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دراسة تأثير مستخلص عود الآراك (المسواك) على بكتيريا عزلت من الفم ومقارنتها ببكتيريا أخرى ممريضة و بالمضادات الحيوية

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Faculty of Science
Department of Botany

Effect of *Salvadora persica* (Miswak) extracts against bacteria isolated from mouth compared with other pathogenic bacteria and antibiotics

A thesis presented to the department of botany faculty of science benghazi university for fulfillment of the requirement for the degree of master of science

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(2016 – 2017)

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

قُلْ هُوَ اللَّهُ أَحَدٌ ﴿١﴾

اللَّهُ الصَّمَدُ ﴿٢﴾ لَمْ يَلِدْ وَلَمْ يُولَدْ ﴿٣﴾

وَلَمْ يَكُن لَّهُ كُفُوًا أَحَدٌ ﴿٤﴾

صدق الله العظيم

Gifting

To the seal of the prophets and messengers honest and custodian prophe

"Muhammad peace be upon him"

To spring tend mess love and her Rahim to her because of her du'aa reason successes

My dear mother

To the pure spirit that long what encouraged me to success

My dear father

To than she taught and lit my way of science and knowledge

Dr. Salha F. Ben-Gwerif

To get tired of and it seeks to rest and it proves difficult

My dear husband

To my soul that walks on the ground It is my hope in life

My beloved Son

To than I love them ,to flowers that fill my life with hope and happiness

My brothers

To lead a spiritual which overwhelmed me science and tender

My friends Mr .Naama aissa

To flowers that fill my life with happiness

My friends

Acknowledgment

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الملخص العربي :

نبات سالفا دورا بيرسيكا المعروف باسم ميسواك (فرشاة الأسنان)، ينتمي إلى عائلة سلفادوريسيا او هو موزع على نطاق واسع في المناطق القاحلة ، وغالبا ينمو على التربة المالحة. وهو شجرة صغيرة دائمة الخضرة ، تؤكل الأوراق الطازجة كسلطة وتستخدم في الطب التقليدي للسعال والربو، الاسقربوط، الروماتيزم،وغيرها من الأمراض. إن استخدام الاراك هو عرف إسلامى الذي التزم به العرب القدامى للحصول على أسنان بيضاء وبراقة

درس تأثير مستخلصا نبات الآراك *Salvadora persica* المائي والكحولي على ثمان عزلات من البكتيريا الفم لعدد من الرجال والنساء وهى :

Staphylococcus aureus, Streptococcus mutans, Streptococcus faecalis ,Streptococcus pyogenis, Enterococcus faecalis and *Staphylococcus mutans* .

وانواع اخرى تحصل عليها من مختبر علم الأحياء الدقيقة في مركز بنغازي الطبي ومستشفى الاطفال الطبى نذكر منها على سبيل المثال :

Escherichia coli , Pseudomonas aeruginosa ,klebsiella pnemoniae algala , Staphylococcus albus ,Streptococcus pnemoniae, Acinetobacter baumannii urine, proteus mirabilis algala and Staphylococcus epidrimidis urine. M*icococcus lylae,* B*acillus stearothermophilus,*

وذلك باستعمال طريقة الانتشار في الأطباق .

وقد أبدا كلا المستخلصين المائي والكحولي تأثيراً تثبيطياً واضيحاً ضد كل العزلات عند التراكيز 30 %, 50% , 100% ,باستثناء المستخلص المائي للماء المغلى أنه لم يمنع نمو جميع بكتيريا وكان المستخلص الكحولي قد سجل أعلى منطقة تثبيط من المستخلص المائي إذ بلغ قطر منطقة تثبيط 25.33 ملم عند تركيز 100% فى حين منطقة تثبيط المستخلص المائي 21:33 ملم عند تركيز 100% أقل قليلاً من المستخلص الكحولي أما أقل منطقة تثبيط المستخلص الكحولي كان قطرها 12:00 ملم وكان أقل منطقة تثبيط المستخلص المائي كان قطرها 9.67 ملم وكان أفضل تأثير للمستخلصين على البكتيريا كان عند التركيز 100% لكلا المستخلصين المائي والكحولي إى كلما كان التركيز عالى كلما كان التأثير أفضل .

كما اظهرت الدراسة بأن تأثير كلا المستخلصين لنبات الآراك المائي البارد والكحولي كانت

تأثيرها أعلى من تأثير المضادات الحيوية وبذلك فإن هذه الدراسة قد اظهرت بالإمكان إستخدام مستجلصات الآراك كبدائل دوائية منخفضة الكلفة وخالية من التأثيرات الجانبية بدلآ من المضات الحيوية ذات التأثيرات الجانبية .

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Abstrsct

Salvadora persica , commonly known as miswak (tooth brush), belongs to the family Salvadoracea It is widely distributed in the arid regions of India and often on saline soils. It is an upright evergreen small tree or shrub, The fresh leaves are eaten as salad and are used in traditional medicine for cough, asthma, scurvy, rheumatism, piles, and other diseases. The use of miswak is a pre-Islamic custom, which was adhered to by the ancient Arabs to get their teeth white and shiny.

Studied the effect of the extracts of *Salvadora Percica* (alcohol and water) against eight types of bacteria isolated from the mouth men and women *Staphylococcus aureus, Streptococcus mutans, Streptococcus faecalis ,Streptococcus pyogenis, Enterococcus faecalis* and *Staphylococcus mutans* **.**

And other types obtained from the laboratory of microbiology at the Benghazi Medical Center and Children's Hospital medical as follows *Escherichia coli , Pseudomonas aeruginosa ,klebsiella pnemoniae algala , Staphylococcus albus ,Streptococcus pnemoniae, Acinetobacter baumannii urine, proteus mirabilis algala and Staphylococcus epidrimidis urine. Micococcus lylae, Bacillus stearothermophilus,* by using disk diffusion method in the dishes.

