UNIVERSITY OF NAPLES "FEDERICO II"

DEPARTMENT OF AGRICULTURAL SCIENCES

AND

AN-NAJAH NATIONAL UNIVERSITY

FACULTY OF GRADUATE STUDIES

MASTER DEGREES IN

FOOD SCIENCE AND TECHNOLOGY

AND

NUTRITION AND FOOD TECHNOLOGY

EXPERIMENTAL THESIS

BIOLOGICAL ACTIVITY OF PLANT EXTRACTS FROM MIDDLE EAST AREA ON FOOD BORNE PATHOGENS

Supervisor:
Dr. Samer Mudalal
Co- Supervisor:
Prof. Gianluigi Mauriello

Candidate:
Nouraldin Shtaya
Matr. N06/682

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Dedication

To my dear parents father and mother who

Supported me all the way since the beginning of my life.

to my brothers and sisters who have been a great source of

Motivation and inspiration, with love and respect.

Acknowledgments

Firstly, I would like to express my sincere gratitude to my thesis supervisor Dr. Samer Mudalal for the continuous support of my thesis study and related research, for his patience, motivation, and immense knowledge. His guidance helped me in all the time.

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My special thanks for all technicians in Department of microbiology at university of Naples Federico II for their help and cooperation.

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

BIOLOGICAL ACTIVITY OF PLANT EXTRACTS FROM MIDDLE EAST AREA ON FOOD BORNE PATHOGENS

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: _____

Signature: _____

Date: _____

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BIOLOGICAL ACTIVITY OF PLANT EXTRACTS FROM MIDDLE EAST AREA ON FOOD BORNE PATHOGENS

By

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ABSTRACT

there's many research investigate of high activity of plants extract and microbes and during the past few decades there has been a notable increase in the demand natural preservatives. The aim of our study to evaluate six methanolic extracts of medicinal plants from Palestine against 18 bacterial indicator strains (gram positive & gram negative).

Six methanolic extracts were prepared from different parts of plants used in traditional medicine in Palestine. We prepared the crude extract by using methanol and distilled water, and used rotary evaporator, Testing by using two method paper disc diffusion and agar well diffusion.

In our study has shown the antibacterial activity for all methanolic extract of plants we tested *Origanum syriacum L. Var. syriacum,* Rhus coriaria L., Rumex acetosa L, Salvia hierosolymitana Boiss, Teucrium capitatum L, Cyclamen persicum mill at different amount range (5µL-50 µL).

In addition the results we got it, can be first step for various future research.

Chapter One

Introduction

1.1 Brief history of use plants in medicine

A lot of evidence proves the history of plants use across human life stages, Since thousands of years the humans used the plants in a meal, and by experiments he learned about the usage of plants in Food and healing diseases, there was evidence prove the uses of herbal remedies since 60,000 years in a burial site northern in Iraq. (Solecki et al.1975).

The usage of herbs going with humans through centuries, the religious books were mentioned many of plants that, for example in the Bible, (Vedas), (Žarko Šantić et al.2017) *Zabur* and Quran in addition every plants mentioned in this book have argued on its benefits . Later on thousands of years, many of these benefits of plants evidence of scientists and researchers.

When we talked about the history of herbal medicine plants we must refer to four historical periods its classified to four famous periods, in different time ad different culture: Traditional Chinese Herbalism, Ayurveda Herbalism, Western Herbalism, which originally came from Greece and Rome to Europe and then spread to North and South America, and Arab traditional medicine, which forms the basis for alternative and herbal medicine in Arab countries.

(WHO. 2001) and every period has its properties with rich information about healing by herbal plants.

The herbal medicine used for a long time to heal many diseases (disease of respiratory system, digestive system, blood pressure, diabetes mellitus type 2, allergy, anti-obesity, anti-cancer, (Ali-Shtayeh MS et al. 2008, Ali-Shtayeh MS et al.2011).

On the other hand the percentage of people usage of traditional remedies including herbal medicine According World Health Organization about 80% of the world population rely chiefly on indigenous medicine and that the majority of traditional therapies involve the use of plant extracts or of their active compound (WHO, 1993, 2002).

Also the use of traditional medicine, and folk medicine widespread throughout the Middle East, including Palestine (Abu-Rabia, 1999; Ali-Shtayeh and Jamous, 2006).

1.2 Plants as antimicrobial

During stages of human life attempt to eliminate and combat with pathogenic microorganisms in human health and food safety human, naturally the Plant have substance in many cases, these substances serve as plant defence mechanisms against predation by microorganisms, insects, and herbivores, (Cown ,1999).

A few decades ago until now the methods of decrease spoilage improved by using many novel techniques, the was used plant products as natural preservation, to decrease or eliminate the various of spoilage on food physical microbial and chemical ((Burt, 2004; Moriera et al, 2007; Simitzis et al., 2008).

In literature, there are many studies related about the effect of types extract of plants against microbial, to keep the high quality of food, or keep economic situation and feeding a high number of population There are around 500 000 plant species worldwide, of which only 1% has been phytochemical investigated, there is great potential for discovering novel bioactive compounds. (Palombo, 2011).

Instead of using chemical preservative and have their bad effect on human health, the way for scientists and researcher in food technology to give attention for natural antimicrobial substance, for example: aromatic plants (mint, sage, rosemary, lavender, basil, thyme, Organum) and other many plants classified a high antimicrobial (Gutierrez, 2008).

in different types of food we can use it the extract of plants either essential oil or crude extract for example uses of (clove oil, cinnamon oil and star anise oil) to Control of human enteric pathogens in fruit & vegetable juices by the use of essential oils.in concentration 0.1mm tested against *E. coli O157:H* the effect was 5 log CFU/ml from a) 24 h, b) 48 h, and c) 72 h respectively.(Pan, et.al 2014).

In addition effect of antimicrobial on various types of meat products either fresh, cooked or processed. for example in chicken slice The effect of EOs of thyme and balm to inhibit growth of Salmonella and *E.coli* in raw chicken slices stored aerobically at 4°C for 21 days (Fratianni et al. (2010).

In that same study, thyme EO show clearly a marked growth inhibitory effect on E. coli compared with balm EO. After 21 days, the viable count *E.coli* in chicken samples surface-treated with balm and thyme were, respectively, about 0.30 and 1.46 log less than that of control. (Fratianni et al. 2010).

Another example of different type of meat the effect of in the essential oil addition of thyme EO (0.6%) to ground beef resulted in growth inhibition of *E.coli O157:H7* during storage of the beef at 10°C but not at 4°C the special properties for thymol because content carvacrol and thymol, were deemed responsible for their high antibacterial effect. (Burt and Reinders 2003, Burt et al., 2005).

Also, there are many examples Explain the real role of plant activity on meat product without a doubt on the effect of EOs of coriander, clove, oregano, and thyme EOs were strongly inhibitory to L.monocytogenes, A.hydrophila, and indigenous microbiota in meat products at levels of 5–20 ml/g.(Skandamis and Nychas, 2001).

also there is many information in literature review show effect of plants as antimicrobial of different types of meat we mention it previously, pork, goat or sheep, sausage and many studies also in effect of plant antimicrobial on Dairy products, fish and seafood…etc.

There is theoretical mechanisms, interaction between the active substance from plants and food matrix and microorganism these mechanisms that could explain synergism among antimicrobial components. First, an antimicrobial may interact with the cell wall or the cell membrane of a microbial cell and enhance the uptake of other antimicrobials, second, numerous antimicrobials may interact with the cell wall. A third theory proposed is that sequential inhibition may occur whereby many steps in a common biochemical pathway are inhibited, thus producing synergistic effects. The fourth theoretical mechanism hypothesized that synergy results from the inhibition of enzymes that degrade antimicrobials (Espina, et al 2013).

1.3 Methods of plants extraction

Essential oils and other extracts of plants have evoked interest as sources of natural products. The content of these active compounds enhanced scientific and specialized persons for extracting by many methods, during few decades ago the method developed

year after year, the traditional methods, for example: maceration and Soxhlet extraction are commonly used at the small research setting or in Small Manufacturing Enterprise.

Some examples of the modern extraction methods, microwave-assisted (MAE), ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SFE), the properties of modern if we compare old method, the modern will keep the quality and quantity of extracts, and short time. (Kothari et al., 2010).

Related to (Tonthubthimthong et al., 2001) depends The supercritical fluid extraction (SFE) an old technique of solvent extraction but its commercial application happened slowly due to the sophisticated and expensive high pressure equipment and technology required.

Also, the amount of extraction and characteristic depend on many factors, pre-extraction, and after extraction, some of these factors weather, season of collection, fresh or dry plant material, during extraction Suitable conditions for each extraction methods are also important such as temperature, light, type of solvent and time (Hijazi et al, 2013, Evans, 2002).

1.4 Composition of plant extracts

The active compounds in plants are different from one to the other, then these differences of active compound (phytochemical) It can also have an effect on effect on shelf life, plant qualities, color, and taste of the plant, More than 8000 phenolic compound were known from plant source, phytochemicals in their cells including saponin, tannins, flavonoids, cyanogenic, phenolic compounds, lignin, lignin, alkaloids and glycosides (Okwu, 2004).

The most common phytochemicals are phenolic compounds, including phenolic acids and flavonoids, also its have more interesting for researcher from a few decades ago due to free radical scavenging properties. (Meneses, et al.2013).

Researchers found phytochemical in vegetables, fruits, beans, whole grains, nuts and seeds, herbs, shrubs, for example:

Carotenoids Flavonoids Polyphenols Terpenes in red fruits and vegetables, Carotenoids Flavonoids Indoles Glucosinolates Isothiocyanates (in green fruit & Vegetables, The composition of plant extract, range from species to another.

1.5 Use of plant extracts in food for Antimicrobial purposes

Food spoilage can occurs in different steps of production such as pre- processing, processing, storage and handling .the cause of spoilage from many source Chemical, physiological, biological (Gould, G.W 1996).

The purpose of extraction from plants source to use extract in food for many reason, such as improve the taste, colour, texture and odour, also to increase a shelf life of food we add for it. (Burt, 2004).

As we mention previously the plant produces chemicals substances to protect themselves from external factors may be will effect on it, but the Research demonstrate that they can also protect humans against diseases (Badugu, 2012).

Related to The FDA defines antimicrobial agents as "substances used to preserve food by preventing growth of microorganisms and subsequent spoilage, including fungi stats, mould and rope inhibitors. (Branen et al., 2002). Depend on many studies the Plants and herbs can achieve and effect against food borne pathogen for example Edible, medicinal and herbal plants and spices such as oregano, Sumac, Onion, garlic, rosemary, thyme, sage, Mint, basil, Lavender turmeric, ginger, garlic, nutmeg, clove, mace, and fennel, have been usefully alone or combinations to inhibition the growth of Food borne pathogen . Also exert direct or indirect effects to extend foodstuff shelf life or as antimicrobial agent against a variety of Gram-positive and Gram-negative bacteria. (Nair et al., 2005).

In another studies of test the effect or extract of 30 plants, show the high activity of (Clove, sage, celery, Rosemary, oregano) against Escherichia coli, Listeria innocua, Staphylococcus aureus, and Pseudomonas fluorescents (Witkowska, et al.2013).

Also the usage of plant keep from long time in folk medicine, related on (Yaghmuor R.M.at al 1998) of studied the extract from 20 plants in Palestine, against five strains bacteria, and one yeast, the study show the significant activity of 10 plants at least .

1.6 Objectives of this study

The main aim of the study were:

- Investigate the antimicrobial activity of methanol crude extract of plants selected specially against many bacterial indicator strains were caused food deterioration.
- Investigate the antimicrobial activity of microcapsulation of some Methanolic extracts of plants we studied.

Chapter Two

Material and Method

2.1 Bacterial Strains

All of the indicator strains were provided by Department of food science, Division of Microbiology - University of Naples Federico II. The indicator strain we used in Table 2.1

It was routinely cultured in Tryptone Soya Broth (TSB) supplemented with 5 g/L Yeast Extract Powder at 20 C for 24 h. the strains was inoculated in TSB agar (TSBA; TSB with addition of 30 g/L agar and 5 g/L Yeast Extract Powder). All media were purchased from Oxoid (Oxoid S.p.A.,Milan, Italy).

Table 2.1

All of the indicator strains were provided by Department of food science, Division of Microbiology - University of Naples Federico II.

Indicator strains	Source	Gram +/-	Growth conditions
Brochothrix thermosphacta 7R1	Meat	+	TSB 24h a 25°C
Enterococcus faecalis 21		+	TSB 24h a 30°C
Brochothrix thermosphacta D274	Meat	+	TSB 24h a 25°C
Listeria innocua 1770	milk	+	TSB 24h a 30°C
Carnobacterium maltaromaticum 9P	Meat	+	TSB 24h a 25°C
Carnobacterium maltaromaticum $D_1$203	Meat	+	TSB 24h a 25°C
Carnobacterium maltaromaticum $F_1$201	Meat	+	TSB 24h a 25°C
Carnobacterium maltaromaticum $H_1$201	Meat	+	TSB 24h a 25°C
Staphylococcus saprophyticus 3S		+	TSB 24h a 37°C
Staphylococcus aureus GB1		+	TSB 24h a 30°C
Pseudomonas gessardii SA33B	Soil	-	TSB 24h a 28°C
Pseudomonas fragi 6P2	Soil	-	TSB 24h a 28°C
Escherichia coli 32	Meat	-	TSB 24h a 30°C
Enterococcus faecalis 226B		+	TSB 24h a 30°C
Streptococcus bovi ES1		+	TSB 24h a 30°C
Enterococcus faecalis 21		+	TSB 24h a 30°C
Streptococcus salivarus		+	TSB 24h a 30°C
Serratia proteamaculans 20P	Meat	-	TSB 24h a 30°C
Bacillus clausii *	Soil	+	TSB 24h a 30°C

2.2 Plant Sample Collection

The plants were collected at winter season January & February 2018, from Mountain IN Til village near Nablus city – *Teucrium capitatum L*. (BERC-BX-C0167) , *Origanum syriacum L. var. syriacum* (BERC-BX-C0026) , Cyclamen persicum Mill.(BERC-BX-C0351) , *Salvia hierosolymitana Boiss* (BERC-BX-C0156) , *Rumex acetosa L.*

(BERC-BX-C0294*), Rhus coriaria L*. (BERC-BX-C0037) this plant from collected from mountain in Hebron Voucher specimens were deposited in the Herbarium were identified by Prof. M. S. Ali-Shtayeh from the Biodiversity and Environmental Research Centre, BERC, Til Village, Nablus.

