-brown color indicates the presence of terpenoids, through the addition of chloroform (2ml) and concentrated sulfuric acid (3 ml) to 5 ml of plant extract.

1.6.6.Reducing compound

Add Fehling's liquor (1ml of reagent A and 1ml of reagent B) to the extract and incubate the whole in a boiling water bath, the appearance of a brick-red precipitate indicates the presence of reducing sugars.

1.6.7. Saponins

In a test tube, introduce 5ml of extract, mixed with 5ml of distilled and with vigorous manual agitation. The formation of a steady foam indicates the presence of saponins.

I.7. Total phenols and flavonoids compounds

I.7.1. Total phenols

The total phenolic contents of the crude extracts were determined according to the Folin-Ciocalteu's phenol reagent method of **(Chouikh *and al* , 2020)** with some modification ; we mixed 0.2 ml of the extract with 1 ml of Folin-Ciocalteu reagent (10%), then we added 0.8 of sodium carbonate solution (7.5%). After stirring the test tubes, we let them rest for 30 min, the absorbance was measured at 765 nm using the spectrophotometer UV. The total phenolic content was expressed as (mg of Gallic acid equivalents in gram of extract).

I.7.2. Total flavonoids

The determination of total flavonoids was carried out according to the method described by**(Chouikh*et al* , 2020)** with some modification: 500 µl of each extract, 500 µl $AlCl_3$. The mixture is stirred and then incubated in the dark and at room temperature for 30 minutes. The blank is made by replacing the extract with 95% methanol and the absorbance is measured at 415 nm using a UV spectrophotometer. The results are expressed in mg equivalent quercetin / g of dry vegetable material with reference to the quercetin calibration curve. The quercetin calibration curve is performed by quercetin at different concentrations (20 - 40 - 60 - 80 - 100 – 120 µg/ml) under the same conditions and the same steps of the assay.

1.8. Antioxidant activity

I.8.1. The reducing power of ferric ion (FRAP)

Take 500µl of sample and Add 1.25ml of the buffer solution (0.2 M, PH = 6.6). Add to 1.25 potassium fericianure**.**Then Incubation during 20 min in a water bath at 50 ° C. After cooling, add 1.25ml of the aqueous TCA solution (10%) to stop the reaction. Centrifugation at 3000 rpm for 5 minutes. Then take1.25 ml of supernatant are then mixed with 1.25 ml distilled water and 250 µl $FeCl_3$ (0.1%). The absorbance was measured at700 nm against a blank. The results expired by IC50, after calculating of the inhibition percentage values according to (**BENHAMMOU NABILA , 2011).**as follows :

$$\mathrm{IP}(\%) = \mathbf{100} - \frac{\mathbf{OD\ controle}}{\mathbf{OD\ controle}} \times \mathbf{100}$$

I.8.2.Free radical scavenging activity, DPPH assay

The free radical scavenging activity was measured by a modified DPPH assay. Briefly, the DPPH assay was carried out as described by **(Popovici*et al.,* 2009).** 500µL of various concentrations of (*Zingiber officinale*) and *(Cinnamon verum*) in methanol was added to 1 mL of a methanol solution of DPPH (0.004 %). The mixture was shaken and then allowed to stand at room temperature for 30 min in the dark. The absorbance was measured at 517 nm. The scavenging activity on the DPPH radical was expressed as inhibition percentage using the following equation:

$$\text{DPPH scavenging-radical } (\%) = [(A0 - As) / A0] \times 100$$

A0: is the absorbance of control reaction

As: is the absorbance of sample solution containing the test compound.

BHT were used as a positive control. The IC50 of extract was calculated from the graph of inhibition percentage plotted against extract concentrations.

I.8.3. Total antioxidant capacity (TAC)

In an Eppendorf tube mix 25 µl of plant extracts with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). Leave the reaction tubes incubated at a temperature of 95°C for 90 min. After the specified time has elapsed, the tubes are cooled to room temperature and the absorbance is measured with the spectrophotometer at 695 nm with a blank containing 25 µl methanol instead of the extract .The total antioxidant capacity was expressed in milligrams of ascorbic acid equivalent per gram of extract (mg EAA/g extract) from an ascorbic acid calibration curve.**(I. MALIKI *and al .,* 2021).**

I.9. Anti-inflammatory activity

I.9.1.Albumin denaturation test

Inhibition of protein denaturation has been used as a method todetermine the anti-inflammatory activity of extract in vitro according to the protocol described by**Rahman *and al.* (2012)** using bovine serum albumin (BSA) protein.

Test solution (0.5ml): composed of 0.45ml of the aqueous solution of BSA 0.5% (w / v) and 0.05 ml of extract withvarious concentrations (0.1, 0.2, 0.4 mg/ml).

Control solution (0.5ml): composed of 0.45 ml of the aqueous solution of BSA 0.5% (w/v) and 0.05 ml of distilled water.

Product control solution (0.5ml): composed of 0.45 ml of distilled water and 0.05 mlof extract with various concentrations (0.1, 0.2, 0.4 mg/ml).

Standard solution (0.5ml): composed of 0.45 ml of the aqueous solution of BSA 0.5% (w/v) and 0.05 ml of Diclofenac Sodium standard solution withvarious concentrations (0.1 , 0.2 ,0.4 mg/ml).

The solutions are incubated at 37° for 20 min then the incubation temperature isincreased to 57° for 3 min. After incubation 2.5ml of phosphate buffered saline (PBS)was added to the solutions. Absorbance was measured at 255 nm with a spectrophotometerUV-Visible.

The percent inhibition of protein denaturation was calculated by the formulafollowing:

The percentage of inhibition

[(Ac - At) / Ac)* 100]

At = Absorbance of test solution (extract tested).

Ac = Absorbance of the control.

I.9.2Anti hemolytic activity

The antihemolytic activity is based on the power of the aqueous extracts of the two plants studied and prevented the destruction of red blood cells. To do this, we first kept the erythrocyte cells in incubation with phenolic compounds at different concentrations, then added hydrogen peroxide (H2O2) and then evaluated the release of hemoglobin by determining the absorbance of the supernatant at 540 nm.

A standard calibration curve was obtained from the solutions of ascorbic acid (vitamin C) of different concentrations ranging from 5% to 100% from the stock solution prepared in the phosphate buffer at a concentration of 0.1 mg /mL.

The anti-haemolytic activity is carried out in vitro according to the protocol described by **MANNA *and al,.* (2002),** with a few modifications. The human blood used in this test is obtained by venous sampling from a non-smoking volunteer. The blood collected in a heparinized tube is centrifuged at 1500g/5min, after elimination of the plasma, the pellet is washed three times with certain volumes of phosphate buffer (PBS without potassium, pH 7.4 and 0.1 M).

During each wash, the suspension is homogenized by simply inverting the tube, the supernatant and the interface layer are removed immediately after centrifugation. At the end of the last centrifugation, the cell pellet obtained is reduced with the same phosphate buffer to obtain a hematocrit of 4%. Two milliliters of the erythrocyte suspension are mixed with 5 mL of PBS solution.