The extracts of water and alcohol have never been inhibited against all isolates at concentrations of 30%, 50% and 100%, with the exception of the water extract of boiled water that did not inhibit the growth of all bacteria.

The alcohol extract recorded the highest inhibitory zone of the water extract, 25.33 mm at 100% concentration while inhibition zone 21:33 mm at 100% less concentration than alcohol extract. The lowest inhibitory zone was 12 mm diameter and the lowest inhibitory zone was 9.67 mm diameter and was the best effect For extracts on bacteria at 100% concentration for

both water extracts And alcohol. whenever a high concentration effect whenever the better.

The study also showed that the effect of both extracts of cold and alcoholic aqueous plants was higher than the effect of antibiotics. Therefore, this study showed that it is possible to use abstractions As a low-cost drug substitute and free from side effects instead of biogas with side effects.

Chapter One

1.1 Introduction

Salvadora persica Linn., commonly known as miswak (tooth brush), belongs to the family Salvadoracea] It is widely distributed in the arid regions of India and often on saline soils. It is an upright evergreen small tree or shrub, seldom more than 1 ft in diameter reaching a maximum height of 3 m. The fresh leaves are eaten as salad and are used in traditional medicine for cough, asthma, scurvy, rheumatism, piles, and other diseases. The use of miswak is a pre-Islamic custom, which was adhered to by the ancient Arabs to get their teeth white and shiny. The beneficial effects of miswak in respect of oral hygiene and dental health are partially due to its mechanical action and partially due to pharmacologic action. There is investigation of its different chemical constituents, which are responsible for these activities.(Farooqi *et al*.,1968) isolated benzyl-isothiocyanate from *Salvadora persica* root, and they claimed to have found saponins along with tannins, silica, a small amount of resin, trimethylamine, and alkaloidal constituents. Ray *et al*. isolated β-sitosterol, m-anisic acid, and salvadourea. Lewis and Elvin-Lewis report a high content of minerals in the a 27.06%. (khatak *et al*,(2010))

In recent years, human pathogenic microorganisms have developed resistance in response to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This situation, the undesirable side effect of certain antibiotics, and the emergence of previously uncommon infections, has forced scientists to look for new antimicrobial substances from various sources, such as medicinal plants. The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new antiinfective agents. (AL.Bayat and D.Sulaimsn .,2008).

The toothbrush tree, *Salvadora persica,* L., locally called miswak, is a member of the Salvadoraceae family has been used by many Islamic communities as toothbrushes and has been scientifically proven to be very useful in the prevention of tooth decay, even when used without any other tooth cleaning methods. Chewing sticks that are made from the roots, twigs, or stems of *S.persica* are commonly used in the Middle East as a means of maintaining oral hygiene. Studies indicate that *S.persica* extract is somewhat comparable to other oral disinfectants and anti-plaque , such as triclosan and chlorhexidine gluconate, if used at a very high concentration. (AL.Bayat and D.Sulaimsn.,2008).

The use of a wood stick *Salvadora persica* (meswak) for brushing the teeth continues to be an important tool for oral hygiene care in many Afro-Asian communities. It is inexpensive, customary and used for religious reasons as well. The pharmacological studies revealed that *Salvadora persica* is effective against dental caries, bacterial and fungal growth. Anti-plaque activity of *Salvadora persica* is comparable with Chlorhexidine gluconate and it is also found to reduce gingival bleeding. Phytochemical screening revealed the occurrence of glycosides, sterols, terpenes, flavonoids and alkaloids. Fluoride, calcium and phosphorus, minerals required for dental health are also present in *Salvadora persica*. Present review is written with objective to renew interest in use of *Salvadora persica* toothbrushes as environment friendly and cheap tool for dental care and oral hygiene.(Manoj *et al*., 2011) .

Research has identified several anionic components of *S. persica*m miswak that are known to have antimicrobial effects (Darout *et al.*, 2002). hypothesized that these components had potent promoter effects on salivary peroxidase thiocyanate and hydrogen peroxidase antimicrobial systems. Furthermore, (Darout *et al.* ,2002)examined the salivary levels of 25 oral microorganisms in a study conducted

among Sudanese adults, and suggested that *S. persica* miswak may have a selective inhibitory effect on the levels of certain bacteria. They observed that miswak users had significantly lower numbers of cariogenic bacteria, except *Streptococcus mutans*, in their saliva, whereas toothbrush users had lower salivary levels of periodontal pathogens *Aggregatibacter actinomycetemcomitans*, a predominant periodontal pathogen, and other bacterial species were found to be present in significantly higher numbers in the saliva of miswak users than in that of tooth brush users (Darout *et al.*, 2002). Furthermore, another study reported that miswak users harbored significantly higher plaque levels of *Staphylococcus intermedius, A. actinomycetemcomitans*, *Veillonella parvula*, *Actinomyces israelii*, and *Capnocytophaga gingivalis* and significantly lower levels of *Selenomonas sputigena*, *Streptococcus salivarius*, *Streptococcus oralis*, and *Actinomyces naeslundii* than did toothbrush users (Darout *et al*., 2003).

In a single-blind, randomized, crossover study involving 15 Saudi Arabian volunteers, (Al-Otaibi *et al.* ,2004) demonstrated that the effect of chewing miswak on the levels of subgingival plaque microbiota was similar to that of regular toothbrushing without toothpaste, and that the level of *A. Actinomycetemcomitans* was significantly more reduced by using miswak than by toothbrushing. (Almas and Al-Zeid.,2004) investigated the immediate antimicrobial effects of *S. persica* miswak and its extract on *Streptococcus mutans* and *Lactobacillus*, and found a significant decrease in *Streptococcus mutans* count, but not in *Lactobacillus* count, in miswak users.