Table 2.2

The plants used in this study

Scientific name	Common name	Part uses	Voucher number
Cyclamen persicum Mill	Cyclamen	Leaves	BERC-BX-C0351*
Origanum syriacum L. Var. syriacum	Za'atar, Syrian Oregano or Syrian marjoram	Leaves	BERC-BX-C0026
Rhus coriaria L.	Sumac	Fruit pericarp	BERC-BX-C0037
Rumex acetosa L.	Sorrel , garden sorrel	Leaves	BERC-BX-C0294
Salvia hierosolymitana Boiss	Jerusalem sage	Leaves	BERC-BX-C0156
Teucrium capitatum L.	Cat thyme	Leaves (Arial part)	BERC-BX-C0167

BERC : Biodiversity & Environmental Research Center , Til, Nablus – Palestine

2.2.1 Cyclamen persicum Mill

The common name Cyclamen, Arabic name :(ziemtut, bikhawr maryam زعمطوط , بخور مريم) belongs to the family *Primulaceae, wild plant ,* had several medicinal, nutritional and cultivated as ornamental in many counties (Primorac *et al.*, 1985). Besides of uses in herbal medicine it's used as food for long time, (Saad and Said, 2011).

Figure 2.1

Flowers and leaves of Cyclamen persicum Mill, Palestine, Nablus, Til, 2018

2.2.2 Origanum syriacum L. Var. syriacum

Common name :(Za'atar, Syrian Oregano or Syrian marjoram) Arabic name,:(Za'atar زعتر, صعتر), belong to Lamiaceae family, it's one of famous important aromatic plants, known in many countries (Palestine , Jordan , Syria , and Lebanon (Ali-Shtayeh and Jamous, 2008), its one of common important recipes mixing with Sumac, olive oil and sesame) its was collected from wild, but now a days cultivted because it have a good medicinal and nutrient properties (Ali-Shtayeh et al., 2013).

Figure 2.2

Leaves of Origanum syriacum L. Var. syriacum plant Palestine , Nablus ,Til ,2017

2.2.3 Rhus coriaria L

Common name: sumac, Arabic name: (Sumacسُماق) ,belongs to Anacardiaceae family, known in many countries (Palestine Jordan, turkey, Iran, Africa, Western Asia and others. it's the one of most important plants usage in traditional medicine for many purposes anti diabetic, bowel pain, fever, enhance appetite and decrease the hypertension (Ali-Shtayeh and Jamous, 2008, Kosar et al., 2007,Shafiei et al., 2011) also its mixed with other foods as spices it's have acidic tasty fruits.

Figure 2.3

Fruits and leaves of Rhus coriari L

2.2.4 *Rumex acetosa L*

Common name (sorrel, or garden sorrel), Arabic name: Humaed حُميض) wild plant, the family name: POLYGONACEAE, its was known in Palestinian traditional folk medicine, used in recipes (Ali-Shtayeh MS 2008). Generally used after boiling .it's have sour taste. Also R.acetosa known in traditional remedies for some countries in Europe such as Hungary and Romania (Dénes et al., 2013) the same usage in folk medicine for Korean , Britain and Ireland (Allen and Hatfield, 2004).

Figure 2.4

Leaves of Rumex acetosa L. Palestine, Nablus, Til, 2018

2.2.5 Salvia hierosolymitana Boiss

The common name is Jerusalem sage, the Arabic name qasaeayn muqdisi قصعين مقدسي, لسيّنة or Lisâna,) belong to Lamiaceae family , in traditional Arabic Palestinian herbal medicine (TAPHM), a common use for digestive system, urinary tracts, and for treatment of hypertension (Ali-Shtayeh, M.S.& Jamous 2008) also used as food one of famous way for cooking the leaves of this plant with rice and meat . Related on website Flora of Lebanon and (Ali-Shtayeh, M.S.& Jamous 2008: its endemic plant in Lebanon, Palestine and Syria.

Figure 2.5

Leaves of Salvia hierosolymitana Boiss. Palestine, Nablus,Til ,2018

2.2.6 Teucrium capitatum L

Teucrium polium L.(synonym) the common name: cat thyme, the Arabic name: Jaada جعَدة belong to belong to Lamiaceae family , it's one of aromatic plants, known in many countries (Palestine, Jordan, Syria and Turkey) (Öztürk et al. 2017 ,Ali-Shtayeh, M.S.& Jamous 2008). In traditional medicine the purpose of usage for Stomach pain, respiratory system, antidipatic, ant-inflammatory, antimicrobial. (Azaizeh H et al. 2006).

Figure 2.6

Arial part of Teucrium capitatum L. Palestine, Nablus ,Til ,2018

2.3 Prepartion the plants Sample for extraction

After collection the fresh plants, we cleaned it from soil or dust and keep it to dry at room temperture in shade, the drying period still about 15 days, after that's packaging the dry plants and label it.

2.4 Preparation of crude extract

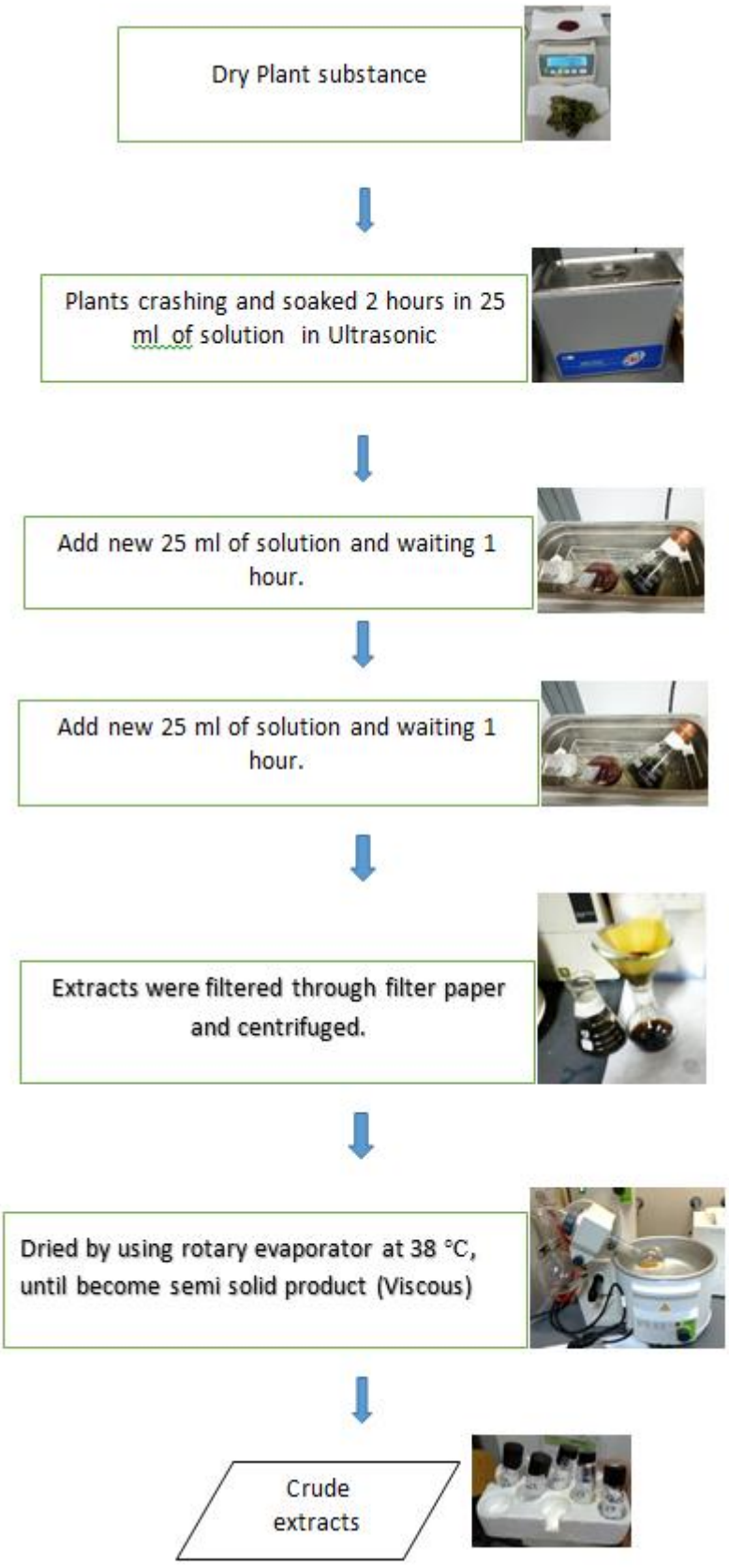
First protocol:

Five grams of dry plant was ground using a mortar and pestle, the resulting powder was extracted by continuous stirring with 25 ml 99% methanol with distilled water (aqueous methanol 2:1v) respectively at 24°C for 2 hours in ultrasonic after that add another 25 ml (aqueous methanol solution) and waiting one hour another 25 ml and another one hour in ultrasonic a. then keep the bottle of soaking solution overnight in incubator shaker, Extracts were filtered through filter paper, then centrifuge at 6500 RPM for 15 minutes , The combined methanol extracts were dried using rotary evaporator at 38 °C, Until become semi solid product (Viscous) the residue was then washed with dimethyl-sulphide (DMSO).then Stored in different bottle (without mixed with totally crude extract (pure) , followed stored at 4°C for future use. (it's more clear in the figure 7)

Note: related on this protocol we will double the volume of solution if we used double amount of dry Plant.

Figure 2.7

Show the steps of extraction crude extract from plants



2.5 Culture Media and Chemicals

Types of media was required for carrying out this study Tryptone soya agar (TSA) and tryptone soya broth (TSB) Also methanol was used for extraction process , Dimethyl sulfoxide (DMSO).

2.6 Preparation of inocula

According to (Jayaraman et al 2008) to Stock cultures were maintained at 4 C on nutrient agar slants for bacteria. Active cultures for experiments were prepared by transferring a loopful of culture to 10 ml of TSB and incubated at ideal temperature for each strain (25 c & 30 c) .

- 0.1 ml overnight culture of each indicator strain in sterile tube of TSB media, Incubation 24 h the necessary for each strain .
- 0.5 ml overnight culture of each indicator strain poured in Petri dishes (60 mm), adding TSA soft waiting a few minutes for Solidification.

2.7 Method of antimicrobial activity test

2.7.1 Agar well diffusion

The common method of testing antimicrobial activity of plant extract or other sources, the principle of this method after we poured the media on petri dish and waiting to dry and cold, then done the hole on media by using cylinder the diameter 6 mm standard, then overnight incubation of petri dish and its can be clear inhibition zone area if the compound we used have this properties, The advantage of this method, available to use more than compound (Obeidat et al 2012).

2.7.2 Paper Disk Diffusion

A suspension of testing microorganisms were spread on (TSA) medium. The filter paper discs (6mm in diameter) was placed on the agar plates which was inoculated with the tested microorganisms and then impregnating with different amount of crude extracts 5, 10, 20, 30 40, 50µl of plant extract. The plates were subsequently incubated at 25 and 30°C for 24h. After incubation the growth inhibition zone were quantified by measuring the diameter of the zone of inhibition in mm (Kumar et al., 2009).

2.8 Microencapsulation

A few years ago the Microencapsulation technique widespread in pharmaceutical and medicine sector for many reasons:

- protect unstable active compound from its surrounding environment.
- keep a safety for the user from the side-effects of the encapsulated compound.
- trap a compound (aromas, organic solvents, pesticides, essential oils, etc).
- modify the density of a liquid, change material from liquid to solid (Munin ,A and Edwards-Lévy, F 2011).

2.8.1 Preparation of Microencapsulation of Crude extract of *Rhus coriara L* & Teucrium capitatum L

Microencapsulation of crude extract was carried out by using the Encapsulator B-395 Pro (BUCHI, Switzerland) equipped with an 80 µm nozzle and the syringe pump. A scheme of the Buchi Encapsulator is reported in (De Prisco et al., 2015). The feeding solution was prepared mixing 5 ml of crude extract of *Rhus coriria L.*, in 20 ml of a 16 g/L alginic acid sodium salt (Sigma) solution previously degassed and sterilized by autoclaving at 121 C for 15 min. This mixture was loaded in the syringe and forced into the pulsation chamber to be further extruded through a nozzle, we prepared previously as we mention the procedure in section (2.3).

The microencapsulation conditions used were: flow rate 2.91 ml/min, vibration frequency 2000 Hz, electrode voltage of 950 V. These conditions were chosen on the basis of results obtained in preliminary experiments carried out with different usage conditions of the equipment. Suspension was recovered in batch and waiting a few minutes to separate the liquid from microcapsule. Finally, hardening solution was discarded to obtain a final microcapsule we want to use.