The radical attack is induced by adding 0.5 mL of H2O2 to the erythrocyte suspension previously incubated (15 min) with 1 mL of each daughter solution of ascorbic acid or of the

extract at different concentrations. After two hours of incubation with H2O2 at 37C°, the reaction mixture is centrifuged at 1500g/10min and the hemolysis (release of hemoglobin) is specified in the absorbance of the supernatant at 540 nm.

The concentration of H2O2 in the reaction mixture was adjusted to cause 90% hemolysis of red blood cells after 2 h of incubation.

The resistance of the blood to radical attack is expressed by the percentage of inhibition:

Inhibition of hemolysis % = ((Abs 540 control – Abs 540 sample) / Abs 540 control) x 100

Or :

- % hemolysis inhibition: percentage of hemolysis inhibition;
- Abs 540 control: control absorbance at 540 nm;
- Abs 540 samples: sample absorbance at 540 nm.

I.10.Antifungals activity

In order to study the antifungal activity of *Zingiber Officinal* and *Cinnamon Verum* extract against the mycelial strain of *Fusarium solani* ., we decided to apply biological control by plant extracts on this species.

The bio-assays are carried out in the agronomy laboratory of the Faculty of Nature and Life Sciences of El ChahidHammaLakhder El-Oued University.

Fusarium solani orotrichoide is a mycotoxin-producing fungal strain of the Hypocreaceae family **(Simic*and al*., 2003).** *Fusarium solani* orotrichoide is widespread on plants and in the soil (Pittj et al., 2000). It causes yield losses and contaminates theharvesting with toxic substances **(Anonymous., 2008).**

I.10.1. Preparation of the culture medium

We used nutrient agar culture medium (sabouraud), which is used for research and enumeration of fungi, as well as for maintaining strains for collection and transplanting.

I.10.2. Reading Colonies

The identification of a fungal strain is carried out by two conventional techniques: by macroscopic and microscopic observation of the strains. These two techniques are largely sufficient to determine the genus of isolated molds (Bourgeois and Leveau, 1980).

❖ **Study of macroscopic characters**

The morphological and cultural characters are determined after inoculation of the pure strains on the solid culture medium specific for peas. The identification is done with the naked eye, it is mainly based on the following characters: the speed of growth, the appearance of the aerial mycelium (diffuse, ears), the color of the back of the colony.

❖ **Study of microscopic characters**

It requires the assembly of microscopic preparations (optical microscope coupled to a computer). The method consists of placing a drop of distilled water on the slide, then bringing and dissociating in the drop a sample of the hyphae of the fungus to be observed; the slide is covered by a coverslip and the observation is made at different magnifications (40X then immersion).

Microscopic examination can study the following characters:

- Hyphae partitioned or not
- Colored, colorless mycelium
- Arrangement of conidiospores and conidia

I.10.3. Confrontation Stage

The objective of this study is to test in vitro the plant extract of the two plants studied on the mycelial growth of Fusarium isolated from potato. The method used to assess the antifungal activity of the extracts is the direct contact method. The strains are treated in Petri dishes using Sabouraud agar culture medium with the plant extract in different concentrations.

A.Preparation of culture medium with the plant extract obtained This step consists in liquefying the Sabouraud agar medium then mixing each concentration of plant extract with 15 ml of Sabouraud agar medium in test tubes. Finally, the medium poured into Petri dishes, each mixture was carried out through three repetitions, considered as treatment. Thus, witness boxes were made available to us.

Table 06 : Concentrations used in anti fungals activity

Extract	**Concentration mg/ml**
Z Officnale	0.01
	0.02
	0.08
C verum	0.01
	0.02
	0.08

B.Preparation of mycelial discs After obtaining a pure culture using a Pasteur pipette, mycelial discs of about 5 mm in diameter were made.

Place the discs in the Petri dish containing nutrient agar medium(sabouraud) and the extract testedOnce you solidify in the growth medium that contains the tested extract,remove the mushrooms in a disc using a Pasteur's absorbent. Then it is placedon the medium previously prepared in the middle of a Petri dish. Finally, the boxes then thePetri dishes are closed and left to spread out on the seat.

C.Incubation The inoculated dishes were then incubated at 27°C for 6 days and the development.

B.Reading coloniesthe reading of the results is done by measuring the diameter of the mycelial growtharound each piece until the control growth covers the total area ofthe plate using a ruler graduated in centimeters.

D. Evaluation of antifungal activity of extractthe antifungal effect of the extract tested against fungus is determined by measuringgrowth rate after incubation at 27°C for 6 days using the formula of**(Motiejunaite&Peiculyte, 2004).**

E. Inhibition rate (T%) Mycelial growth was assigned every 24 hours by specifying theaverage of three perpendicular diameters passing through the middle of thewasher. Three replicates were performed for each concentration.

This reading is always carried out in comparison with the control culturesthat they are started on the same day and under the same conditions.

$$\mathbf{T = (DK\text{-}D0)*100}$$

DK: Control fungal colony diameter in (cm)

D0: Diameter of the fungal colony in the presence of the extract in (cm)

T: Rate of inhibition of mycelium growth in percentage

The extract is qualified as Very active when it has an inhibition of between 75%and 100%, the fungal strain is said to be susceptible. It is active when it has an inhibitionbetween 50% and 75%, the fungal strain is said to be sensitive. It is consideremoderately active when it has an inhibition of 50%, the fungal strain is said to belimit. Finally, it is little or not active when it has an inhibition of between 0% and25%, the fungal strain is said to be not very sensitive or resistant (Motiejunaite&Peiculyte, 2004).

F. Growth rate (VC) The rate of mycelial growth of each concentration isdepends on the formula:

$$V = D/T$$

V = Growth rate in cm / day

T = Growth time in days

D = Growth diameter in cm.

Chapter II: Results & Discussion

II.1.Phytochemical study for aqueous extract of *Z.officinale* and *C.verum*

II.1.1.Qualitative phytochemical analysis

The results of the preliminary phytochemical screening tests are presented in the table below.

Table 07: Preliminary Phytochemical Results of aqueous extract of *Z.officinale* and *C.verum*

Compound	Alkaloids	Flavonoids	Terpenoids	Phenols	Tannins	Reducing Compound	Saponins	Steroids Unsaturated	Steroids Derivatives
Aqueous extract Of Z.o	+	+	++	+	+	+	+	++	_ _
Aqueous extract Of C.v	++	++	++	++	++	+	+	++	_ _

(+): Present, (-): Absent

The table above shows test results on Preliminary phytochemical screening of extracts, the phytochemical analysis revealed the presence of flavonoids, alkaloids, tanins, terpenoids, phenols, reducing compounds, saponins and steroids unsaturated in all the extracts, except steroids derivatives.

These phytochemical analysis results agree with those obtained by **S. Prasad (2015)** for the *Z Officinale* extract and by **A. Kenza (2020) and hamza A (2020)** for the *C verum* extract.

Photochemical screening of Z officinale and C verum extracts. Revealed the presence of several chemical compounds. The richness of these plants in secondary metabolites justifies their great effectiveness in traditional therapeutic use.

II.1.2.Quantitative phytochemical analysis

A.Determination of polyphenol contents

The total polyphenol content of the various extracts was determined through using the method using the Folin-Ciocalteu reagent. The curve shows a linearity of absorbance as a function of concentration. The amounts of the corresponding extract polyphenols were reported in mg gallic acid equivalent per gram of extract (mg GAE/g)**(figure 17 and18) (table 08)** and it is determined by the type equation : $y=5{,}5262\ x + 0{,}025$, $R^2 = 0{,}9988$.