Based on the results of their experimental research, recommended the use of *S. persica* miswak extract in mouthrinses and toothpastes due to its remarkable antimicrobial effects. This research comprised three *in vitro* studies that tested the effect of *S. persica* miswak extract on selected bacteria, compared the paraclinical

effects of Iranian toothpaste containing *S. persica* miswak extract and placebo toothpaste on dental plaque, and compared the antibacterial effect of the Iranian toothpaste with that of a Swiss toothpaste on dental plaque and also with penicillin. The results of all three studies showed the positive paraclinical effects of *S. persica* miswak extract on dental plaque.(Poureslami *et al.* ,2007)

1.2 Aims of this study

1 - Effect of Salvadora persica extract against bacteria isolated from mouth, bacteria cause infection of chiidren(UTI),bacteria urinary tract infection, bacteria multi drag resistance (MDR) *and* bacteria infection after the operation

2- Comparison effect between Aqueous extracts , Ethanol extracts and antibiotics

Chapter Two

Review of literature 2.1

Miswak (chewing stick) was used by the Babylonians some 7000 years ago; it was later used throughout the Greek and Roman empires, and has also been used by ancient Egyptians and Muslims. It is used in different parts of Africa, Asia-especially the Middle East- and South America. Chewing sticks are used for oral hygiene, religious and social purposes. Dental caries and periodontal diseases are the two main afflictions to mankind. Bacterial plaque is solely responsible for the initiation and progression of periodontal diseases. The methods available for the maintenance of oral health are mainly mechanical and chemical. Toothbrushes and dentifrices are widely used for cleaning teeth. The traditional toothbrush or chewing stick is deeply rooted in Islamic culture. This article gives a brief cultural and historical background of the subject and review current literature on Miswak . Pencil-sized sticks of various plants are fashioned from certain plant - parts and are chewed on one end until they become frayed into a brush. The brush-end is used to clean the teeth in a manner similar to the use of a toothbrush. When used in this manner, they are commonly referred to as chewing sticks or Miswak. The conventional meaning of Miswak is 'stick used on teeth and gums to clean them.' Its various names are Miswak and Siwak as used in the Middle East, Mswaki in Tanzania, Mefaka in Ethiopia and Datun in India and Pakistan.1 Although Siwak or Miswak is used to describe Arak *(Salvadora persica)*, the stick which the Prophet Muhammad - Peace and Blessings of Allah be upon Him (PBUH) - used to clean his mouth with, miswak is a more general term which includes all types of sticks used as toothcleaning aids.(Al Sadhan, Almas.,1999)

2.2 Salvadora persica

Salvadora persica (kharijal) is a large, well-branched, and evergreen shrub or a tree resembling *Salvadora oleoides* (meethijal) found in the dry and arid regions of India. Chewing sticks have been used for centuries for tooth cleaning, and are recommended by the World Health Organization in areas where their use is customary. *Salvadora persica* has enormous reported activities.

It has potential medicinal and research activities. *Salvadora persica* is a promising product and is useful to produce antiplaque, analgesic, anticonvulsant, antibacterial, antimycotic, cytotoxic, antifertility, deobstruent, carminative, diuretic, astringent, and also used in biliousness, and rheumatism. This review highlights the pharmacologic effects and therapeutic effects of *Salvadora persica*. The chemical constituents present in different parts of the plant are also discussed. (Hassan., (2012) .

Salvadora persica Linn., commonly known as miswak (tooth brush), belongs to the family Salvadoracea. It is locally called as kharijal; BENG—Jhal; Mah—Khakhin Kickni, Miraj, Pelu, Pilva; GUJ—Kharijal, Piludi; TEL—Ghunia, Varagogu; TAM—Kalawa, kakkol, vivay; KAN—Goni-mara; and ORIYA—Kotungo, piluIt is widely distributed in the arid regions of India and often on saline soils. It is an upright evergreen small tree or shrub, seldom more than 1 ft in diameter reaching a maximum height of 3 m. The fresh leaves are eaten as salad and are used in traditional medicine for cough, asthma, scurvy, rheumatism, piles, and other diseases. The use of miswak is a pre-Islamic custom, which was adhered to by the ancient Arabs to get their teeth white and shiny. The beneficial effects of miswak in respect of oral hygiene and dental health are partially due to its mechanical action and partially due to pharmacologic action. There is investigation of its different chemical constituents, which are responsible for these activities. Farooqi *et al*.,1968) isolated benzyl-isothiocyanate

from *Salvadora persica* root, and they claimed to have found saponins along with tannins, silica, a small amount of resin, trimethylamine, and alkaloidal constituents. Ray *et al*. isolated β-sitosterol, m-anisic acid, and salvadourea. Lewis and Elvin-Lewis report a high content of minerals in the root, 27.06% [Figure 1]. (Hassan., 2012).

Figure 1 *Salvadora persica* (chewing stick)

2.3 Botanical description

Salvadora persica is a large, well-branched evergreen shrub or small tree having soft whitish yellow wood, bark is of old stems rugose, branches are numerous, drooping, glabrous, terete, finely striate, shining, and almost white. Leaves are somewhat fleshy, glaucous, 3.8–6.3 by 2–3.2 cm in size, elliptic lanceolate or ovate, obtuse, and often mucronate at the apex, the base is usually acute, less commonly rounded, main nerves are in 5–6 pairs, and the petioles 1.3–2.2 cm long and glabrous. The flowers are greenish yellow in color, in axillary and terminal compound lax panicles 5–12.5 cm long, numerous in the upper axils, pedicels 1.5–3 mm long, bracts beneath the pedicels, ovate and very caducous. Calyx is 1.25 mm long, glabrous, cleft half-way down, lobes rounded. Corolla is very thin, 3 mm long, deeply cleft, persistent, lobes are 2.5 mm long, oblong, obtuse, and much reflexed. Stamens are shorter than corolla, but exserted, owing to the corolla lobes being reflexed. Drupe is 3 mm in diameter, globose, smooth and becomes red when ripe. It is widely distributed in the drier parts of India, Baluchistan, and Ceylon and in the dry regions of West Asia and Egypt Figures 2,3 and4 (Hassan, (2012)) .