Figure 2.8

Microcapsule of Teucrium capitatum L. Under microscope

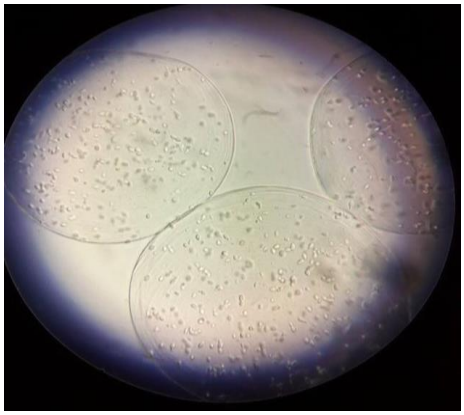


Figure 2.9

Microcapsule of Teucrium capitatum L. Under microscope

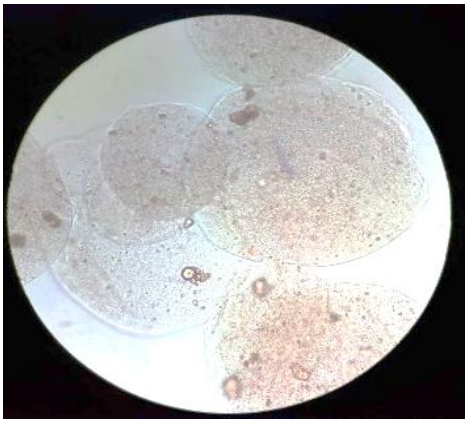


Figure 2.10

No.1 Antibacterial test of Rhus coriaria L. microcapsulation against Streptococcus salivarius

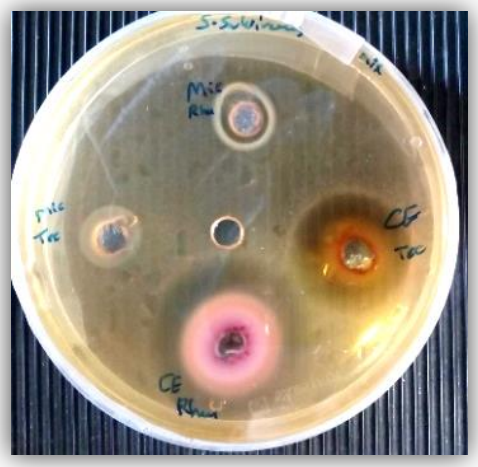
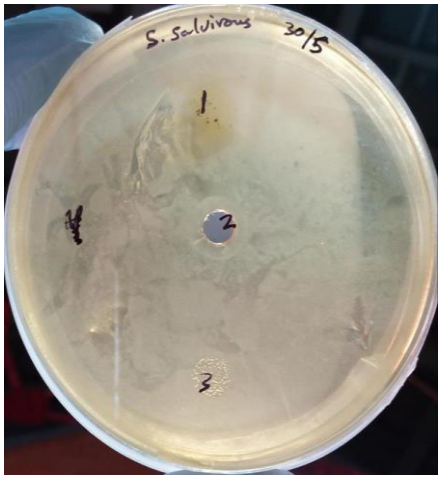


Figure 2.11

(No.3&4) Antibacterial test of Teucrium capitatum L. microcapsulation against Streptococcus salivarius. 10 µl



Chapter Three

Result and Discussion

The selection of the appropriate preservation system for a particular food product. Should be more careful, because should identified pathogen or spoilage microorganisms, and then the possible preservation systems must be evaluated via model studies and studies in the food product in question. (Leistner, 2000) Generally, a combination of chemical preservatives and other preservation methods is needed more test and studied, in our study here we are determine the antibacterial activity of each Plants against food spoilage microorganisms. So next of this test we can identify possibility of use this plants as natural preservatives , to extension a shelf life of food products or enhance tast and smell , so this natural preservative will have many benefits for health , Economic , food safety .

In addition the method we following in all extracts, preparation media, preparation the indicator strains inoculated in the Plate, and the incubation of plates were been the same way we were tested all extracts. the Error bar in all graphs represented the standard deviation.

3.1 Antibacterial activity of Cyclamen persicum Mill

In our test of activity of extract of Cyclamen persicum Mill during different time of storage (0 days storage, 7days, 10days ,and 14 days) In the following Table (3) the result of tested show the antimicrobial as a following, in addition we.

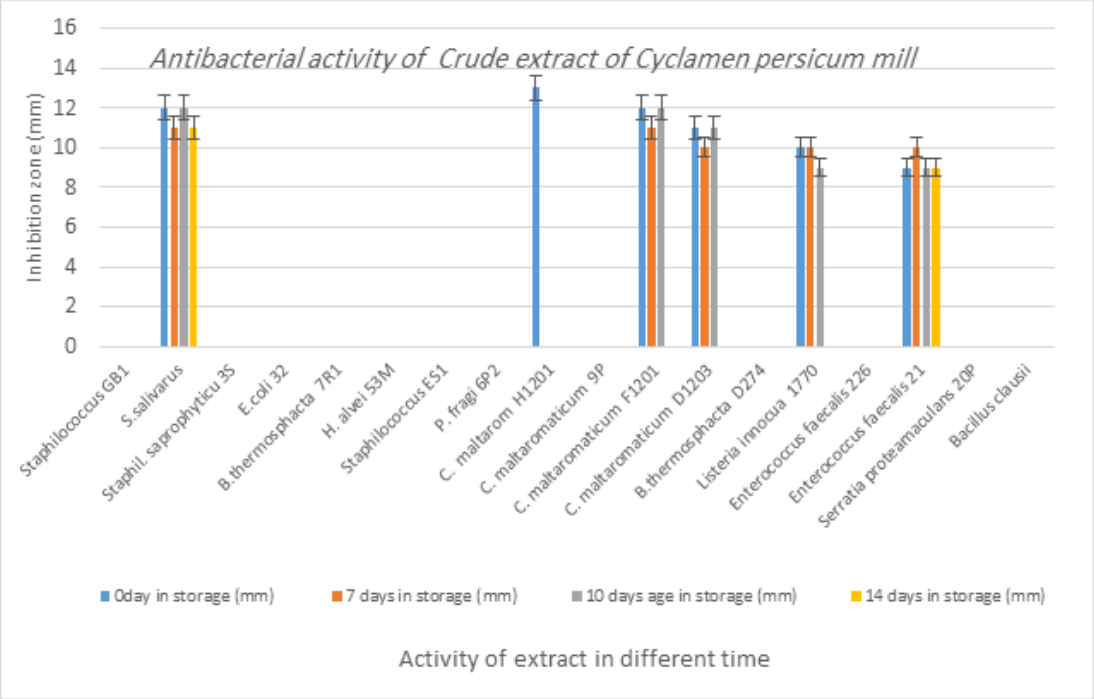
Table 3.1

The Result of Antibacterial activity of Crude extract of Cyclamen persicum mill in different time of storage

Indicator strains	0day in storage (mm)	7 days in storage (mm)	10 days age in storage (mm)	14 days in storage (mm)
Staphylococcus aureus GB1	0	0	0	0
Streptococcus salivarius	12	11	12	11
Staphylococcus saprophyticu 3S	0	0	0	0
E.coli 32	0	0	0	0
B.thermosphacta 7R1	0	0	0	0
*H. alve*i 53M	0	0	0	0
Staphilococcus ES1	0	0	0	0
P. fragi 6P2	0	0	0	0
C. maltarom $H_1$201	13	0	0	0
C. maltaromaticum 9P	0	0	0	0
C. maltaromaticum $F_1$201	12	11	12	0
C. maltaromaticum $D_1$203	11	10	11	0
B.thermosphacta D274	0	0	0	0
Listeria innocua 1770	10	10	9	0
Enterococcus faecalis 226	0	0	0	0
Enterococcus faecalis 21	9	10	9	9
Serratia proteamaculans 20P	0	0	0	0
Bacillus clausii	0	0	0	0

Figure 3.1

Antibacterial activity of Crude extract of Cyclamen persicum mill in different time of storage (0, 7,10 and 14 days)



Mostly the activity of Crude extract of Cyclamen persicum mill leaves, has been very low or not clear against many indicator stains in our study.

But different parts of Plants will have different antimicrobial activity, furthermore the testing of tuber extracts show the antibacterial activity

Figure 3.2

No.6 Inhibition zone of extract Cyclamen persicum mill, against C. maltaromaticum D1203

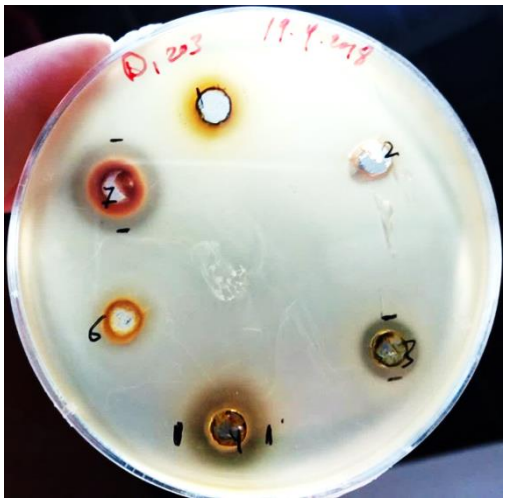
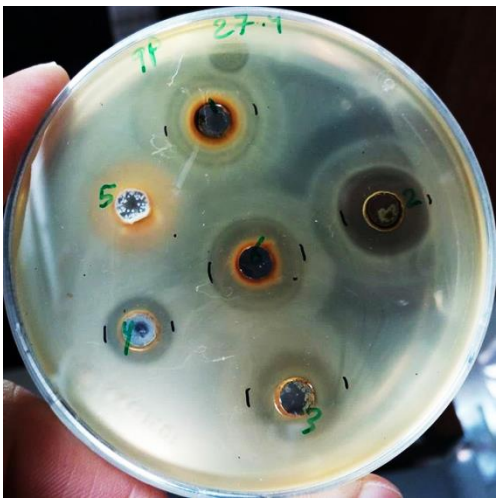


Figure 3.3

No.4 Inhibition zone of extract Cyclamen persicum mill against C. maltaromaticum



3.2 Antibacterial activity of Origanum syriacum, L. Var. syriacum

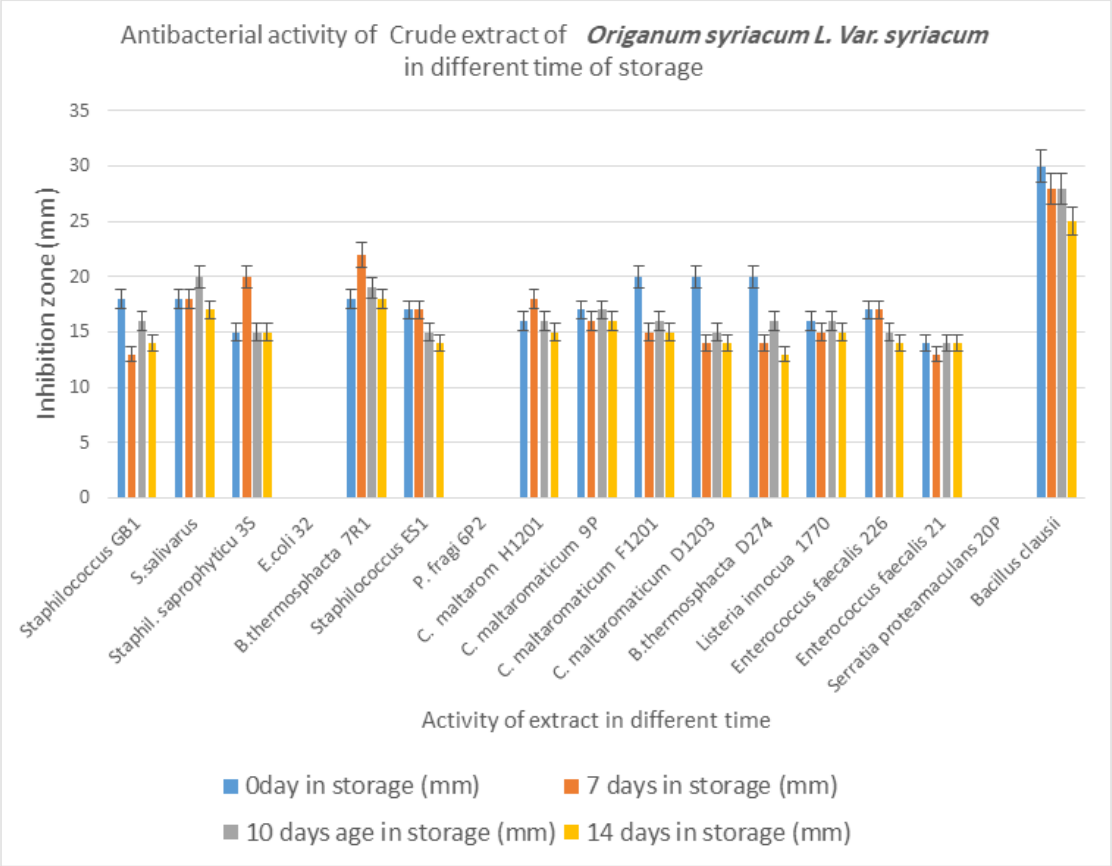
Table 3.2

Antibacterial activity of Crude extract of Origanum syriacum, L. Var. syriacum in different time of storage

Indicator strains.	0day in storage (mm)	7 days in storage (mm)	10 days age in storage (mm)	14 days in storage (mm)
Staphilococcus GB1	18	13	16	14
Streptococcus salivarius	18	18	20	17
E.coli				
Staphil. saprophyticu 3S	15	20	15	15
B.thermosphacta 7R1	18	22	19	18
Staphylococcus ES1	17	17	15	14
P. fragi 6P2	0	0	0	0
C. maltarom $H_1$201	16	18	16	15
C. maltaromaticum 9P	17	16	17	16
C. maltaromaticum $F_1$201	20	15	16	15
C. maltaromaticum $D_1$203	20	14	15	14
B.thermosphacta D274	20	14	16	13
Listeria innocua 1770	16	15	16	15
Enterococcus faecalis 226	17	17	15	14
Enterococcus faecalis 21	14	13	14	14
Serratia proteamaculans 20P	0	0	0	0
Bacillus clausii	30	28	27	25

Figure 3.4

Antibacterial activity of Crude extract of Origanum syriacum, L. Var. syriacum (leaves) in different time of storage (0,7, 10 and 14 days) at 4 C°



The studied of antibacterial activity of O. syriacum, L. crude extract against Bacterial indicator strains by using well diffusion method, the amount of crude extract used 50 µl, standard well 6 mm . The maximum activity on clear against Bacillus clausii, in four time of tested and next high activity at against

The Range of activity against gram positive bacteria between (14 – 23 mm) at 0 day storage, (13 – 25mm) at 7 days storage, (15- 30 mm) at 10 days storage and (13- 28 mm) at 14 days storage, moreover O. syriacum have activity against all indicator strains, expect P. fragi 6P2

Also in literature review O. syriacum, shown one of highest antibacterial activity against the high activity of this Plant related on the bioactive compound for example and this may phenols, thymol and carvacrol as major constituents of thyme oil in the plant (Abu-Lafi et al., 2007).