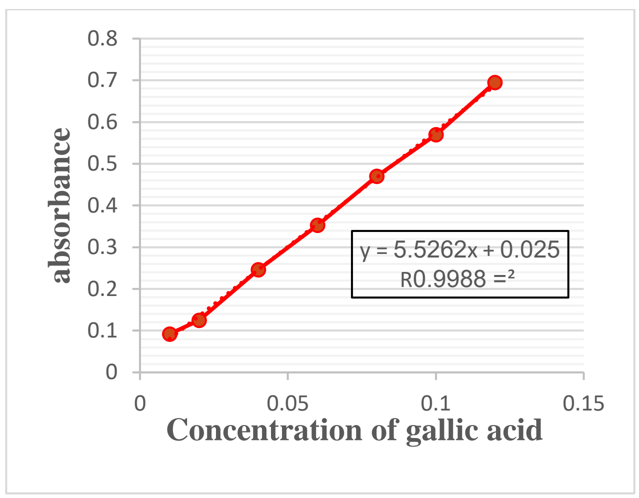


Figure17. Calibration curve of polyphenol.

Table08. Total polyphenol contents

Extracts	**Polyphenol Content (mg GAE/g E)**
Aqueous Extractof *Z.officinalle*	1,073 ± 0,343
Aqueous Extractof *C.verum*	3,133±0,809

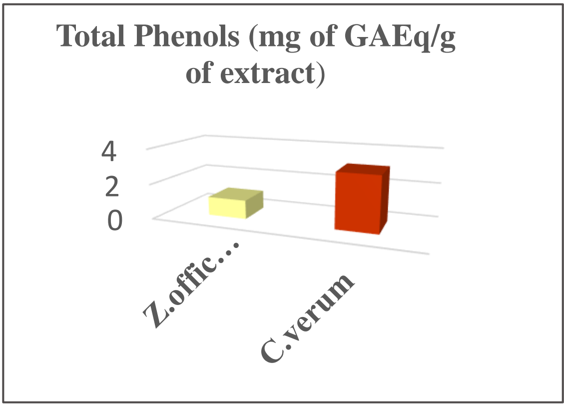


Figure 18. Total polyphenol contents of aqueous extract of *Z.officinale* and *C.verum* .

The content of total polyphenols in the extract of Cinnamomum verum obtained in the study carried out by ILHAMI G. *and al* (2019) is (0.153 mg E.A.G. / g ES) and by A. Kenza (2020) which is (0.365 mg E.A.G. / g ES) lower than the results obtained during our study (3.133 mg GAE/g).

Concerning the *Z Officnale* extract, our results for the polyphenol content (1.073 ± 0.343 mg GAE/ge extract) are lower than those found by Oussama .B*and al* (2022) showed values corresponding to 18.48 ± 1, 14 mg GAE/g extract .

The results obtained by **Beggas**and **Bendoukhane (2017)** showed that the content of total polyphenols is 3.56 µg (EAG)/g of *Zingiber officinale* extract.

These differences in results may be due to the low specificity of the Folin reagentCiocalteau" which is the main drawback of this color dosage. It has been shown thatthe reagent is extremely sensitive to the reduction of any non-hydroxyl groupsonly those of phenolic compounds, but also of certain sugars and proteins. Therehe phenolic content of a plant also depends on a number of factors such as, the conditionsclimate, time of harvest, extraction solvent, storage conditions **(SAIDI, 2019).**

B. Determination of flavonoid content

The determination of flavonoids was carried out using the aluminium trichloride (AlCl3) method and the standard is quercetin. The flavonoid content is expressed in milligram quercetin equivalent per gram of plant extract (mg QE/g) **(figure 19 and20) , (table 08)** . The flavonoid content of extracts was obtained from the calibration curve following a type equation :

$$y = 2,5937x + 0.0011, R^2 = 0.9989.$$

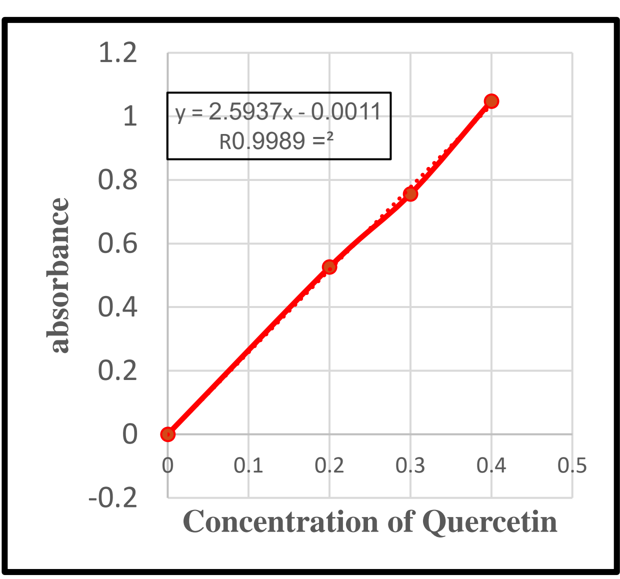


Figure19 :Calibration curve of flavanoid.

Table 09 . Flavanoidcontents

Extracts	**FlavanoidContent (mg QE/g E)**
Aqueous Extractof *Z.officinal*	0,602±0,089
Aqueous Extractof *C.verum*	0,761±0,018

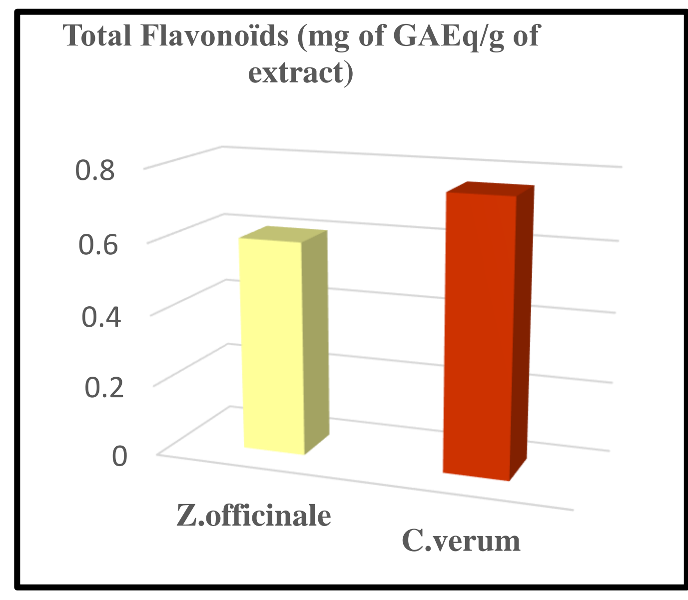


Figure20.Flavanoid contents of aqueous extract of *Z.officinale* and *C.verum*

The content (75.56 mg EAG/g) of flavonoids from Z Officinale was obtained by **Khadidja F and al (2007) and Bendoukhane.M (2017),** which is very high compared to Our results are (0.602 mg EAG/g).