Figure 2 *Salvadora persica* tre

Figure3 *Salvadora persica* leaves

Figure 4 *Salvadora persica* fruit

2.4 Scientific classification

Kingdom : Plantae

Division : Magnoliphyta

Class : Magnoliopsida

Order : Brassicales

Family : Salvadoraceae

Genus : *Salvadora*

Species : *persica oleoides*

Binomial name : *Salvadora persica* (Khari Jaal) *Salvadora oleoides* (Meethi Jaal) (Hassan.,2012) .

2.5 Traditional uses

2.5.1 Leaves

The leaves are eaten as a vegetable in the eastern tropical Africa and are used in the preparation of a sauce, and tender shoots and leaves are eaten as salad. Leaves are bitter in taste, corrective, deobstruent, astringent to the bowels, tonic to the liver, diuretic, analgesic, anthelmintic, useful in ozoena and other nose troubles, piles, scabies, leukoderma, lessening inflammation, and strengthening the teeth. Leaves are pungent and are considered in Punjab as an antidote to poison of all sorts and in south of Bombay as an external application in rheumatism. The juice of the leaves is also used in scurvy.

2.5.2 Fruits

Fruits are sweet and edible. A fermented drink is reported to be made from the fruits. Fruits possess deobstruent, carminative, diuretic, lithontriptic, and stomachic

properties and are used in biliousness and rheumatism. In Sind, it is believed that fruits have a good effect on snake bite.

2.5.3 Root bark

Root bark is used as a vesicant and is employed as an ingredient of snuff. A paste of the roots is applied as a substitute for mustard plaster and their decoction is used against gonorrhea and vesical catarrh. A decoction of the bark is used as a tonic in amenorrhea and the dose of the decoction is half a teacupful twice daily and as a stimulant in low fevers and as an emmenagogue.

2.5.4 Stem bark

Stem bark is used as an ascarifuge and also in gastric troubles.

2.5.5 Seeds

Seeds have bitter and sharp taste. They are used as purgative, diuretic and tonic seed oil is applied on the skin in rheumatism (Hassan, (2012)) .

2.6 Chemical profile of *salvadora persica*

On phytochemical investigations, its stem yielded octacosanol, 1-triacantanol, β-sitosterol, and β-sitosterol-3-O-β-D-glucopyranoside. On thin layer chromatography examination, it was found to be a mixture of 2 compounds, which were separated by column chromatography. Compound A had a melting point (m.p.) -136-7ºC, *m*/*z* = 414 (mass) and molecular formula $C_{29}H_{50}O$ (C = 83.75%, H = 12.25%). It gave positive Salkowski, Liebermann, Burchard reaction, Noller reaction, Brieskron, Tschagajew, and yellow color with tetranitro methane Peaks in the infrared spectrum at V_{max}^{KBr} 3500, 1450, 1470, and 1145 cm^{-1} showed its identity as compound β-sitosterol in

white needle form. Compound B was found to be the white crystalline compound, with the molecular formula $C_{35}H_{60}O^{6}$, C = 72.9%, H = 14%, m.p. 265-68ºC *m/z* $[\alpha]_D^{29}$ -36.2 gave positive test for saponin and on hydrolysis yielded β-sitosterol and a sugar glucose thereby identified it as β-sitosterol-3-O-β-D-glucopyransoside. Essential oil contained α- and β-thujones, camphor, cineole, β-cymene, limonene, β-myrcene, borneol, linalool, and bornyl acetate and nonvolatile fraction contained humulene, caryophyllene, β-santatol, and farnesol. (Hassan., 2012) .

2.7 Pharmacologic activities

2.7.1 Hypolipidemic activity

The stems of *Salvadora persica* are widely used as tooth cleaning sticks in Arabic countries and decoctions show hypocholesterolemic properties. The effects of prolonged administration of a lyophilized stem decoction of *Salvadora persica* were evaluated in diet induced rat hypercholesterolemic. The preparation was administered for 15 and 30 days and cholesterol, HDL, LDL, and triglycerides plasma levels were assayed.

The results showed that the *Salvadora persica* decoction significantly lowered cholesterol and LDL plasma levels in the rats, proving to be more active at 30 days of treatment. The systemic administration of Triton resulted in a rise in plasma cholesterol and triglyceride levels. The results showed that *Salvadora persica* decoction was inactive at 18 h after treatment, whereas at 27 h it was able to reduce cholesterol and LDL plasma levels; in all the experiments HDL and triglycerides were unchanged.(Hassan., 2012) .

2.2.7 Release of calcium and chloride into saliva

Gazi *et al*. investigated the immediate and medium-term effect of miswak on the composition of mixed saliva. They reported that miswak produced significant increases in calcium (22-fold) and chloride (6-fold), and significant decreases in phosphate and pH, saturation of saliva with calcium inhibits demineralization and promotes demineralization of tooth enamel, whereas high concentration of chloride inhibits calculus formation. (Hassan., 2012).

2.3.7 Analgesic effect

Mansour *et al*. studied the analgesic effect of miswak decoction when injected into mice. They found that miswak was more effective against thermal stimuli than against chemical stimuli and also acts as an analgesic. (Hassan., 2012) .