Otherwise many studied focus on this plant for many reason, most of these reason because the popularity for this plants as food and spices, other reason the composition of this Plants of bioactive compound

In fact the activity of essential oil different from activity of crude extract of the same plant, also will be a different of essential oil of same plant but different region,

In this study in Turkey show the different activity of different species of Origanum.

According to (Kizil et al, 2014) the different species of Origanum, show significantly the antibacterial activity of essential oil against gram positive and negative.

In addition in all the studies (Abu-Lafi et al., 2008, Ali-Shtayeh and Jamous, 2008, Imelouane et al .,2009). The Origanum syriacum, L. Var. syriacum has shown clear antimicrobial activity against many indicator strains.

Figure 3.5

No.3 Inhibition zone of extract Origanum syriacum, L against C. maltaromaticum D1203

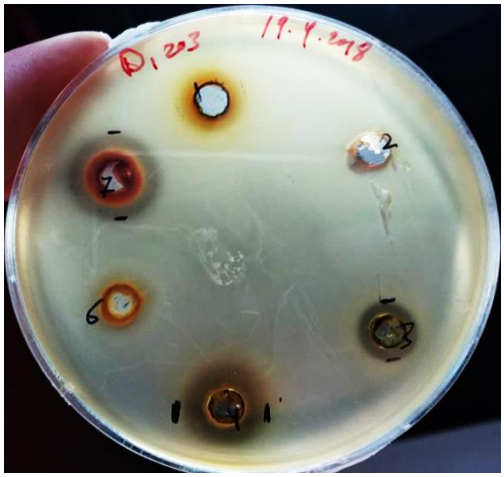


Figure 3.6

No3. Inhibition zone of extract Origanum syriacum, against B.thermosphacta 7R1

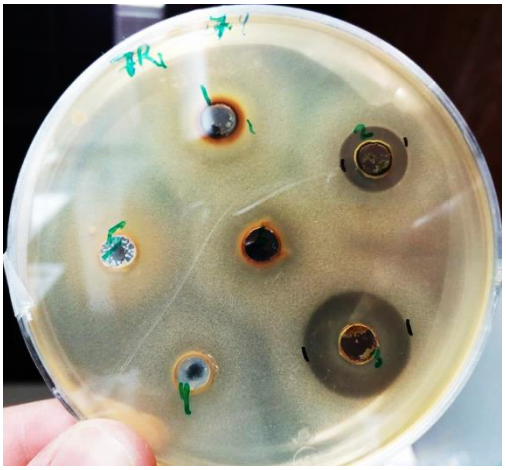


Figure 3.7

No.4 Inhibition zone of extract Origanum syriacum .L against Listeria innocua 1770

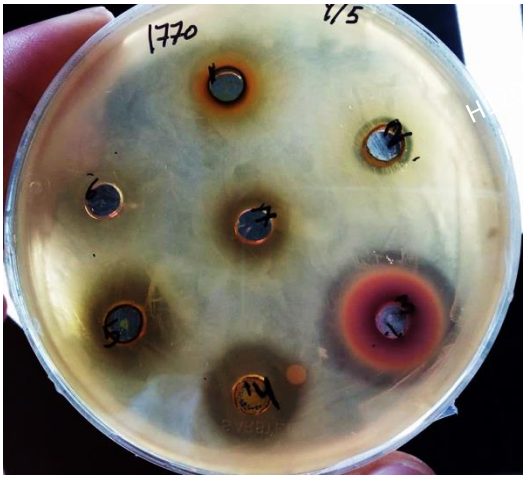
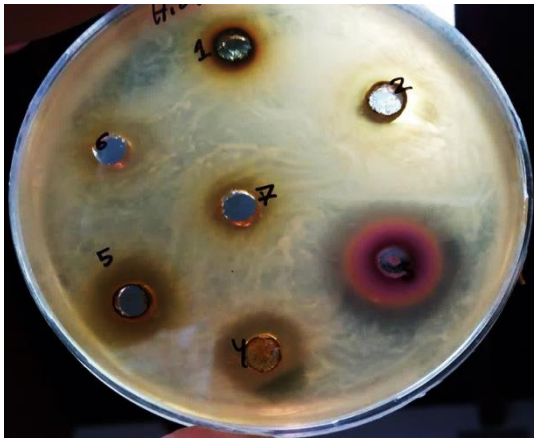


Figure 3.8

No.4 Inhibition zone of extract Origanum syriacum .L against C. maltarom H_1201



3.3 Antibacterial activity of *Rhus coriaria L*

Antibacterial activity of the Rhus coriaria L. extracts were assessed using the agar well-diffusion method .the bacterial indicator strains were cultured in Tryptone soya agar (TSA)plates were used for antibacterial susceptibility tests. Bacterial strain were uniformly mixed with these media in separate plates .Wells (6 mm diameter) were created in these plates, and 50 µl of plant extracts were pipetted into the wells and allowed to diffuse at room temperature for 30 min. Plates were incubated at 25 & 30 °C for 18-24 h . The zone of inhibition for each extract was measured and expressed in mm.

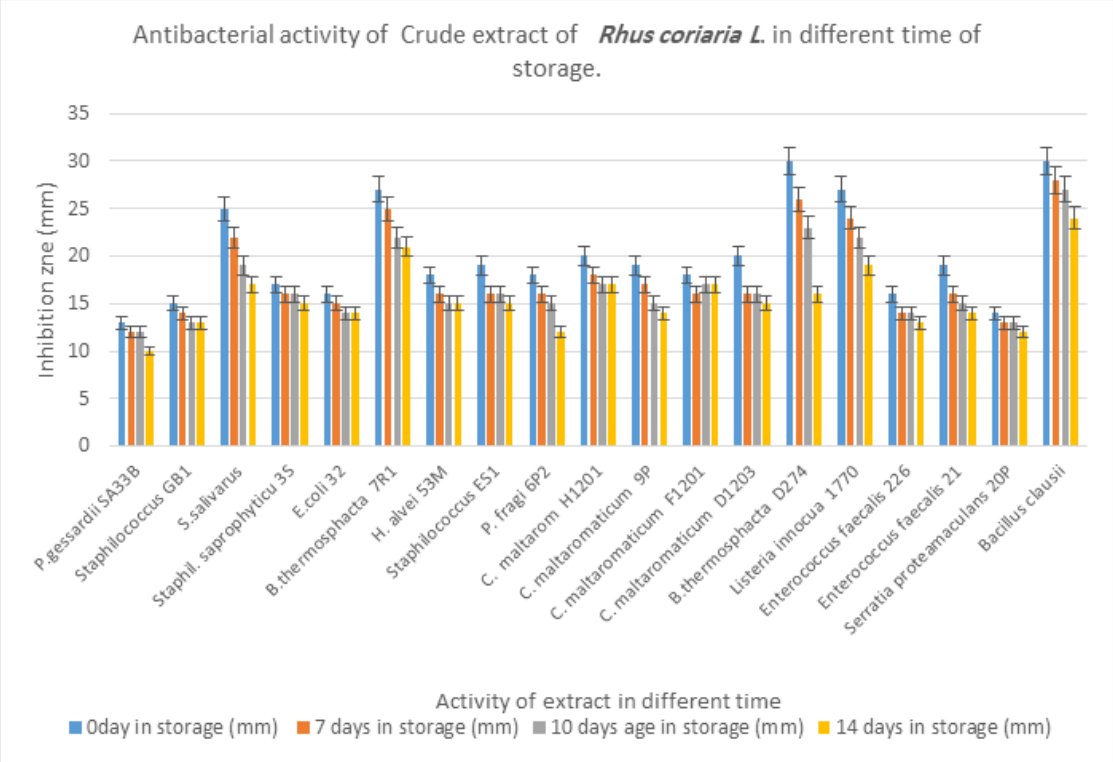
Table 3.3

Antibacterial activity of Crude extract of Rhus coriaria L. in different time of storage

Indicator strains	0 day in storage (mm)	7 days in storage (mm)	10 days age in storage (mm)	14 days in storage (mm)
P.gessardii SA33B	13	12	12	10
Staphylococcus GB1	15	14	13	13
Streptococcus salivarius	25	22	19	17
Staphylococcus saprophyticus 3S	17	16	16	15
E.coli 32	16	15	14	14
B.thermosphacta 7R1	27	25	22	21
*H. alve*i 53M	18	16	15	15
Staphylococcus ES1	19	16	16	15
Pseudomonas fragi 6P2	18	16	15	12
C. maltarom $H_1$201	20	18	17	17
C. maltaromaticum 9P	19	17	15	14
C. maltaromaticum $F_1$201	18	16	17	17
C. maltaromaticum $D_1$203	20	16	16	15
B.thermosphacta D274	30	26	23	16
Listeria innocua 1770	27	24	22	19
Enterococcus faecalis 226	16	14	14	13
Enterococcus faecalis 21	19	16	15	14
Serratia proteamaculans 20P	14	13	13	12
Bacillus clausii	30	28	27	24

Figure 3.9

Antibacterial activity of Crude extract of Rhus coriaria L. in different time of storage (0 day,7days, 10days, and 14 days)



The tested of antibacterial activity of methanol extract of fruit *Rhus coriaria L.* show the high activity for the extract against gram positive and gram negative bacteria, Obviously the high activity of *Rhus coriaria L.* related to the composition of this plant, such as active compound .the range of inhibition zone were range from (10 to 30 mm) including the standard dimeter of well 6 mm. additionally the Error bar in all graph represented the standard deviation.

Moreover the investigated of *R.coriaria L.* extract and fifty six plants in Palestine. Among these study, the *R.coriaria L.* had the greatest antibacterial activity against *Propionibacterium, acnes* Staphylococcus *aureus, Escherichia coli* and *Pseudomonas aeruginosa*. (Ali-Shtayeh et al., 2013).

In addition the analysis of composition of *R.coriaria L.*by by using high-performance liquid chromatography-diode array detector-hyphenated with tandem mass spectrometry HPLC–DAD–ESI-MS/MS screening of bioactive components from *Rhus coriaria* L.

(Sumac) fruits found about 211 Compounds, also about 180 phytochemicals (tannins, (iso)flavonoids, terpenoids, etc.) are reported in *R.coriaria L*.(Abu-Reidah et al., 2015) .

Our research and other research will prove the claim about health benefits from side inhibition growth of food borne pathogens, and food safety, it's one of natural preservative.

Related on (Nasar Abbas et al 2004) was performed studied on the growth of 12 bacterial strains (six Gram positive strains and six Gram negative strains), mostly food borne including pathogens. Its show to be effective against all the test organisms with Gram positive strains being more sensitive than Gram negative strains.

Also in another study showed that the fruit extract of R. coriaria had the strongest antimicrobial effect with an inhibition zone of 35–51 mm against all the bacteria used (Digrak et al 2007).