Gaber El-Saber Batiha (2020) in his study on C Verum obtained a content of 54.77 mg catechin equivalent / g DM of flavonoids. These results differ from those found in the present study 0.761 mg EQ/g)

It should be noted that flavonoid content is often expressed by different standards Equations (quercetin, rutin, catechin) and the nature of the standard used can thus, in More of the above factors affect the final result..

II.2Antioxidant activity

II.2.1 DPPH radicals scavenging activity and IC_{50} value

The DPPH test is one of the most widely used tests to determine the antiradical activity of plant extracts . DPPH is a free radical allowing us to determine the scavening potential of our extracts to its sensitivity to detect active components at low concentration . Antiradical activity was estimated spectrophotometrically by following the reduction of DPPH at 517nm . This reduction capacity is determined by a decrease in absorbance induced by antiradical substances . In this test ascorbic acid is used as the standard, the obtained results (percentage of inhibition I %) are represented in the calibration curve **(Figure 21),** having the equation: y=3376,5x- 0,9094 with a correlation coefficient R^2 = 0.9854

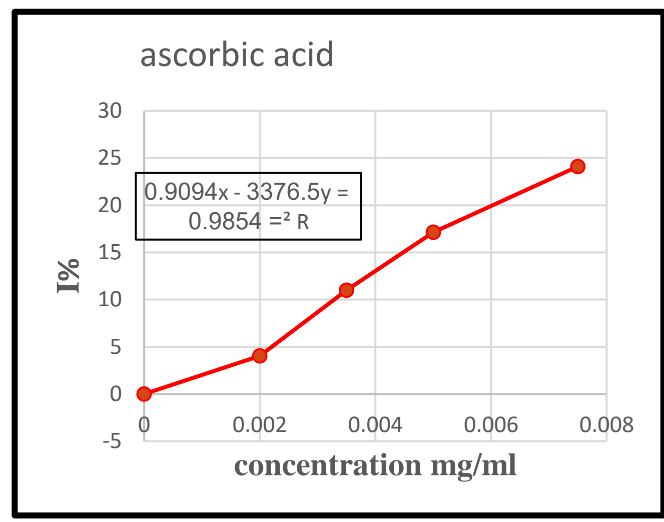


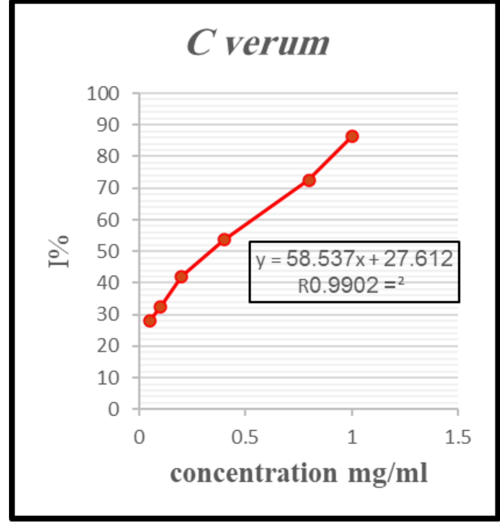
Figure 21. Calibration curve of ascorbic acid

To characterize the antioxidant power, we have introduced the IC50 parameter. IC50: it defines the effective concentration of the substrate that causes the 50% reduction of DPPH in solution.

The IC50 values of the different extracts were estimated through using the linear regression curve: y = ax + b. The IC50 values, presented in **table (10).**

Calculation of IC50 The antioxidant capacity of our different extracts is determined from IC50, parameters commonly used to measure antioxidant activity. It is the concentration of extract necessary to reduce 50% of the DPPH radical in a defined period of time. A low value of IC50 corresponds to a higher antioxidant activity of the extract. The IC50 are therefore calculated from the graphs shown in **Figure (22).**

The variability of antiradical activity is shown below:



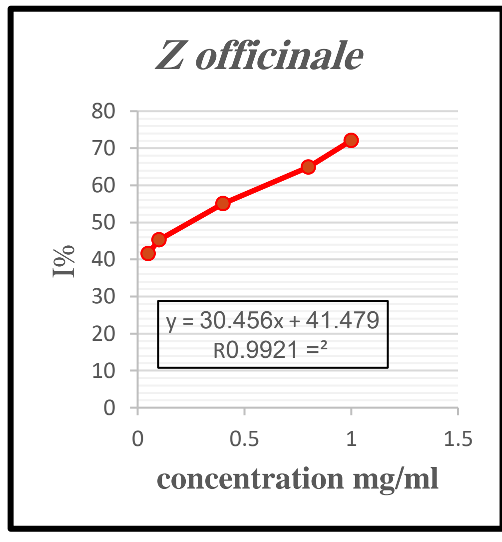


Figure22 . Variation in inhibition power as a function of the concentration of extract.

Table 10 . IC50 values of sample extracts of *Z.officinaleand C.verum* using DPPH

Sample	DPPH method		
	Equation	**R2 values**	**IC50 (mg/mL)**
Z.officinale	y =30,456x + 41,479	0,9921	0,285
C.verum	y =58,537x + 27,612	0,9902	0,386
Ascorbic acid	y=3376,5x- 0,9094	0.9854	0,014

From the curves , the value of percentage of inhibition of free radical scavenging activity of of sample extracts of *Z.officinaleand C.verum*varied from 41,6159 to 64,939% and 27,8963 to 72,561 % respectively. Furthermore, **the table (10)** exhibited the moderate capacity to neutralize DPPH radicals was found for the extracts of *Z.officinale*which neutralized 50% of free radicals at the concentration of 0.285 mg/mL while a lowest activity was found for***C.verum*** 0.386 mg/ml. In comparison to IC50 value of ascorbic acid (0.014 mg/ml).

Our results are lower than those found by **Amari (2016)** who demonstrated that *Zingiber officinale* has considerable antioxidant activity with an IC 50 of 1.29 mg/Ml.

Similarly for the results obtained by **NesrineAissani (2016),** they showed powerful anti-free radical activity with IC 50 of around 4.04 against an IC 50 of 2.51 for the positive control.

The work carried out by Liliane Bezerra et al. (2021), showed that the aqueous extract of C Verum has an IC50 of 29.01 mg/ml, which is higher compared to our results.

II.22.The reducing power of ferric ion (FRAP)

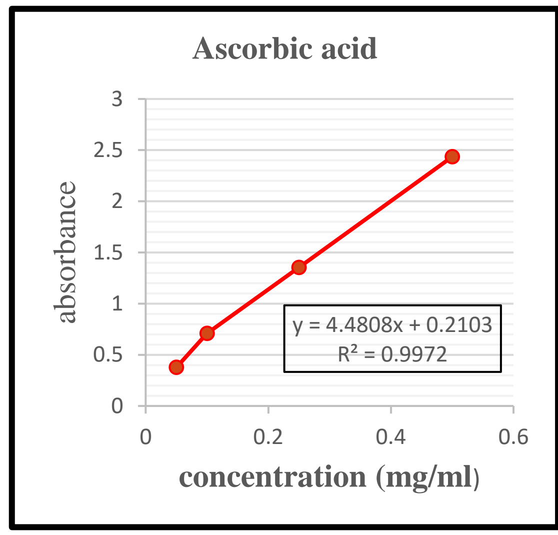
The FRAP of aqueous extracts of Z *officinale* and *C verum* were compared to the FRAP of ascorbic acid. This technique uses the direct reduction of hexacyanoferrate(III) anion [Fe(CN)6] 3- into hexacyanoferrate(II) anion [Fe(CN)6] 4-. As shown in Table11, The extract of ***C.verum***showed, exhibited a higher reducing power than the extracts of ***Z.officinale***. However, these antioxidant activities were inferior to that of ascorbic acid.