2.4.7 Cytotoxicity

Mohammad *et al*. investigated the cytotoxic potential of *Salvadora persica* on gingival and other periodontal structures, using the agar overlay method. Results showed no cytotoxic effect by a freshly cut and freshly used miswak. However, the same plant used after 24 h does contain harmful components. Based on these findings they recommend cutting the used portion of the miswak after it has been used for one day and preparing a fresh part. The cytotoxicity in this study became evident only after 24 h because the agar overlay method depends on the diffusion of the medicament to the agar material.(khatak *et al*., 2010).

2.5.7 Effects on dental plaque, gingival health, and periodontal status

Many reports have revealed that *S. persica* miswak effectively reduced gingivitis and dental plaque. Studies among Ethiopian school children (Olsson., 1978) and Saudi Arabian dental students (Char *et al*., 1987) showed that miswak removed

plaque more effectively than did toothbrushing. The study involving schoolchildren required the provision of instructions and supervision because most children were unfamiliar with the proper technique of miswak use.(Olsson ,1978) recommended the use of miswak in preventive dental programs because it is economical and familiar to older people.(Moustafa *et al*., 1987) reported 75% plaque reduction after the use of *S. persica* miswak for 8 days. A study conducted among two groups of students in Kenya (Danielsen *et al.*, 1989) reported that no additional method was required to remove dental plaque in the group that used toothpaste in combination with chewing sticks.

Although miswak may effectively remove dental plaque, an association between excessive miswak use and gingival recession was demonstrated in Saudi schoolchildren (Younes and El Angbawi, 1983). (Eid *et al*., 1991) also reported many cases of gingival recession among miswak users, which may be due to mechanical trauma. An earlier study by the same investigators (Eid *et al*., 1990a) found no significant difference in gingival indices or bleeding between miswak and toothbrush users. However, (Gazi *et al*.,1990) found gingival indices to be significantly lower following the use of *S. persica* miswak in comparison with the use of a conventional toothbrush without toothpaste.

kSeveral toothpastes containing *S. persica* miswak extract are commercially available (Al Sadhan and Almas, 1999 and Guile *et al*., 1996). One such toothpaste was found to be significantly more effective in removing dental plaque when compared with Oral-B toothpaste (Hattab, 1997). The combined effect of mechanical cleansing and enhanced salivation achieved with the proper use of *S. persica* miswak was found to be more efficient than toothbrushes in removing dental plaque (Wu *et al*., 2001).

In a randomized crossover study among 15 Saudi Arabian male volunteers,(Al-Otaibi *et al*. ,2003) found that miswak use, significantly reduced plaque and gingival indices and was more effective than toothbrushing when preceded by professional instruction regarding its correct application. Rinsing with a slurry of toothpaste containing *S. persica* miswak has been shown to reduce gingival inflammation and bleeding on probing. In comparison, chlorhexidine was found to reduce plaque more effectively than *S. persica* miswak, and the anti-plaque effects of both products suggested no definite advantage in using *S. persica* miswak over chlorhexidine (Gazi *et al.*, 1987).

In a similar study investigating a commercial herbal mouthwash containing *S. persica* miswak extract, significant reductions in gingival bleeding were observed in both test and placebo subjects. However, a significant reduction in the load of cariogenic bacteria was observed only in the test subjects (Khalessi *et al.*, 2004).

The efficacy of chewing sticks has been challenged in several studies. (Norton and Addy ,1989) reported more plaque formationm and gingival bleeding in persons using chewing sticks than in toothbrush users. Habitual users of miswak had a significantly higher prevalence of gingivitis than did toothbrush users (Eid *et al.,* 1990b and Norton and Addy, 1989). (Gazi *et al*.,1990) indicated that miswak sticks were less effective than toothbrushes in cleaning interproximal dental areas and lingual surfaces.

Low levels of tooth loss in adults have been found in countries where miswak is widely used (Elvin-Lewis *et al*., 1980). Epidemiological ,studies (al-Khateeb *et al*., 1991 and Younes and El-Angbawi, 1982) suggested that miswak use had beneficial effects on the prevalence of periodontal diseases and caries. Furthermore,(al-Khateeb *et al*. (1991) and Guile (1992)) reported low periodontal treatment needs among Saudi

adults who used miswak. An epidemiological study conducted among certain nomadic groups of the Kaisut Desert region of Kenya showed that dental caries and advanced periodontal disease were rare among miswak users under the age of 50 years (Carl and Zambon, 1993). However, a retrospective study conducted in Saudi Arabia showed conflicting results; miswak users had deeper periodontal pockets and a higher prevalence of periodontal diseases (Eid and Selim, 1994) than did non-users. In a study performed at a medical campus in Sudan, (Darout *et al.* ,2000) reported that the periodontal status of habitual miswak users was similar to or of toothbrush slightly better than that users. (Hassan., 2012) .

2.7.8 Antiulcer activity

Salvadora persica possessed significant protective action against ethanol and stress-induced ulcers. This study was designed to confirm the antiulcer activity of *Salvadora persica* decoction using optical microscopy. The elements of gastric mucosa tended to be reestablished normally in tested rats. (Hassan., 2012) .

2.7.9 Anticonvulsant activity

The effect of *Salvadora persica* as an anticonvulsant was identified by using stem extracts. The stem extracts show the potentiation of sodium pentobarbital activity and on generalized tonic-clonic seizure produced by pentylentertazol (PTZ) on the rat is reported. The extracts of *Salvadora persica* Linn. extended sleeping-time and decreased induction-time induced by sodium pentobarbital, in addition it showed protection against PTZ-induced convulsion by increasing the latency period and diminishing the death rate. (Hassan., 2012) .