Figure 3.10

No.7 Inhibition zone of extract Rhus coriaria .L against C. maltaromaticum D1203

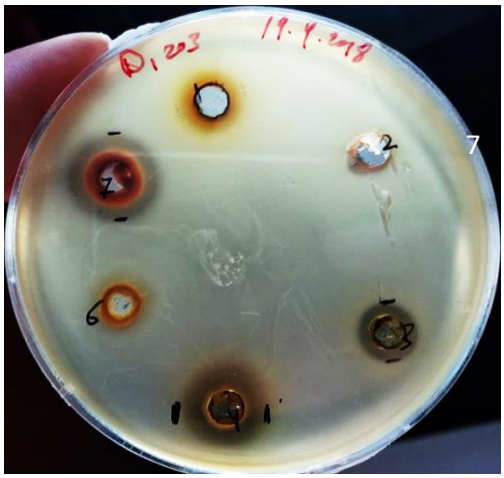


Figure 3.11

No.7 Inhibition zone of extract Rhus coriaria .L against C. maltaromaticum D1203

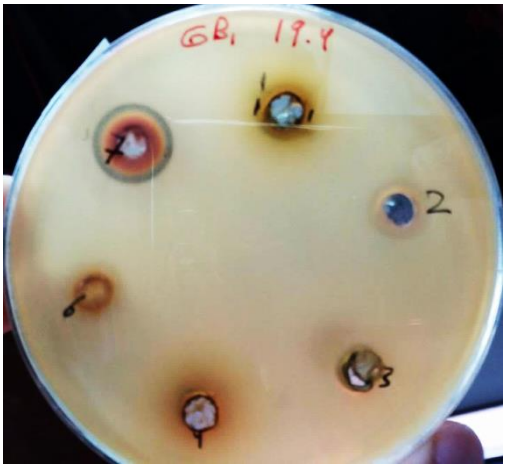


Figure 3.12

No.3 Inhibition zone of extract Rhus coriaria .L against Listeria innocua 1770

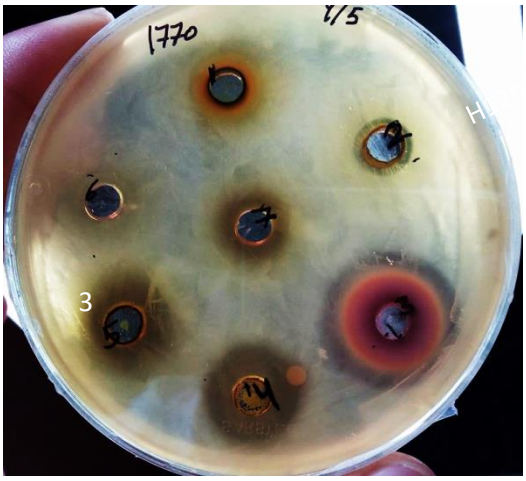


Figure 3.13

No.3 Inhibition zone of extract Rhus coriaria .L against C. maltarom H_1201

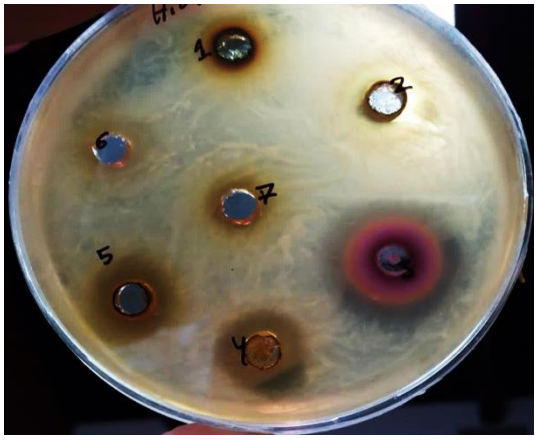


Figure 3.14

No.3 Inhibition zone of extract Rhus coriaria .L against S.salivarius

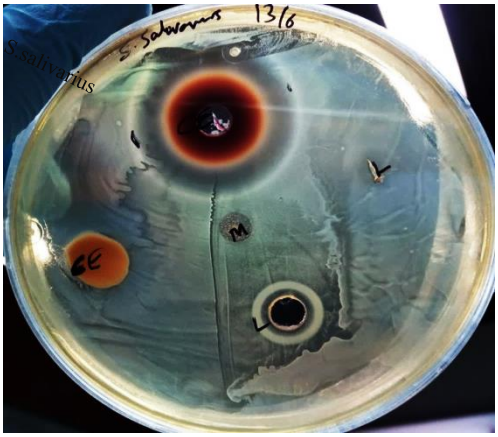


Figure 3.15

No.3 Inhibition zone of extract Rhus coriaria .L against Bacillus clausii.

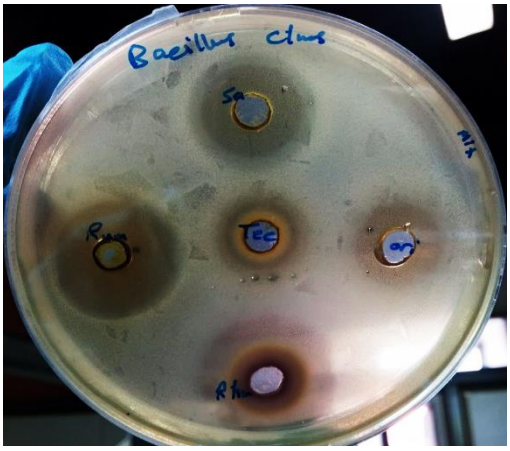


Figure 3.16

No.7 Inhibition zone of extract Rhus coriaria .L against S.saprophyticus 3S

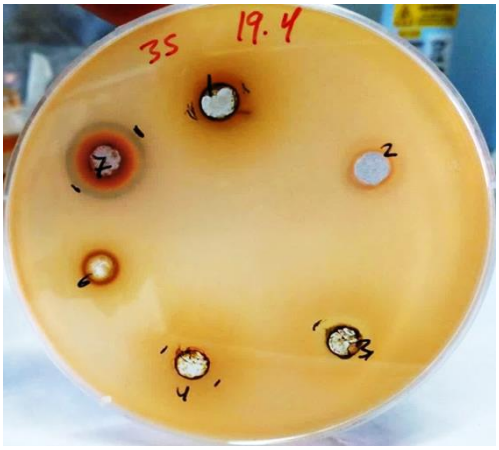
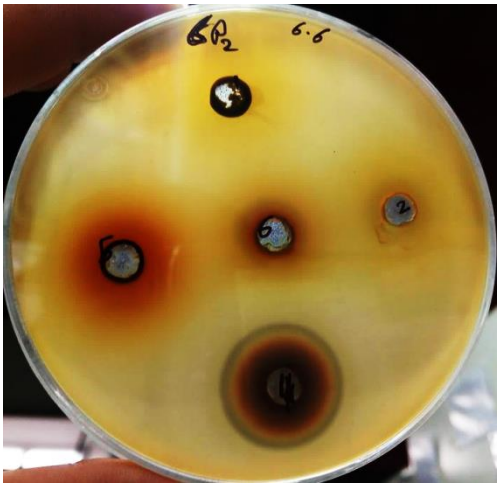


Figure 3.17

No.4 Inhibition zone of extract Rhus coriaria .L against P. fragi 6P2



3.4 Antibacterial activity of *Rumex acetosa L*

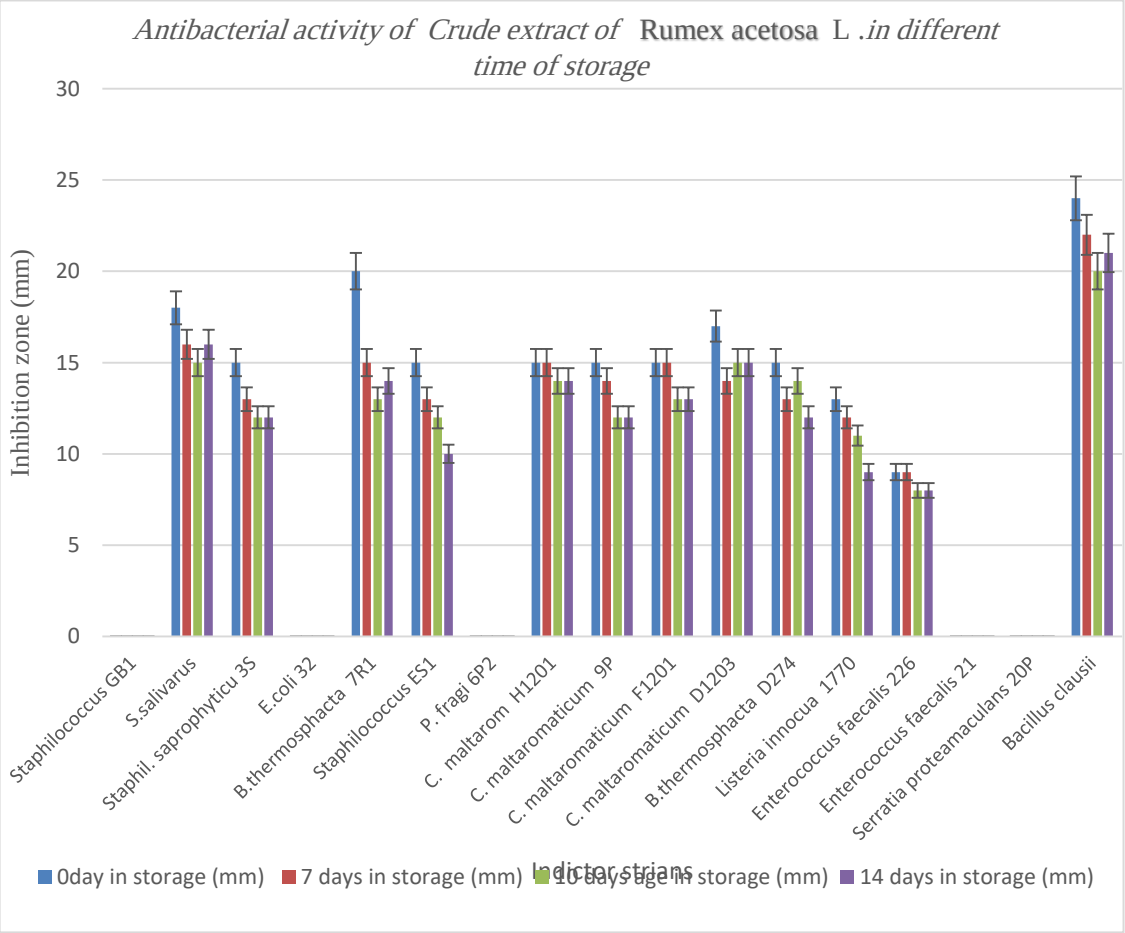
Table 3.4

Antibacterial activity of Crude extract of Rumex acetosa L .in different time of storage

Indicator strain	0day in storage (mm)	7 days in storage (mm)	10 days age in storage (mm)	14 days in storage (mm)
Staphilococcus GB1	0	0	..	0
S.salivarus	18	16	15	16
Staphil. saprophyticu 3S	15	13	12	12
E.coli 32	0	0	0	0
B.thermosphacta 7R1	20	15	13	14
Staphilococcus ES1	15	13	12	10
P. fragi 6P2	0	0	0	0
C. maltarom $H_1$201	15	15	14	14
C. maltaromaticum 9P	15	14	12	12
C. maltaromaticum $F_1$201	15	15	13	13
C. maltaromaticum $D_1$203	17	14	15	15
B.thermosphacta D274	15	13	14	12
Listeria innocua 1770	13	12	11	9
Enterococcus faecalis 226	9	9	8	8
Enterococcus faecalis 21	0	0	0	0
Serratia proteamaculans 20P	0	0	0	0
Bacillus clausii	24	22	20	21

Figure 3.18

Antibacterial activity of Crude extract of Rumex acetosa L. (Leaves) in different time of storage: in different time of storage (0, 7 , 10 and 14 days) at 4 C°



In our study this is the first screening of antibacterial activity of crude extract of *Rumex acetosa L*. leaves against many food borne pathogen (Table 1), its shown the clear inhibition strains (D274 & 7R1) , also the activity against *staphylococcus salivarius and Bacillus clausii* was high .however there is no activity against gram negative bacteria in our study .

Related on (Ladeji, O., et al.1997) the leaves of Rumex acetosa L. have high nutritional value comparison between two concentration of mixing leaves at rats , the rats ate from concentration 50% of leaves with supplemental diet shown significant different of concentration nutrient in serum.

Figure 3.19

Inhibition zone of extract of Rumex acetosa..L against Bacillus clausii

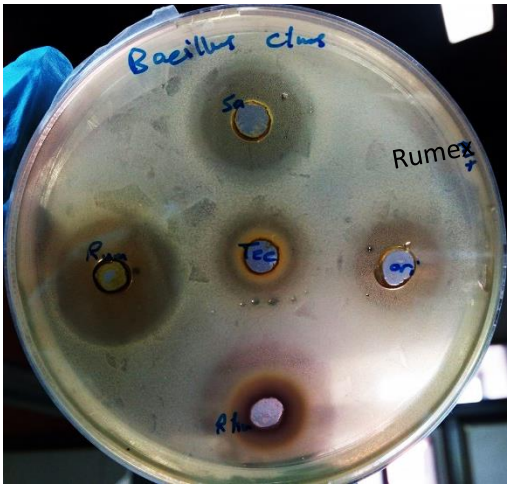


Figure 3.20

Inhibition zone of extract of Rumex acetosa..L against B..thermosphacta

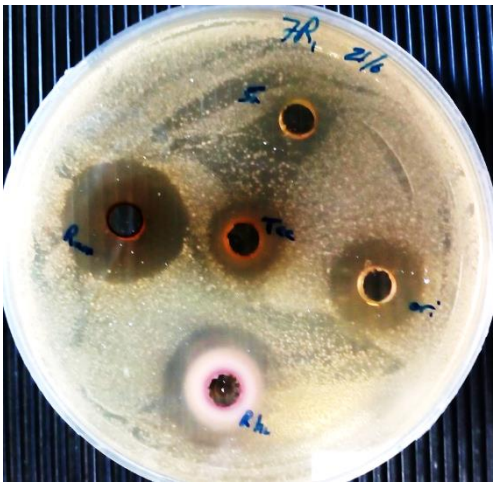


Figure 3.21

Inhibition zone of extract of Rumex acetosa..L against C.maltaromaticum

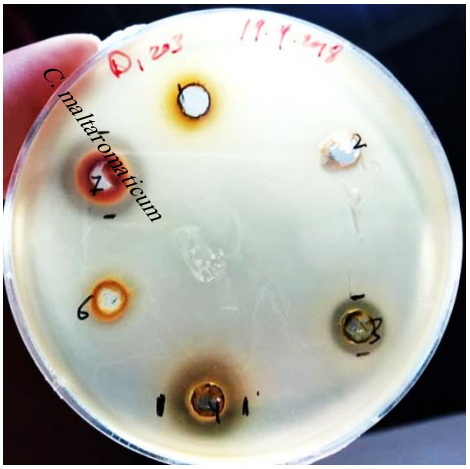
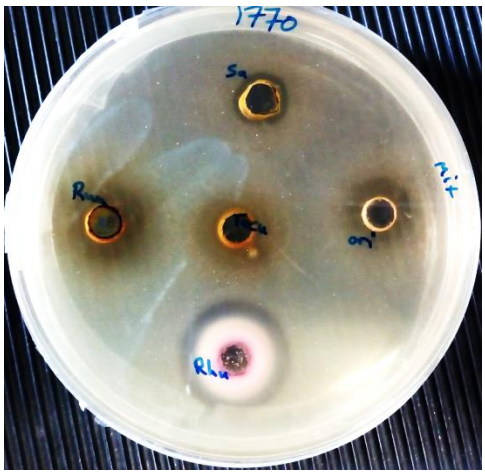


Figure 3.22

Inhibition zone of extract of Rumex acetosa..L against Listeria innocua .