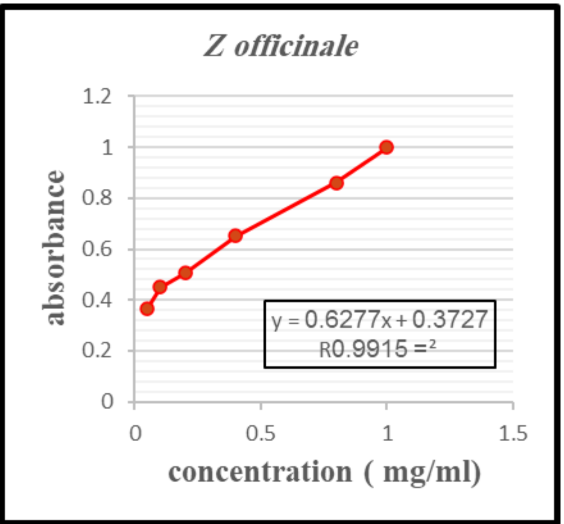
In this work, the extract has a dose –dependent reducing power which reaches a plateau at the concentration 1 mg/ml(**figure 23) .**

The highest FRAP value was observed for ascorbic acid (EC 0,5 0,068 ±0,004 mg/ml), followed by ***C.verum*** with(EC 0,5 0,133 ± 0,006mg/ml), and then the extract from***Z.officinale***with EC (0,5 0,207 ±0,005 mg/ml) .

Table 11:EC 0.5 values of sample extracts of *Z.officinale and C.verum* using FRAP

Sample	EC 0,5 (mg/ml)
Z.officinale	0,207 ±0,005
C.verum	0,133 ± 0,006
Ascorbic acid	0,068 ±0,004





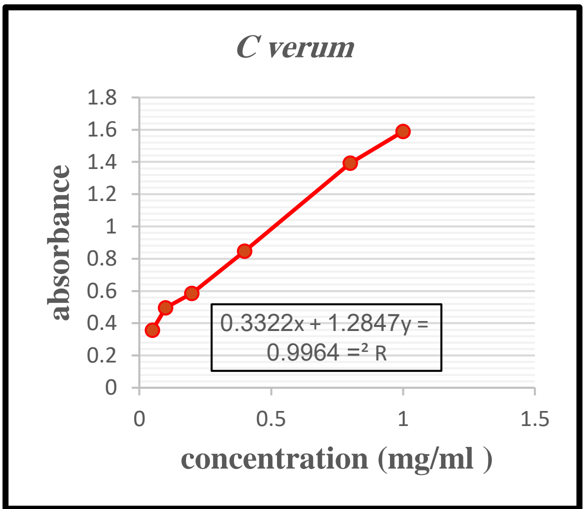


Figure 23:reducing power of ascorbic acid , the aqueous extracts of *Z Officinale* and *C verum*

Our results are approved by **A. Kenza (2020),** who showed a reducing power greater than the 1mg/ml concentration.

II.2.3 .Total antioxidant capacity (TAC)

The last method used in this work to quantify the antioxidant capacity of the aqueous extract of *Z Officinale* and *C Verum* is the total antioxidant capacity using gallic acid as standard at different concentrations expressed in milligrams of Gallic acid equivalent per gram of extract (mg EGA/g EXT) **(Figure 24)**

According to the calibration curve of gallic acid (y = 3,5125 x - 0.0073, R^2 =0.9948), the results show an antioxidant capacity of the order of 0,358 ±0,038 mg/g and 0,087 ± 0,024 mg/g respectively

For all the dilutions (0.04; 0.08; 0.1; mg/ml) the Z Officinale extract showed the greatest antioxidant activity compared to the C Verum extract **(Figure 24).**

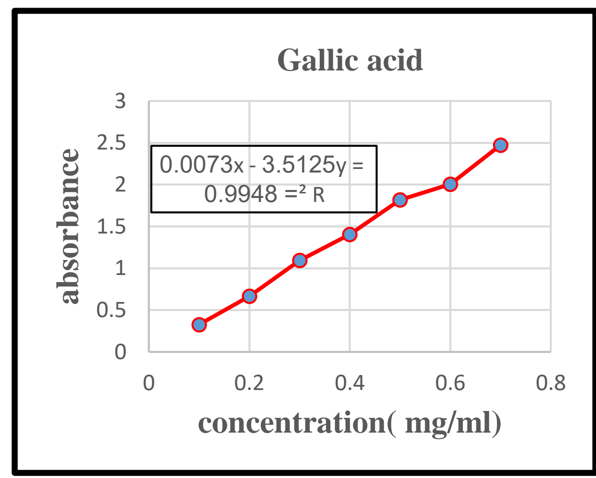


Figure24 :Calibration curve of gallic acid

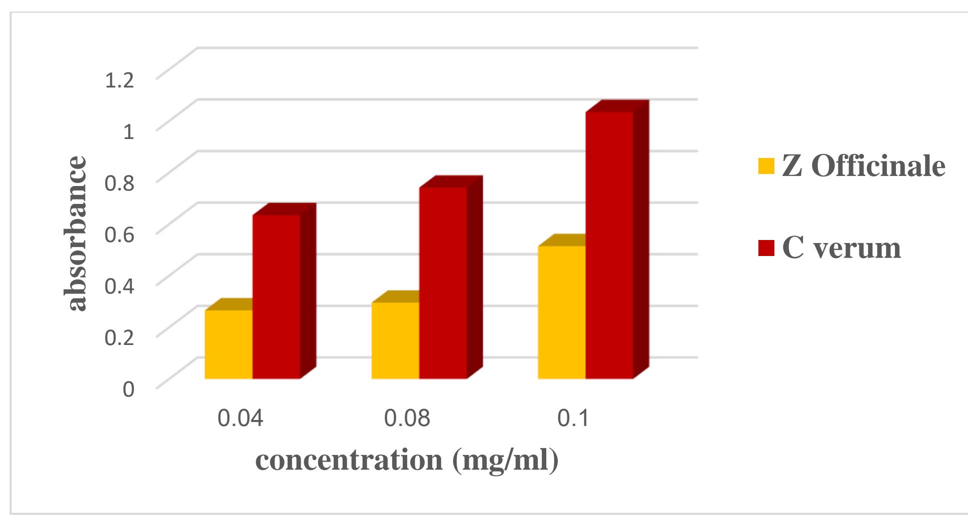
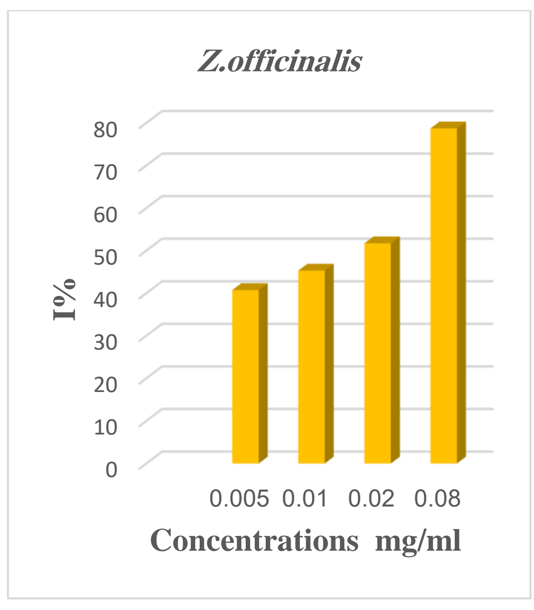


Figure25 :antioxydant activity of the extracts of *Z Officinale* and *C Verum* .