2.7.10 Antifertility activity

Miswak extract did not have much effect on female mouse fertility, although it caused a significant decrease in the relative weights of the ovary and an increase in the uterine weights. Exposure of male mice to miswak resulted in a 72% reduction in pregnancies in untreated females impregnated by test males. The relative weights of the testes and preputial glands were significantly increased and that of the seminal vesicles was significantly decreased in test males. The results indicate that miswak has adverse effects on male and female reproduction systems and fertility. (Hassan., 2012) .

2.7.11 Antimycotic activity

Aqueous extracts of miswak could be used to reduce the growth of *Candida albicans*. Such inhibition lasts for up to 36 h at concentrations of 15% and above.
(Hassan., 2012) .

2.8.12 Anticariogenic effects

Many epidemiological studies revealed that *S. persica* miswak had strong anti-decay effects. In a dental health survey conducted in Sudan, Emslie (1966) reported a lower caries prevalence among miswak users than among toothbrush users. Subsequent studies (Baghdady and Ghose, 1979, Sathananthan *et al*., 1996) and Younes and El-Angbawi, 1982) found similar lower caries incidences among school children using miswak.

(Olsson. ,1978) reported that chewing sticks reduced dental caries more effectively than did conventional toothbrushes. Despite the carbohydrate-rich diet traditionally consumed in Ghana, the incidence of caries and other dental diseases was low among Ghanaian chewing-stick users (Elvin-Lewis *et al*., 1980). A cross-sectional pilot study

among adults in West Africa (Norton and Addy, 1989) also reported a decreased rate of caries and plaque in miswak users in comparison with non-users.

The pungent taste and chewing effects of miswak may increase saliva secretion in the mouth, thereby increasing its buffering capacity (Hattab, 1997).

2.7.13 Antimicrobrial activity

An *in vitro* study showed that the aqueous extract of *S. persica* miswak had an inhibitory effect on the growth of *Candida albicans* that may be attributed to its high sulfate content (al-Bagieh *et al*., 1994). AlLafi and Ababneh (1995) investigated the derivatives of *S. persica* miswak using three different laboratory methods, and demonstrated strong antimicrobial effects on the growth of *Streptococcus* sp. and *Staphylococcus aureus*. In addition,(Alm as *et al*.,1997) showed that *Enterococcus faecalis* is the most sensitive microorganism affected by the use of *S. persica* miswak, and noted no significant difference in the antimicrobial effects of freshly cut and 1-month-old miswak. A comparison of the alcoholic and aqueous extracts of *S. persica* miswak revealed that the alcoholic extract had more potent antimicrobial activity than did the aqueous extract (Al-Bagieh and Almas, 1997). (Almas ,1999) showed that *S. persica* miswak extracts had antimicrobial effects on *Streptococcus mutans* and *E. faecalis*.(Elvin-Lewis *et al*. ,1980) and (Almas .,1999) suggested that this effect may be due to the interaction with bacteria, which prevents their attachment on the tooth surface.(Almas and Al-Bagieh. ,1999) also noted significant differences in the antimicrobial activities of the pulp and bark extracts of *S. persica* miswak.

An investigation of whole (unextracted) *S. persica* miswak pieces embedded in agar or suspended above the agar plate revealed strong antibacterial effects against oral microorganisms associated with periodontitis and dental caries (Sofrata *et al*., 2008). Fluoride, which is a component of *S. persica* miswak, showed a possible interaction

with bacterial glycolytic enzymes and their acids. Moreover, in an earlier study, the BITC component of *S. persica* miswak was suggested to be a bacterial growth inhibitor (Farooqi and Srivastava, 1968).

steadies In Vitro Antimicrobial Activity of Salvadora persica Extracts Against Some Isolated Oral Pathogens in Iraq Aqueous and Ethanol extracts of *Salvadora persica*., was studied in Iraq for the activity *Salvadora persica* , was investigated for its antimicrobial activities against 7 isolated oral pathogens: *Staphylococcus aureus, Streptococcus mutans, Streptococcus faecalis*, *Streptococcus pyogenis*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa*, and *Candida albicans* using disc diffusion and micro-well dilution assays. According to both antimicrobial assays the aqueous extract inhibited all isolated microorganisms, especially the *Streptococcus species*, and was more efficient than the Ethanol extract, which was resisted by Lacto. acidophilus and *Ps.aeruginosa*. The strongest antibacterial activity was observed using the aqueous extract against Strep. faecalis (zone of inhibition: 22.3 mm; MIC: 0.781 mg/ml). Both extracts had equal antifungal activity against C. albicans based on the turbidity test (MIC: 6.25 mg/ml). (Al-Bayati and Sulaiman.,2008) Investigated the aqueous and Ethanol extracts of *S. persica* miswak for antimicrobial activities against seven isolated oral pathogens (*S. aureus*, *Streptococcus mutans*, *Streptococcus pyogenes*, *E. faecalis*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa*, and *Candida albicans*) using two methods. Both antimicrobial assays showed that the aqueous extract inhibited all isolated microorganisms and was more efficient than the methanol extract, which was resisted by *L. acidophilus* and *P. aeruginosa*. The strongest antibacterial activity was shown by the aqueous extract against *E. faecalis*. Turbidity tests showed that both extracts had equal antifungal activity against *C. albicans*. The derivatives of *S. persica* miswak are reported to have pronounced antimicrobial activity, and these heterogeneous components can be extracted using different chemical procedures (Akhtar *et al*., 2011).