3.5 Antibacterial activity of Salvia hierosolymitana Boiss

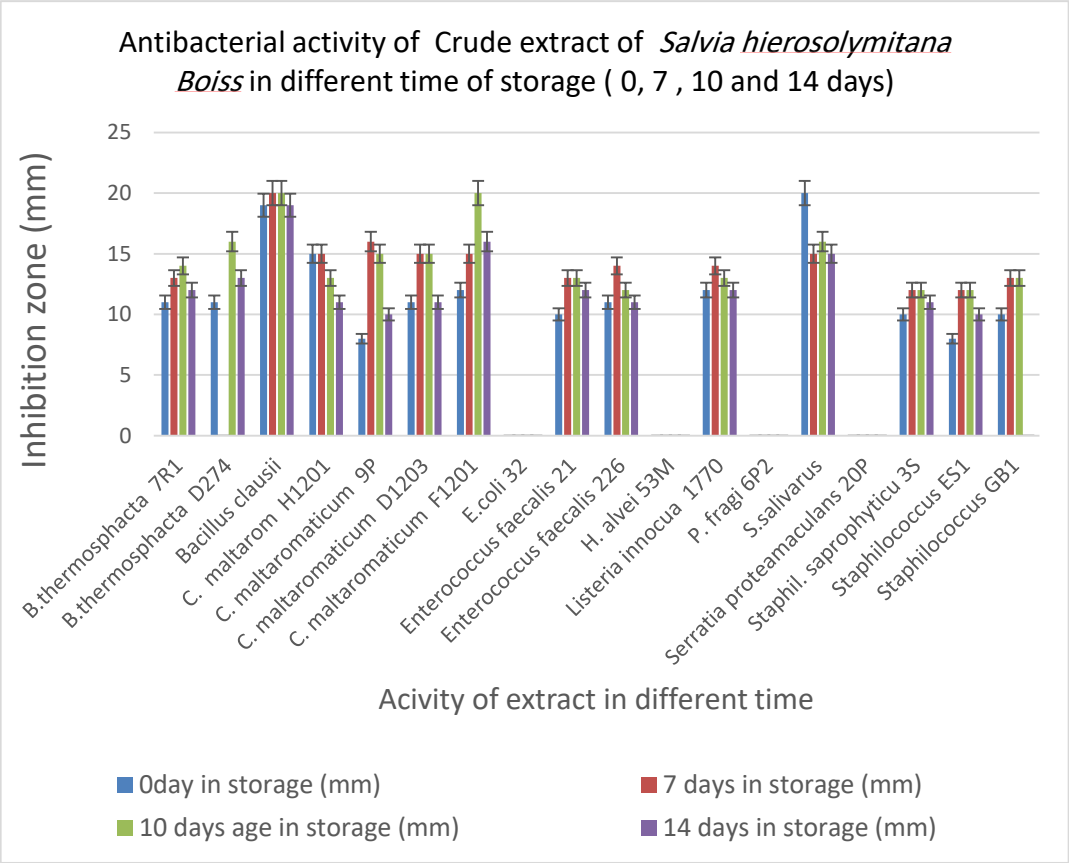
Table 3.5

Antibacterial activity of Crude extract of Salvia hierosolymitana boiss (leaves) in different time of storage

Indicator strains	0day in storage (mm)	7 days in storage (mm)	10 days age in storage (mm)	14 days in storage (mm)
B.thermosphacta 7R1	11	13	14	12
B.thermosphacta D274	11	15	16	13
Bacillus clausii	19	29	20	19
C. maltarom $H_1$201	15	15	13	11
C. maltaromaticum 9P	8	16	15	10
C. maltaromaticum $D_1$203	11	15	15	11
C. maltaromaticum $F_1$201	12	15	20	16
E.coli 32	0	0	0	0
Enterococcus faecalis 21	10	13	13	12
Enterococcus faecalis 226	11	14	12	11
*H. alve*i 53M	0	0	0	0
Listeria innocua 1770	12	14	13	12
P. fragi 6P2	0	0	0	0
S.salivarus	20	15	16	15
Serratia proteamaculans 20P	0	0	0	0
Staphil. saprophyticu 3S	10	12	12	11
Staphylococcus ES1	8	12	12	10
Staphylococcus GB1	10	13	13	0

Figure 3.23

Antibacterial activity of Crude extract of Salvia hierosolymitana boiss (leaves) in different time of storage (0, 7, 10 and 14 days)



The studied of antibacterial activity of *Salvia hierosolymitana boiss*. crude extract against Bacterial indicator strains by using well diffusion method, the amount of crude extract used 50 µl, standard well 6 mm . the maximum activity on clear on Bacillus clausii (29 mm) at 7 days storage, and next high activity at against S.salivarius (20mm) at the same time of storage. Also its show the high activity against *C. maltaromaticum* $F_1$201(20mm at 10 days storage).

In this study we tested the antibacterial activity of *S.hierosolymitana boiss* crude extract against Bacterial indicator (gram positive & gram negative), it's shown the activity against Gram positive bacteria, but not on gram negative.

According to (Al-Assali,. et al. 2013) the antibacterial activity of *S.hierosolymitana boiss*. Shown the moderate activity against bacterial skin pathogen Propionibacterium *acnes* with range of inhibition zone (8.1-9.9 mm).

Otherwise our study and other studies will be Justify the antibacterial activity of *S.hierosolymitana boiss*, also rarely information or research about crude extract of this Plant.

the essential oil of this plant This plant was characterized and shown antinflammatory (Felice De et al .2006) , in another Jordanian study shown the antioxidant properties (Tawaha, et al 2007).

Figure 3.24

Inhibition zone of extract of S.hierosolymitana against C. maltaromaticum

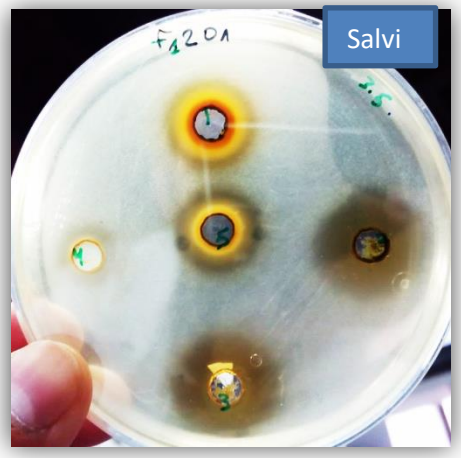


Figure 3.25

Inhibition zone of extract of S.hierosolymitana against C. maltaromaticum

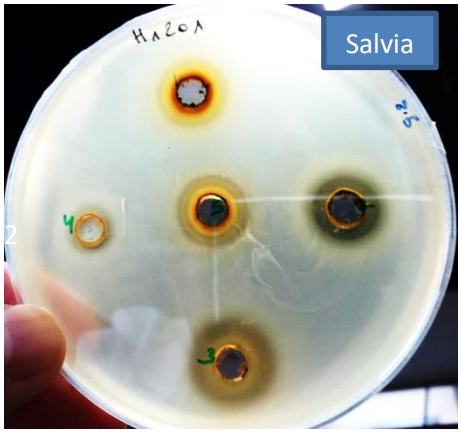


Figure 3.26

No.2 Inhibition zone of extract of S.hierosolymitana against S.salivarius

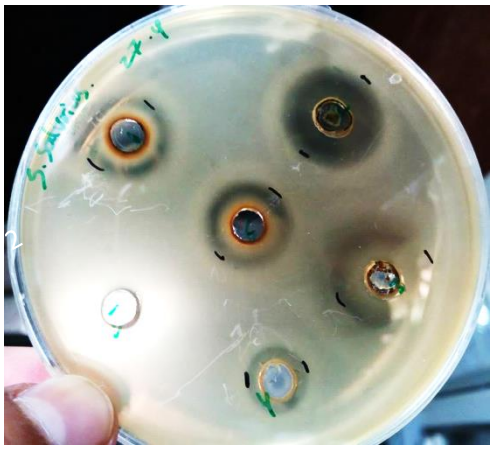
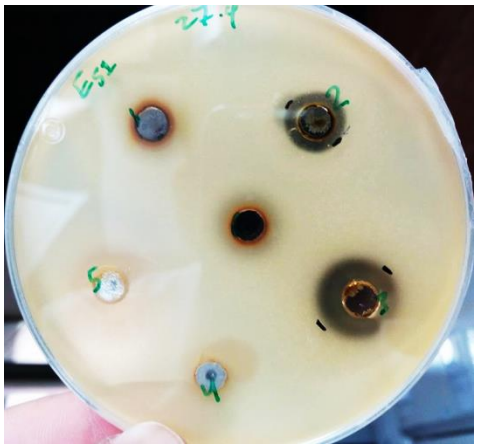


Figure 3.27

No.2 Inhibition zone of extract of S.hierosolymitana against Staphylococcus ES1



3.6 Antibacterial activity of Teucrium capitatum L

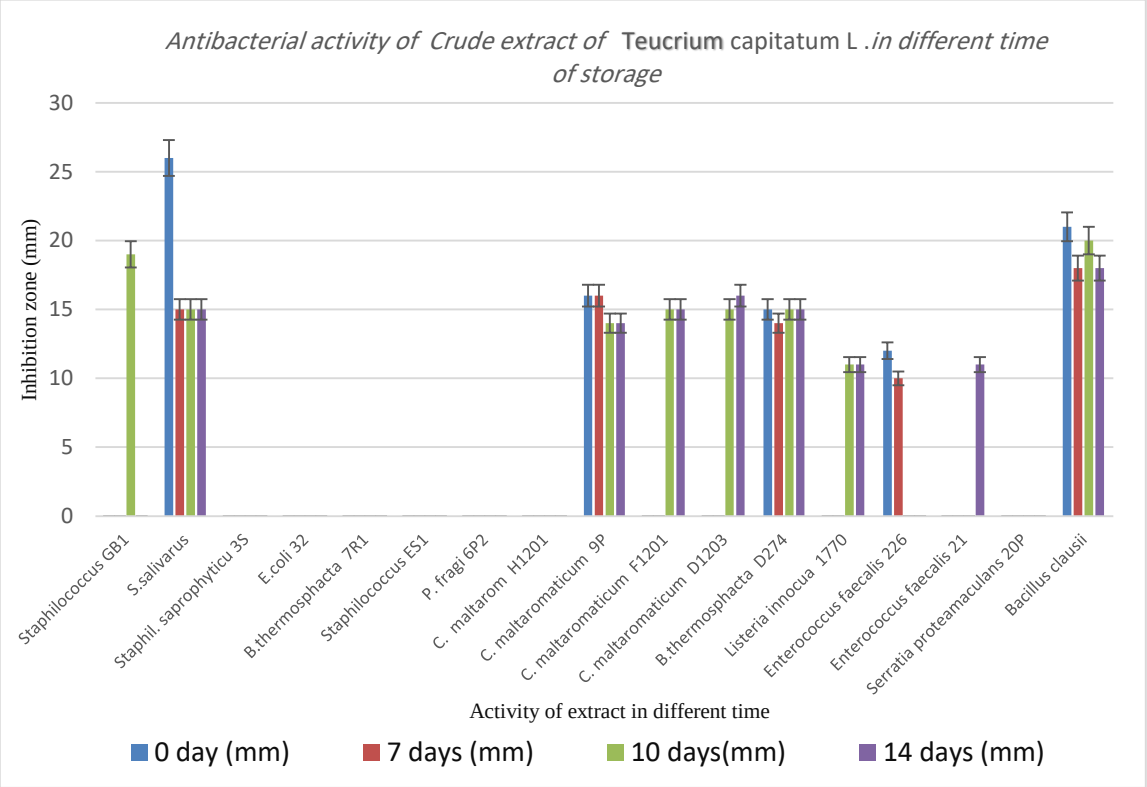
Table 3.6

Antibacterial activity of Crude extract of Teucrium capitatum L .in different time of storage.

Indicator strain	0day in storage (mm)	7 days in storage (mm)	10 days age in storage (mm)	14 days in storage (mm)
Staphilococcus GB1	0	0	0	0
S.salivarus	26	15	15	15
Staphil. saprophyticu 3S	0	0	0	0
E.coli 32	0	0	0	0
B.thermosphacta 7R1	0	0	0	0
Staphilococcus ES1	0	0	0	0
P. fragi 6P2	0	0	0	0
C. maltarom $H_1$201	0	0	0	0
C. maltaromaticum 9P	16	16	14	14
C. maltaromaticum $F_1$201	0	0	15	15
C. maltaromaticum $D_1$203	0	0	15	16
B.thermosphacta D274	15	14	15	15
Listeria innocua 1770	0	0	11	11
Enterococcus faecalis 226	12	10	0	0
Enterococcus faecalis 21	0	11	0	11
Serratia proteamaculans 20P	0	0	0	0
Bacillus clausii	21	18	20	18

Figure 3.28

shown the antibacterial activity of Teucrium capitatum L. crude extract against Bacteial indicator strains by using well diffusion method, the amount of crude extract used 50 µl, the standard well 6 mm



In this study we tested the antibacterial activity of of Teucrium capitatum L. crude extract against Bacterial indicator (gram positive & gram negative), it's shown the activity against Gram positive bacteria, these study beside another studies in literature review , justify the important T.capitatum L. as antimicrobial activity .

Related on (Lograda .et al.,2014) show the different antimicrobial activity of essential oil of Teucrium polium L.(synonym) from Algeria , against bacterial indicator strains, The different of activity of essential oil of T.capitatum L. depend on many factors can effect on the composition of essential oil, one of these factors: genetic, environmental .Also in the same study the antibacterial activity of essential oil has high activity against Bacillus cereus.

In our study the antibacterial activity of T.capitatum L. crude extract Show significant inhibition zone against *(Bacillus clausii*, Streptococcus salivarius) specially after extraction without storage.