II.3Anti Hemolytic assay

The in vitro incubation of erythrocytes isolated from human blood with variable concentrations of the extracts was carried out in order to evaluate the anti-haemolysis activity. The results of the concentrations of the extracts causing the inhibition of haemolysis are mentioned in the **(figures 26).**



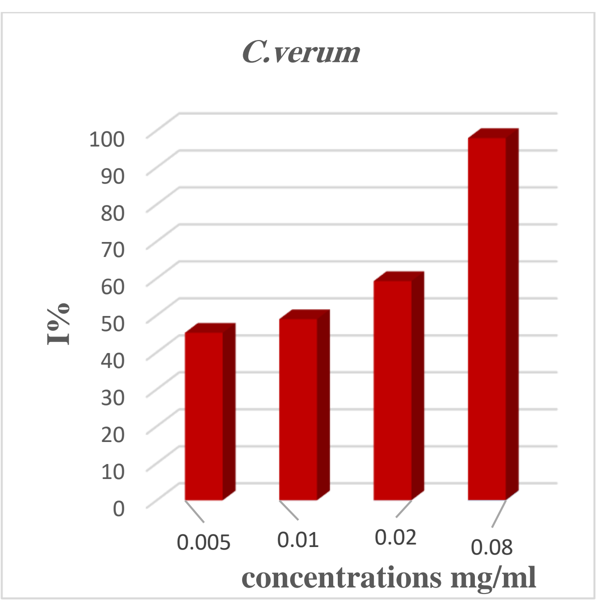


Figure26 :the effect of aqueous extracts of *Z Officinal* and *C Verum* on anti-hemolytic activity.

We found a decrease in the rate of remaining red blood cells depending on the concentration of the extracts.

Indeed, erythrocytes constitute a very adequate cellular model for the study of oxidative stress. Due to their ease of isolation, their simplicity, the richness of their membranes in polyunsaturated fatty acids and the high cellular concentration of oxygen and hemoglobin, these cells are extremely susceptible to oxidative damage **(ARBOS *and al*, 2008)** .

The results of the anti-hemolytic activity of the aqueous extracts of the two plants studied presented in the histograms, showed that the extract of *cinnammomum verum* has a greater inhibitory capacity than that of the aqueous extract of z. officinal. We observed that the extract of *cinnammomum verum* with a concentration of 0.08 mg/l presents the highest percentage of inhibition (97.87%), on the other hand the low percentage of inhibition is detected for the AG extract with a concentration of 0.005 mg/l (40.73%).

The evolution of the antihemolytic activity was proportional to the concentration of the extract that is to say that the more the concentration of the extract increased, the more the percentage of inhibition increased.

Nos résultats sont partiellement similaires à ceux trouves par **AMRANE *and al* ., 2017** Taux d'inhibition de l'hémolyse d'HE de *Menthaspicata* (49.53%, 26.56%,30.47%) pour les faibles concentrations.60, 0.90 et 0.51 mg/ml respectivement.

Muthu and Duraira., 2015. Found that the anti-hemolytic activity of Annona muricata extract is around 85.7% at 500 µg/mL

SUBOH ***and al.,*** **(2004),** reported that Nigella sativa seed extract will enable erythrocytes against oxidative damage induced by hydrogen peroxide (H2O2).

In this biological test, the red blood cells are subjected to oxidative stress conditions by adding H2O2. When the red blood cells are subjected to oxidative stress, the radicals cause membrane alterations and consequently hemolysis and the release of hemoglobin. Compounds possessing antioxidant activity delaying hemolysis relative to the control. Interactions between reactive species and putative antioxidants depend on several biological, physical and chemical parameters such as structure, size, solubility and redox potential.

Under the conditions of this test, free radicals are generated in the aqueous medium and react with the membrane lipids of erythrocytes during hemolysis. Nevertheless, lipophilic antioxidants are the most effective, in fact hydrophilic antioxidants require significantly but they cannot directly protect the membrane of red blood cells. In addition, hydrophobic compounds that insert into the lipid bilayer regenerate other antioxidants and/or enter into various enzymatic processes **(LESGARDS, 2000).**

The anti-hemolytic effect can be attributed to the secondary metabolites present inthese extracts such as flavonoids and polyphenols (Thephinlap et al., 2013). In effect,several studies have proven that some of the phenolic compounds, in particular theflavonoids with anti-free radical properties, by neutralizing or trappingfree radicals (Khalili et al., 2014). Additionally, polyphenols are known aschelators of transition metals, thus capable of the reaction rate of fenton. They canalso prevent oxidations caused by the hydroxyl radical and prevent the passage of H2O2 through the erythrocyte membrane and the generation of free radicals **(Wong** ***et al.,*** **2006).**

The inhibition of hemolysis may also be due to the interaction of the proteins of the extract with that of erythrocytes, which have important biological functions. There may be a protective effect on the erythrocytes either by the interaction of the proteins of the extract with the erythrocyte membrane lipids ; which are the target of oxidation ; which protects them against destruction and oxidation **(Chaudhuri** ***and al.,*** **2007).**

II.4.Anti inflammatory activity

An anti-denaturation study, when heat is given activation of antigen and denaturation of protein occurs as a part of type III hypersensitivity reaction. The assay infers the ability of extract to stabilize the protein from denaturation. The absorbance of test sample with respect to control indicates the stabilization of membrane**.(Dhanik*and al.,* 2017)**

Inflammation is one of the defense mechanisms against infections, tissue damage or injury. Non-steroidal anti inflammatoryagents (NSAIDs) are commonly used in the treatment of inflam-mation. Most NSAIDs act inhibiting the cycloo-xygenase pathway. Main causes of inflamma-tion in certain disease states such as arthritis, cancer and other inflammatory conditions are protein denaturation and lysosomal membrane lysis. One of the reasons for protein denatur-ation is production of auto antigens **(Gunathilake *and al.,* 2018)**.