Phytochemical and Antimicrobial Activity of *Salvadora persica* (Miswak) against Some Animal Pathogen. The aqueous extract is the most effective against *Ps. aerogenes* and *B.* cereus followed by *Enterococcus , K. pneumonia and S.epidermis* cold aqueous extract showed significant antibacterial activity against, .While the ether extract did not show any significant antibacterial *S. typhimurium ,S.pyogens , and E. coli*. Also the extract showed high antifungal effect against *C. albicans* .Phytochemical screening indicated that the aqueous extract most abundantly contained only. (EL.Desoukey. ,2014)

Studied the antimicrobial effect of water extraction of *Salvadora persica* (Miswak) as a root canal irrigant The aim of this study was to evaluate the antimicrobial effect of 10% water extraction of *Salvadora persica* (Miswak) when used clinically as an endodontic irrigant. Twenty four uniradicular teeth with necrotic pulps were chosen. The patients were divided randomly into 2 groups: Experimental group, in which water extract of *Salvadora persica* (10%) was used as a root canal irrigant; and control group, in which distilled water was used as a root canal irrigant. Bacteriological samples were obtained from the canal at the step of working length determination (before the canal was subjected to instrumentation and irrigation procedures), and at the end of the biomechanical instrumentation procedures by using a sterile K–file. The file was separated from the handle using a sterile wire cutter, and the severed portion was placed in a sterile screw–capped vial containing 5 ml of thioglycollate broth as a transport media. A 0.1 ml of thioglycollate broth was inoculated on each of two brain–heart infusion agar plates: One plate was incubated under aerobic conditions, and the other was incubated under anaerobic conditions using anaerobic jar and gas pack anaerobic system. Both plates were incubated at 37 ºC for 24 hours; then, the number of bacterial colonies was counted. The results revealed that 10% water extraction of *Salvadora persica* is an effective antimicrobial agent when utilized clinically as an irrigant in the endodontic treatment of teeth with necrotic pulps. (Talal *et al*. ,2005)

2.7.14 Antibacterial activity

Salvadora persica contain substances that possess plaque inhibiting and antibacterial properties against several types of cariogenic bacteria, which are frequently found in the oral cavity. The growth and acid production of these bacteria is thus inhibited.A comparison of alcohol and aqueous extract of miswak was also made. It was found that alcoholic extract is more effective than aqueous extract for antibacterial activity.

In another study, miswak pieces were standardized by size and weight and tested against *Streptococcus mutans, Lactobacillus acidophilus, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis*, and *Haemophilus influenzae*. Results found that the strong antibacterial effects against all bacteria tested is due to the presence of a volatile active antibacterial compounds .

The effects of the extracts of *Salvadora persica* and derum were examined on the proliferation of Balb/C 3T3 of fibroblast and viability of carcinogenic bacteria. For this, aqueous extracts of miswak and derum were prepared and their effects investigated on the growth of Balb/C 3T3 mouse fibroblast by measuring the mitochondrial dehydrogenase activity.

Also the effect on the viability of various cariogenic bacteria was also determined. From the obtained results, it is concluded that miswak and derum have adverse effects on the growth of cariogenic microorganisms, with derum as more active than miswak; they show cell proliferation by 156% and 255%, respectively. (Hassan., 2012) .

steadies a review on miswak (*Salvadora persica*) and its effect various aspects of oral health Plants have been used for centuries to improve dental health and to

promote oral hygiene, and this practice persists in several communities throughout the world. “Miswak” is an Arabic word meaning “tooth-cleaning stick,” and *Salvadora persica* miswak has a wide geographic distribution. It was used by ancient Arabs to whiten and polish the teeth. This review discusses the history and chemical composition of *S. persica* miswak and its influence on oral health, including the advantages and disadvantages of its use . (Hassan., 2012)

found that Anti dental caries effect of *Salvadora persica:* Anti dental caries effect of *Salvadora persica* studied by many researchers. demonstrated efficacy of Miswak in preventing dental caries in the clinical trial carried out on three hundred eighty second years students of high school in the city of Yazd, Iran. The data showed a significant increase (55%) in the rate of dental caries in control group compared to case group. The risk of dental caries for each tooth in control group was 9.35 times more than case group (9.14 and 0.98%, respectively).

The antibacterial effect special against Streptococcus mutans is responsible for anti-caries effect of *Salvadora persica* .

Antibacterial effect of *Salvadora persica:* Antibiotics and antiseptics have been used successfully to treat moderate-to-severe periodontal diseases. It appears that the local application of antimicrobials that are effective against periodontal pathogens(Manoj *et al*. ,2011)

The uses Aqueous and Ethanol extracts of *Salvadora persica* was investigated for its antimicrobial activities against 7 isolated oral pathogens: *Staphylococcus aureus. Streptococcus mutans*. *Streptococcus faecalis, Streptococcus pyogenis, Lactobacillus acidophilus***,** *Pseudomonas aeruginosa* and *Candida albicans* using disc diffusion and micro-well dilution assays. Aqueous extract inhibited all isolated microorganisms, especially the Streptococcus species and was more efficient than the methanol extract

which was resisted by *Lactobacillus acidophilus* and *Pseudomonas aeruginosa***.** The strongest antibacterial activity was observed using the aqueous extract against *Streptococcus faecalis*. Both extracts had qeual antifungal activity against *C. albicans* based on the turbidity test The antimicrobial effects of bark and pulp and entire *S. persica* extracts at 1, 5, 10 and 50% concentrations were tested against five different micro-organisms. Result revealed that 10 and 50% concentrations extracts were effective against *Streptococcus faecalis*. At 5% concentration only bark and olewh miswak extracts were effective against *S. faecalis*. The study revealed that miswak is more effective compared with bark or pulp separately