Figure 3.29

Inhibition zone of extract of Teucrium capitatum L. against Streptococcus salivarus.(1):Crude extract 100% . (2):50% crude extract

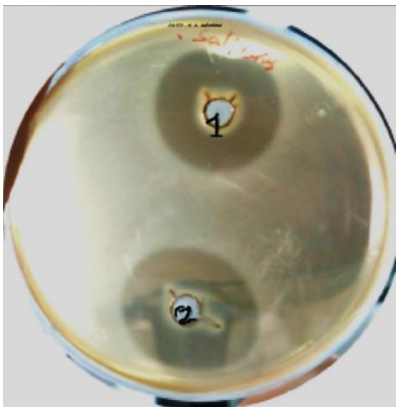


Figure 3.30

Inhibition zone of extract of Teucrium capitatum L. against Bacillus clausii.(1):Crude extract 100% . (2):50% crude extract

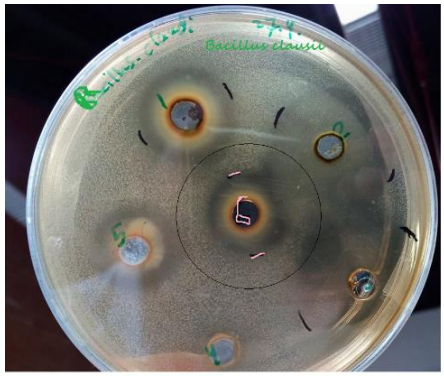


Figure 3.31

NO.(6) Inhibition zone of extract of Teucrium capitatum L against Brochothrix thermosphacta D274. by using well diffusion method

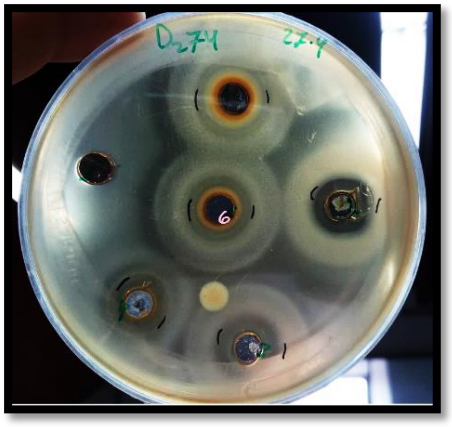
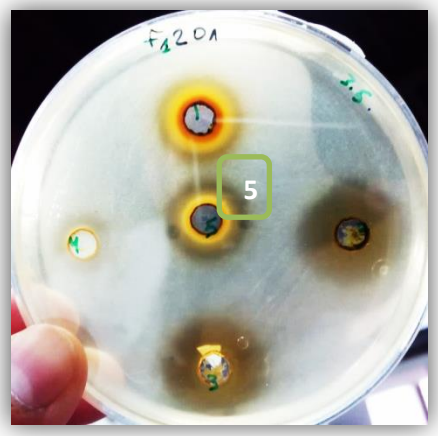


Figure 3.32

NO.(5) Inhibition zone of extract of Teucrium capitatum L. against Carnobacterium maltaromaticum F1201 by using well diffusion method



3.7 Comparison between Agar disc diffusion & Paper disc diffusion

Agar well diffusion and paper disc diffusion the two method show various results, The methanol extract of Salvia hierosolymitana show antibacterial activity by using well diffusion method the same amount of crude extract were used 20µl versus paper disc diffusion, but the activity of test by paper disc diffusion was more higher in *Bacillus calusii* at same amount of crude extract of S. hierosolymitana.

In generally the inhibition zone of well diffusion method more than paper disc diffusion method for five methanol extracts against against three indicator strains, but the paper disc diffusion shown higher inhibition zone against Bacillus clausii at same amount of crude extract for two methods 20µl , the results of this test in the following table No.9.

Table 3.7

Comparison between the two methods of testing antimicrobial activity of Plants (Agar disc diffusion & Paper disc diffusion by using same amount of crude extract 20 µl

Indicator strains	Salvia hierosolymitana		Origanum syriacum		Rhus coriaria L.		Rumex acetosa L.		Teucritum capitatum L.	
	*Agar well .d	*Paper. disc.d	Agar well .d	Paper. Disc.d	Agar well .d	Paper. disc.d	Agar well .d	Paper. disc.d	Agar well .d	Paper. disc.d
Listeria innocua 1770	11	0	14	0	15	18	12	0	10	0
Pesudomonas fragi 6P2	0	0	0	0	20	12	0	0	0	0
Brochothrix thermosphacta7R1	12	0	17	15	23	20	22	15	13	0
Bacillus clausii	16	20	17	15	24	28	23	20	16	12

Agar disc diffusion: 20 µl Paper disc diffusion: 20 µl

In addition we known from previously sections about the antibacterial activity of crude extract for each Plant, we chosen four indicator strains (Table 1) to compare between Paper disk diffusion and agar well diffusion on five plants extracts (Table2).

firstly in S. hierosolymitana the agar well diffusion shown the activity in (two indicator strains (1770, 7R1) and not in paper disc diffusion, also in Testing crude Origanum syriacum, the agar well diffusion method have more activity than paper disc diffusion.

Otherwise the antibacterial activity of *Rhus coriaria L*. extract shown high activity in agar well diffusion against two indicator strain (6P2 & 7R1) but the paper disc diffusion shown Higher activity in another two indicator strain (Bacillus clausii & Listeria innocua 1770), one of attractive and important results in this test that especially R.coriaria L. show high antimicrobial activity against Gram negative Bacteria.

Also the extracts of Teucritum capitatum L. & Rumex acetosa L. in well disk diffusion show the more activity against indicator strains . (Especially for gram positive).

Figure 3.33

Comparison between the two methods of testing antimicrobial activity of Plants (Agar disc diffusion & Paper disc diffusion by using same amount of crude extract 20µl

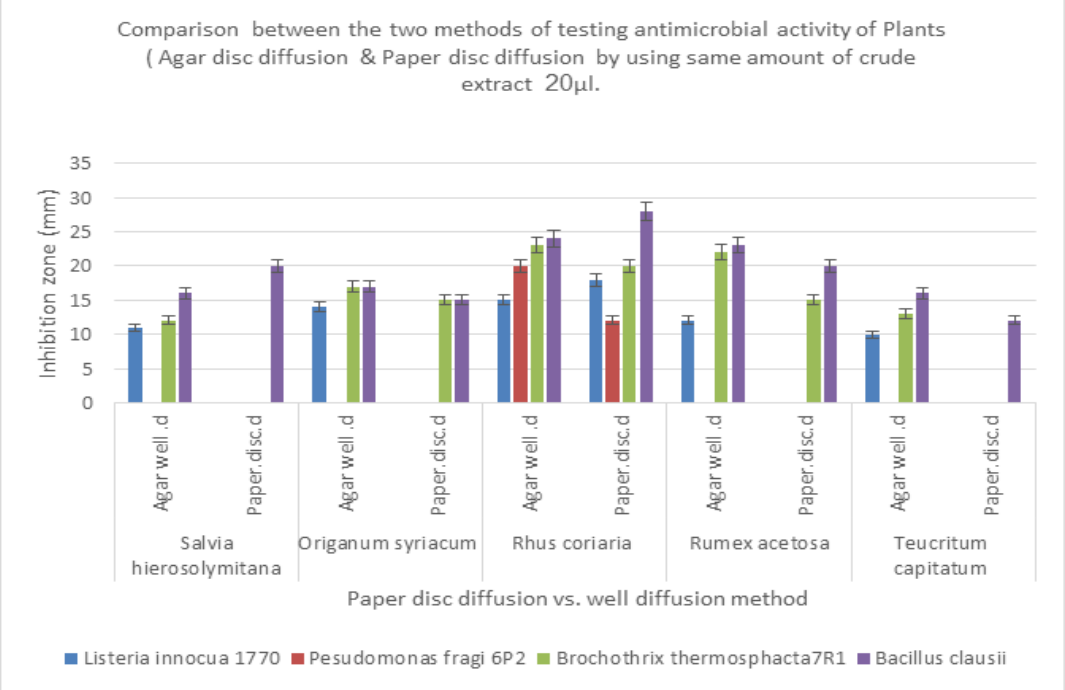


Table 3.8

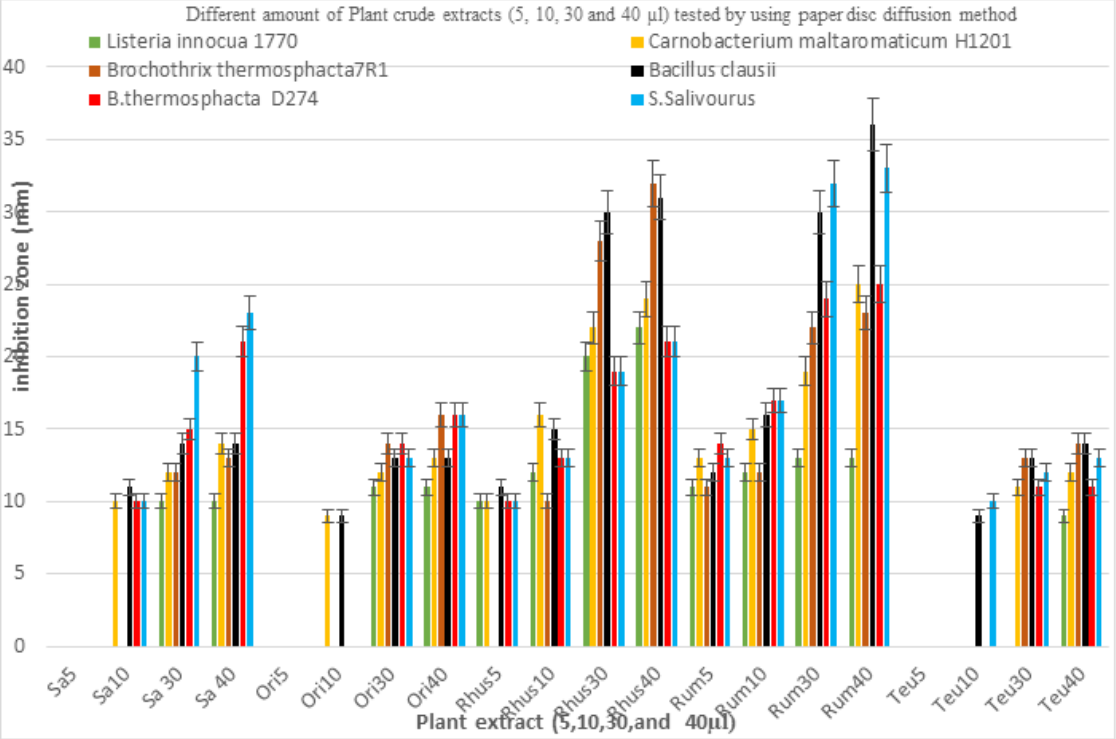
Different amount of crude (extract 5,10,30 and 40 µl) tested by using paper disc diffusion method

Indicator strains	Salvia hierosolymitana amount in µl				Origanum syriacum amount in µl				Rhus coriaria amount in µl				Rumex acetosa amount in µl				Teucritum capitatum amount in µl			
	5	10	30	40	5	10	30	40	5	10	30	40	5	10	30	40	5	10	30	40
Listeria innocua 1770	0	0	10	10	0	0	11	11	10	12	20	22	11	12	13	13	0	0	0	9
C. maltaromaticum H1201	0	10	12	14	0	9	12	13	10	16	22	24	13	15	19	25	0	0	11	12
B.thermosphacta7R1	0	0	12	13	0	0	14	16	0	10	28	32	11	12	22	23	0	0	13	14
Bacillus clausii	0	11	14	14	0	9	13	13	11	15	30	31	12	16	30	36	0	9	13	14
B.thermosphacta D274	0	10	15	21	0	0	14	16	10	13	19	21	14	17	24	25	0	0	11	11
S.Salivourus	0	10	20	23	0	0	13	16	10	13	19	21	13	17	32	33	0	10	12	13

The result for the test more explained in figure.28, it has comparison between all extracts, In this experiment we tested the different amount of extraction of each plant, it was range from 5, 10 , 30 and 40 µl .

Figure 3.34

Different amount of Plant crude extracts (5, 10, 30 and 40 µl) tested by using paper disc diffusion method



From our Result of tested antibacterial activity of plant extracts by paper disc method, the methanolic extract of *Rumex acetosa. L*. shown the majority of bacterial inhibition zone the range was (11 to 36 mm), next the Rhus coriaria L shown the range from (0 to 32 mm), then the Range of *Salvia hierosolymitana boiss* from(0 to 23mm) after there *Origanum syriacum* & Teucrium capitatum L. shown The range (0 to 16 mm) and (0 to 14 mm) respectively.

Generally all plant extracts here show antibacterial at minimum level 5µl and more for *R.acetosa* & *R.coriaria L*, but for *S. hierosolymitana, O.syriacum and T.capitatum L*. at 10 µl shown the inhibition of growth.