C.verum and*z.officinal* extracts are capable of anti-inflammatory activity with protein denaturation inhibition percentage higher than 20% (**figure 27).**

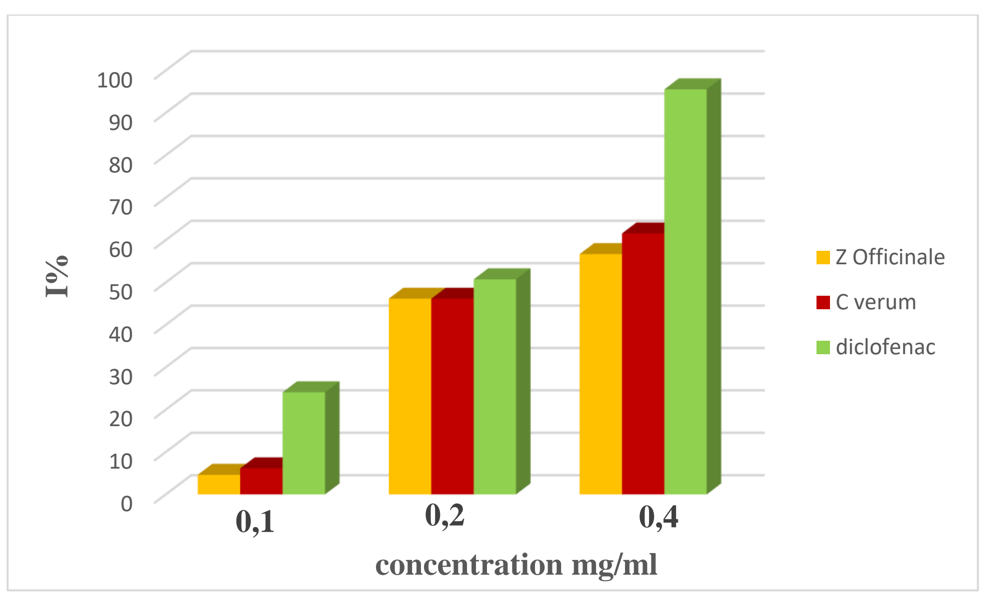


Figure 27:the effects of Z Officinale and C verum on Albumin denaturation test

The effect *c. verum* water extract as anti-inflammatory agent can be seen from the Figure. The pourcentage islower than the diclofenac sodium as positive control which only showed 24.08%, 50.72 % and 95.53% from 0.1, 0.2 and 0.4 mg/ml concentration respectevly.

The effect of z. officinal water extract as anti-inflammatory agent can be seen from **the Figure 27**. At 0.1, 0.2 and 0.4 mg/ml concentration, the extract of*z. officinal* has shown 4.61%-46.15 and .56.66 % inhibition percentage while the diclofenac sodium as positive control showed 24.08%, 50.72 % and 95.53% from 0.1, 0.2 and 0.4 mg/ml concentration respectevly.

This resultats ishigher than those obtained by **Ambily*and al.,(*2022),** for the waterextract of *z.officinal* with36.80% at 0.2 mg/ml concentrations. While,

Kavitha *and al.,* (2018)found that the percentage inhibition of protein denaturation of *z.officinal*tea is 91% at 0.25 mg/ml .

The leaf extract of ginger did show significant anti-inflammatory activity may be due to the presence of shagaol and gingerol that act by suppressing the prostaglandin and leukotriene

pathway by inhibiting the enzymes responsible for inflammation **(Dugasani*and al.,* 2010)**or probably by inhibiting inducible nitric oxide synthase(iNOS) which is also a mediator of inflammation **(Bischoff-Kont, *and al*., 2021).**

Protein denaturation is the protein's secondary, tertiary and quaternary structure change without the breaking of covalent bonds and is one of the causes of inflammation. The factors which cause protein denaturation are stress, temperature, pH, agitation, electricity, chemical substance, alcohol and reducing agents. Heat is one of the causes of protein denaturation because heat can disrupt the hydrogen bond so its capability of bonding the wa-ter decreased (Aditya *et al.*, 2015). It happens be- cause high temperature increases the kinetic energy and causes the molecules to vibrate so rapidly and violently that the bonds are disrupted. Protein dena- turation occurs steadily and unchanged. Denatured protein loses its solubility so it is easy to settle **(*Nijveldtand al.*, 2001).** When the protein denatures, some of its biological function might be lost. In ad-dition, Autoantigen produced by certain illnesses related to inflammation may be caused by denatur-ation of protein. Protein denaturation is the loss of a structure and protein function caused by stress, chemical substance or heat. There is also loss of bio-logical function when the protein denatures **(Aditya *and al.*, 2015).**

The anti-inflammatory activity mechanism can be done through some ways, Cyclooxygenase (COX) and lipoxygenase (LOX) en-zyme inhibition, leukocyte accumulation inhibition, neutrophil degranulation inhibition, histamine re-lease inhibition **(Riansyah*and al.,* 2015).** Anti inflam-matory activity from the flavonoid with Cyclooxygenase (COX) and lipoxygenase (LOX) en-zyme inhibition can cause leukotriene and prostag-landin synthesis inhibition which then inhibit mucus secretion which protect the stomach wall. Leukocyte accumulation inhibition during the inflammation process will lower the body response against inflam-mation. This inhibition happens because Cyclooxygenase (COX) is inhibited so thromboxane causing the leukocyte modulation is inhibited. His-tamine release inhibition happens because flavonoid can inhibit mast cell release **(Hidayati*and al.,* 2008).**

Beside flavonoid compound, tannin compound also reported to have anti inflammatory property **(Verma *and al.*, 2011)** and saponin compound has the potential to be anti inflammatory agent by protein denaturation inhibition (Erianti*et al.,* 2015). Proteins of a vulnerable body can experience denaturation caused by free radical formation which causes an inflammation mechanism through inflammatory mediator release. Denaturation of protein is a process where protein loses its secondary or tertiary structure due to exter-nal compounds such as strong acid or base, inor-ganic salt, organic solvent and heat **(Verma *and al.*, 2011).**

II.5.Antifungals activity

To minimize as far as possible the affection of potatoes by fungal diseases and to avoid the toxicity of chemical control (fungicides and heavy metals, etc.) on the one hand and their harmful corollary on the environment and the consumer health on the other hand, we use a control strategy which is based on the use of extracts of two plants at different concentrations, these plants are: *z. officinal* and *c. verum.* The aqueous extracts of these two plants have been tested on the fungal species isolated: *Fusarium solani*

The results are represented as follows:

Z officinal**:** it has been shown that the extract of this plant can effectively reduce the growthof the fungal species tested and which caused a good inhibition of fungal growth for the species *Fusarium solani* (65%) at a concentration of 0.08 mg/ml after 144 hours, but this extract shows an average effect of 29% at a concentration of 0.02 mg/ ml it can be said that *Fusarium solani* resists the effect of the extract (15%) at a concentration of 0.1mg/ml after 72 hours of incubation. This last result is in agreement with **Ghedira and Goetz** (2016) who reported that *Fusarium oxysporum* was found to be resistant when tested.

However**, Hexiang*and al.* (2005**) demonstrated that ginger rhizome is able to exert an inhibitory effect on mycelial growth in several species of fungi *(B. cinerea, F. oxysporum, M. arachidicola, and P. piricola)*. Similarly, **Gayathria a*nd al* (2020)** showed that the effect of antifungal activity of *Zingiber officinale* fresh sample has a maximum zone of inhibition compared to other fungi. According to the same author **(Gayathri *and al*, 2020)** fresh ginger contains more oxygenated compounds such as gingerol compared to dry ginger which makes it more powerful than dry *Zingiber officinale*

c.verum: this extract showed an effect on the fungal species *Fusarium solani* testedand caused a strong inhibition of fungal growth (60%) for the 0.08 mg/ml concentration after 144 hours of incubation, this demonstrated the effectiveness of the extract. But this extract presents weak inhibition of the order of 18% and 20% at a concentration of 0.01 mg/ml and 0.02mg/ml respectively

Yeole*and al.*, (2016), found that the inhibition of F. oxysporum was pronounced more with Hx and MeOH extracts of Cinnamomum zeylanicum with inhibition zone in order to 43.33 mm and 40.67mm.

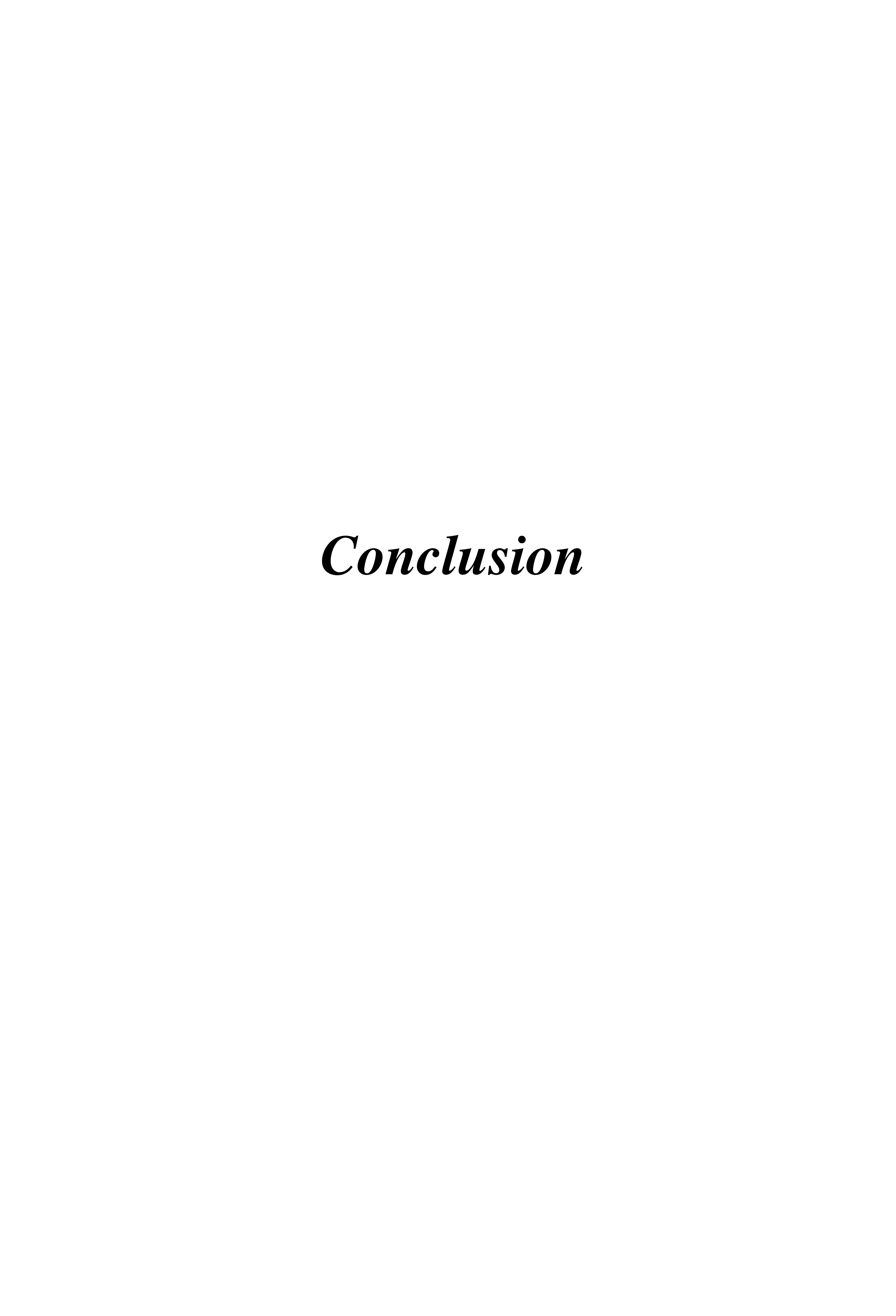
according to **Hussain *and al.*, (2015)** who reported that there are other extracts which are: *Acacia nilotica, Azadirachta indica* and *Eucalyptus camaldulensis* are very effective and suitable forinhibiting mycelial growth of *Fusarium solani*

Our study of the aqueous extracts tested shows an inhibitory activity on the fungal species *Fusarium solani*. there are better activities by certain concentration but other concentrations do not present an interesting activity against the fungal species tested this can be due to the difference in the concentration of the bioactive compounds of the plants used to carry out the extractiont, or by the resistance of the species tested.

The results are summarized in **the table 12**.

Table12: percentage inhibition of plant on *Fusarium solani*

Extract	Concentration mg/ml	72 h	96 h	120 h	144h
C verum	0.01	18	20	21	29
	0.02	20	24	27	30
	0.08	27	30	48	60
Z Officnale	0.01	15	18	22	30
	0.02	20	23	25	37
	0.08	29	35	41	65



Conclusion

The objective of this study is first to reveal some fungal diseases affecting potato cultivation in the wilaya of El Oued. In addition, we carried out a test of biological control in laboratory speaks test of the aquatic extracts of the two plants c.verum and z.officinal against *Fusarium solani* of potato.

Our results revealed significant levels of total polyphenols and flavonoids fromour extracts, particularly the extract of *C verum* than *Z officinal*.these extracts exercised a modesteantioxidant activity in all the tests carried out (the DPPH test, FRAP ,and TAC).

The anti-inflammatory effects of the extracts were evaluated by the protein denaturation test and the H2O2 test as a biological test to evaluate the anti-hemolysis activity.

Our extracts have an anti-inflammatory effect with a very high percentage inhibition of protein denaturation of the order of 61.53% in the aqueous extract c.verum and 56.66% in the aqueous extract z. officinal.

The results demonstrate that the extracts examined have a very significant anti-hemolysis power, and that the aqueous extract of c.verum showed good activity (97.87%), whereas the aqueous extract of z. officinal has an activity of (78.71%).

The antifungal power is always sought in plants to fight against thespread of fungal diseases. *Fusarium solani* is one of the most important fungal diseases affecting potato cultivation. Thus, the properties of aqueous extracts of z.officinal and c.verum were tested on this fungus. Our study reveals a remarkable inhibition by our extracts on the fungus *Fusarium solani* .

Through the results obtained, the aqueous extracts of c.verum and z.officinalhave remarkable antioxidant, anti-inflammatory and antifungal potential justifying the traditional use of these plants.

These results revealed interesting prospects for the future of which it would benecessary to:

- Separate, isolate and identify the phenolic compounds of these species bythe use of chromatographic techniques.
- Study of other biological activities namely: antibacterial properties,antivirals and others.
- Create combinations between these extracts and antifungals and test them on other species of Fusarium and other species of fungus.

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