Figure 3.35

Inhibition zone of different amount of extract of O.syriacum against C. maltaromaticum H1201 by using paper disc diffusion

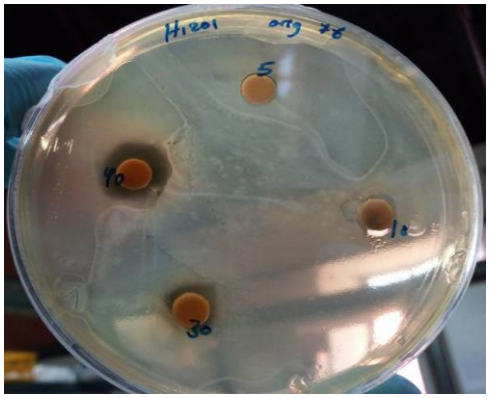


Figure 3.36

Inhibition zone of different amount of extract of S.hierosolymitana against C. maltaromaticum H1201 by using paper disc diffusion

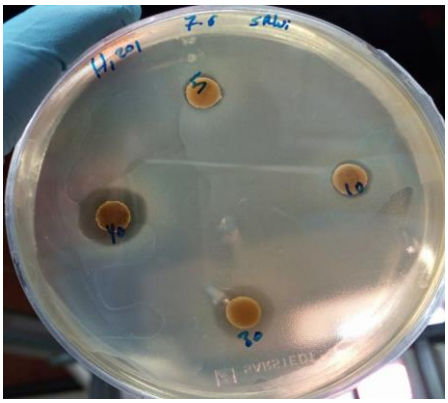


Figure 3.37

Inhibition zone of different amount of extract of T.capitatum against C. maltaromaticum H1201 by using paper disc diffusion

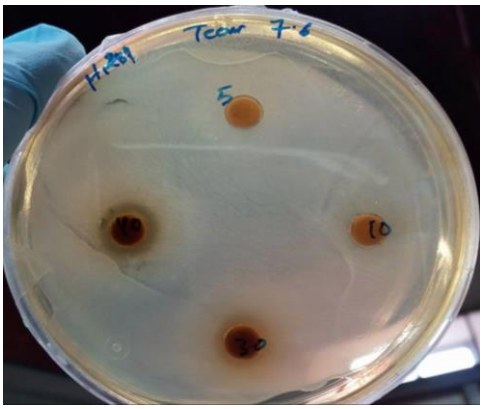


Figure 3.38

Inhibition zone of different amount extract of S.hierosolymitana against S.salivours by using paper disc diffusion

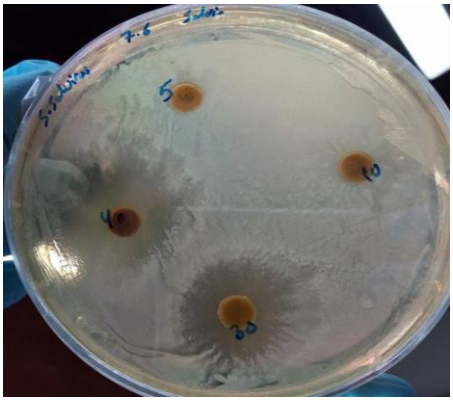


Figure 3.39

Inhibition zone of different amount of extract of O.syriacum against S.salivirus by using paper disc diffusion

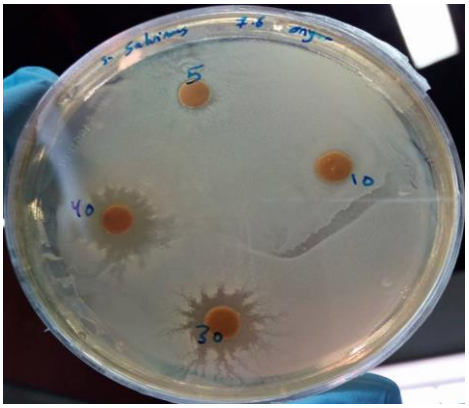


Figure 3.40

Inhibition zone of different amount of extract T.capitatum against Bacillus clausii by using paper disc diffusion

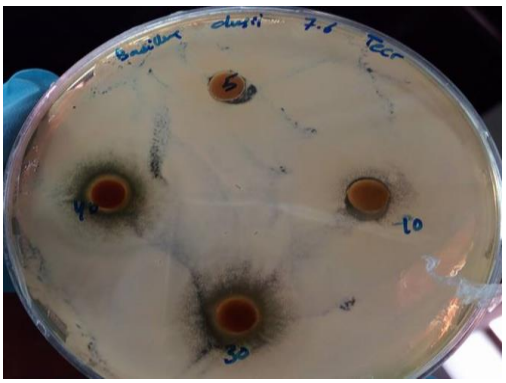


Figure 3.41

Inhibition zone of different amount of extract of S.hierosolymitana against Listeria innocua 1770 by using paper disc diffusion

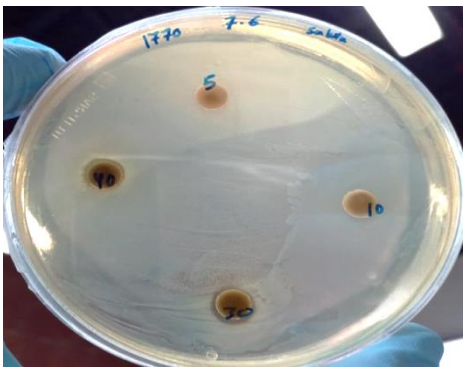


Figure 3.42

Inhibition zone of different amount of extract of O.syriacum against Listeria innocua 1770 by using paper disc diffusion

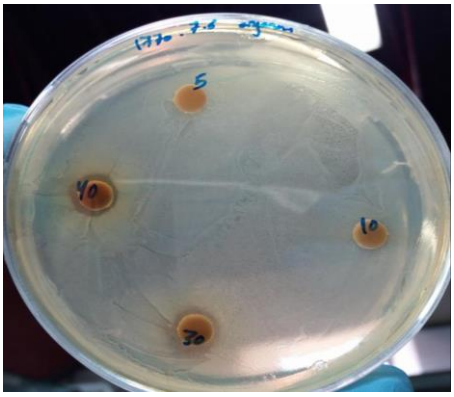


Figure 3.43

Inhibition zone of different amount of extract Rumex acetosa L. against Bacillus clausii by using paper disc diffusion

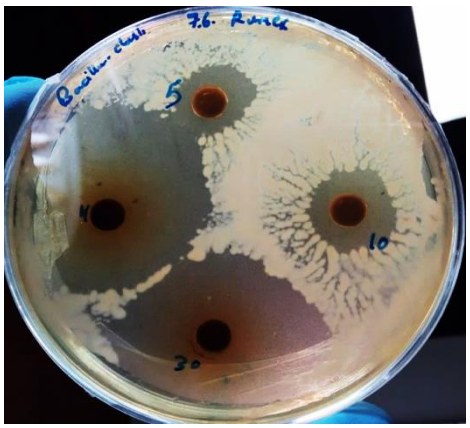


Figure 3.44

Inhibition zone of different amount of extract of Rumex acetosa against Listeria innocua 1770 by using paper disc diffusion

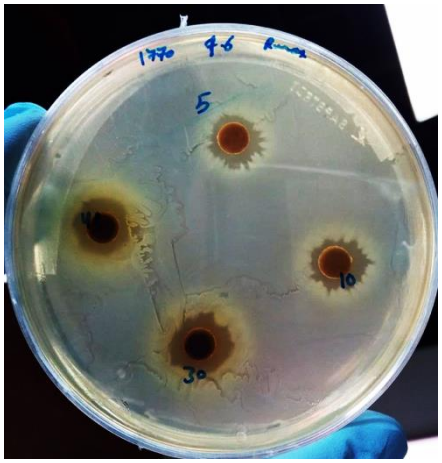
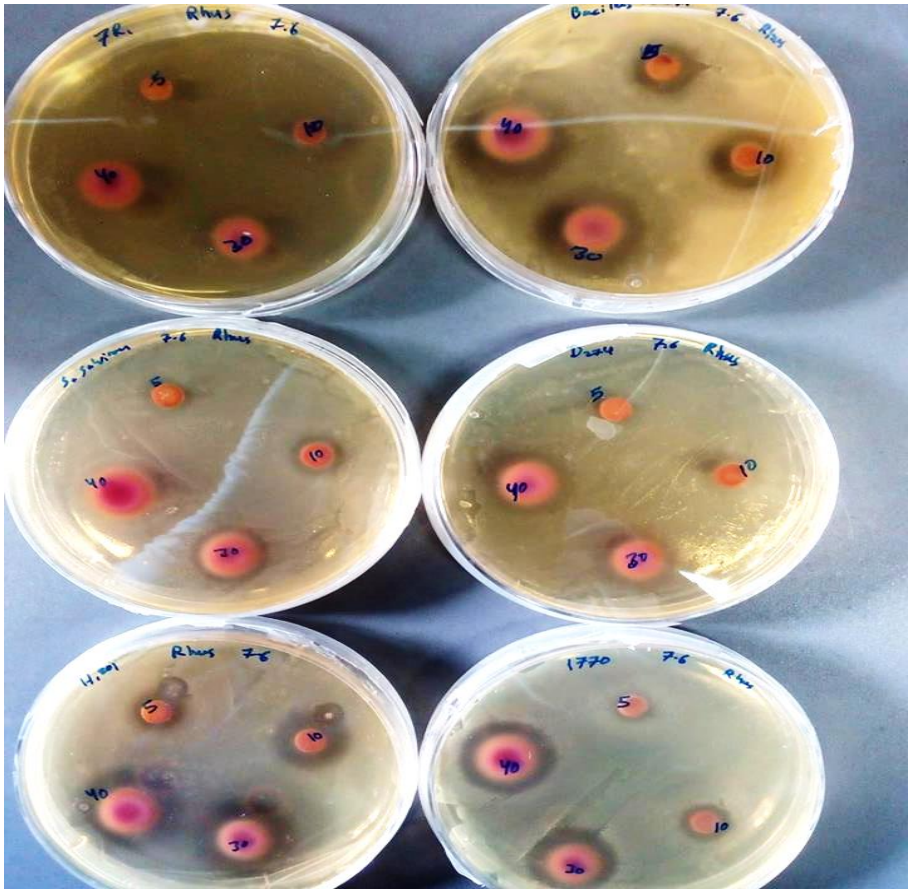


Figure 3.45

Inhibition zone of different amount of extract of Rhus coriaria L. against six indicator strains (1770, H1201, D274, Bacillus clausii, S.salivours, 7R1) by using paper disc diffusion



3.8 Antibacterial activity of Microencapsulation of R.coriaria L and T. capitatum L

Very little information in literature review about microencapsulation of crude extract, as we know just since few years ago started in studied microcapulation and preparation capsule specially in medicine sector, now a days widespread in other sector for example in food technology.

We performed two of crude extracts in microencapsulation separately (Rhus coriaria L. and *Teucrium* capitatum L.

The antibacterial activity of Rhus coriaria L. has high activity, but the Antibacterial activity of Microencapsulation of R.coriaria L shown very low activity. But the antibacterial activity of microencapsulation T.capitatum didn’t show any antibacterial activity.

Chapter Four

Conclusion

The screening of antibacterial activity of crude extract of plant (Table 2) against bacterial indicator strains (Table1) food borne pathogens, in our study has shown the antimicrobial activity for all methanol extract of plants we tested *Origanum syriacum L. Var. syriacum,* Rhus coriaria L., Rumex acetosa L, Salvia hierosolymitana Boiss, Teucrium capitatum L, by using two methods (well diffusion and paper disc diffusion), Obviously the two methods has shown a high inhibition zone, the different of active compound in each Plant gave the plant different activity, so the Rhus coriaria L., shown highest antibacterial activity also in low amount of some extracts. Also one of crude extract have very low or not clear activity (Cyclamen persicum mill). In addition, this study justifies the reality of usage a plant in folk medicine for a long time. we were studying. This study suggested a further advanced study should be focused on the antibacterial agent of these plant parts, which may be used by food enterprises, pharmaceuticals, medicine, and preservatives. Also need more studying of chemical characteristic to determine which the most active compound will inhibit the bacteria growth.

Related to Results of this experiment and other studies we are suggesting to introduce this plants material for enterprises to use it with many products as natural preservatives instead of chemical substances, also we are recommending this Plants for new researcher, because some of its haven't information in literature review, or rare information about plants we studied. In addition the results we got it, can be first step for various future research.

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النشاط البيولوجي للمستخلصات النباتية من منطقة الشرق الاوسط على مسببات الأمراض في الأغذية

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الملخص

هناك العديد من الأبحاث التي تبحث في النشاط العالي لمستخلصات النباتات والميكروبات وخلال العقود القليلة الماضية كانت هناك زيادة ملحوظة في الطلب على المواد الحافظة الطبيعية، الهدف من دراستنا هو تقييم ستة مستخلصات ميثانولية لنباتات طبية من فلسطين على 18 سلالة بكتيرية (موجبة وسالبة الجرام).

تم تحضير مستخلصات من ستة نباتات (السماق، الزعتر، اللسينة، الحميض، جعدة الصبيان، والزعمطوط) مستخلصات ميثانولية من أجزاء مختلفة من النباتات المستخدمة في الطب التقليدي في فلسطين، جهزنا المستخلص الخام باستخدام الميثانول والماء المقطر واستخدمنا المبخر الدوراني، الاختبار باستخدام طريقتين (القرص الورقي وانتشار الاجار).

أظهرت دراستنا النشاط المضاد للبكتيريا لجميع المستخلصات الميثانولية للنباتات التي اختبرناها Origanum syriacum L. Var. syriacum, Rhus coriaria L. Rumex acetosa L Salvia hierosolymitana Boiss, Teucrium capitatum, Cyclamen persicum mill.

من الواضح أن الطريقتين قد أظهرت منطقة تثبيط عالية لجميع المستخلصات خاصة البكتيريا الموجبة جرام باستثناء(Cyclamen persicum mill)، وأظهر Rhus coriaria أعلى نشاط مضاد للجراثيم ضد الجرام موجب وسالب.

من الدراسة التي تم اجرائها توضح أهمية النباتات التي تمت دراستها لكونها لها خاصية مضادة لسلالات بكتيرية مسببة تلف للأطعمة، حيث بالإمكان استخدامها كمواد حافظة طبيعية بديلا عن المواد الكيماوية، مع التركيز على زيادة الابحاث حول هذه النباتات، نقترح تقديم هذه المواد النباتية للشركات العلمة في قطاع الاغذية والبدائل الطبيعية لاستخدامها مع العديد من المنتجات كمواد حافظة طبيعية بدلاً من المواد الكيميائية.