Various authors demonstrated antibacterial action of *Salvadora persica* against different strains of microbes. measured the antibacterial effect of Miswak, Miswak extract, toothbrush and normal saline against *Streptococcus mutans* and *Lactobacilli*. The reduction of *Streptococcus mutans* was significantly greater in group using miswak in comparison to toothbrushing and there was no significant difference for *lactobacilli* reduction.(Manoj *et al*. ,2011)

study miswak *(salvadora persica chewing stick)* and its role in oral health; an update: Miswak, as a cultural and scientific heritage oral hygiene tool, it is now being evaluated on evidence based criteria. Through comparing the naturally-occurring and scientific evolution of *Salvadora persica's* usage, we will be able to better understand the uniqueness of miswak, relative to that of other oral hygiene tools as being a solo oral hygiene tool of a significant part of theWorld population. The review is an update on chemical composition, antimicrobial, anticariogenic, anti plaque, and antigingivtis effects of miswak on oral health in the context of *invitro* experiments and clinical trials. Special emphasize is on how to use and when to use miswak for effective cleaning of teeth and mouth. Recent scientific evidence regarding its probiotic role, cell viability and comparative cytotoxicity and research trends will be highlighted. It is

hoped that the review will help health care professionals to have better knowledge and awareness about miswak, to improve the quality of life of their culturally diverse patients population who are uninitiated for regular oral hygiene measures due to various constraints. The use of miswak on population bases is in line with the theme of primary health care approach (PHCA) and oral health promotion. Miswak has wider acceptance among many communities and populations around the world. (Areej and Khalid. , 2013)

Antioxidant capacity of chewing stick miswak *Salvadora persica* Background: Chewing stick (miswak *Salvadora persica.*) is an effective tool for oral hygiene. It possessed various biological properties including significant antibacterial and anti-fungal effects. In the present study, we evaluated the antioxidant compounds in miswak. Method: Miswak root was extracted with 80% methanol. Methanol extract as antioxidant was evaluated by using and phosphomolybdenum complex assays and analysis by GC-MS. Peroxidase, catalase and polyphenoloxidase assays were performed for crude extract of miswak root.

The reaparted that The methanol extract of miswak contained the highest amount of crude extract among the various solvent extracts. The methanol extract showed a concentration dependent scavenging of radicals with IC50 values 4.8 and 1.6 μg crude extract, respectively. The total antioxidant activities, based on the reduction of molybdenum to molybdenum, increased with increasing crude extract content. The correlation coefficients between total crude extract and scavenging activities and the formation of phosphomolybdenum complex were 0.97, 0.99 and 0.95, respectively. The GC-MS analysis showed that the methanol extract doesn't contain phenolic and flavonoid compounds or under detected limit. After silylation of methanol extract, three compounds namely 2-furancarboxaldehyde-5-(hydroxymethyl), furan-2-carboxylic acid-3-methyl- trimethylsilyl ester and Derythro-pentofuranose-2-deoxy-

1,3,5-tris-O-(trimethylsilyl) were identified by GC-MS analysis. These furan derivatives as they contain hydroxyl groups could be possessed antioxidant activities. The antioxidant enzymes were also detected in the miswak extract with high level of peroxidase and low level of catalase and polyphenoloxidase.

Conclusions: The synergistic actions of antioxidant compounds and antioxidant enzymes make miswak is a good chewing stick for oral hygiene and food purposes. (Saleh and Jalaluddin.,2013)

Studied effect of mouth wash extracted from *Salvadora persic* (Miswak) on dental plaque formation: clinical trail Chewing sticks or Miswak are used for teeth cleaning in many parts of the world, these Miswaks are believed to contain chemical substances which inhibit plaque formation and gingivitis. In the present study, *Salvadora persica* (Miswak) was extracted with 60% ethanol and was examined for its toxic effect, assessed its antibacterial activity and evaluated clinically for its effect on dental plaque formation. 4 day plaque regrowth, double - blind, crossover design was used in which 10 dental students volunteers were rendered plaque free (0.3), ceased tooth cleaning, then, asked to rinse twice daily for 1.5 min each time with 10 ml of chlorhexidine 0.2% mouth rinse and three times daily for 1.5 min each time with 10 ml of *S. persica* 10% solution and placebo mouth rinse. On day five, plaque was scored by the plaque index system. wash out period of 2 days was allowed in which the volunteers returned to self- performed plaque control, then a new test period was initiated. Statistical analysis showed that the mean score were 1.48 for *S. persica* mouth rinse, 0.48 for chlorhexidine and 2.07 for placebo mouth rinse. Acute toxicity test revealed no mortality among the experimental animals which is an indication that *S. persica* crude extract solution is well tolerated, disk diffusion test showed a marked antibacterial effect *in vitro* and this effect is concentration dependent, had an effect in *in vivo,* but this effect cannot be considered absolute. (AL.Bayaty *et al*. ,2010)

In an *in vivo* study, Sofrata *et al*. (2007) found that rinsing with *S. persica* miswak extract stimulated parotid gland secretion, thereby raising the plaque pH; this effect can potentially prevent dental caries by reversing the acid challenge of cariogenic bacteria. In Zanzibar, the caries prevalence rate was found to be lower in rural areas, where miswak use was customary, than in urban areas (Petersen and Mzee, 1998). A comparative study found that the aqueous extracts of miswak and derum (different type of chewing stick obtained from walnut tree *Juglans regia*) were both able to significantly inhibit the growth of cariogenic bacteria (Darmani et al., 2006). The large amounts of fluoride present in miswak maybe a contributing factor to this effect (Ezoddini-Ardakani, 2010).However, an earlier study found that this potential contribution of fluoride was doubtful, due to the negligible total soluble content of fluoride in *S. persica* miswak soaked in water (Hattab, 1997).

CHAPTER Three

3.1 MATERIALS AND METHODS

3.2 Plant material

Dried stems of miswak *Salvadora persica* well purchased from a localy planted in Aojala city, of liaby and identified by an agriculturist and the vendor according to their color and scent.

3.3 Preparation of *Salvadora Persica* Extracts

3.3.1 Aqueous extracts

3.3.1.1 Aqueous extracts (cool)

To prepare the aqueous extract, *Salvadora persica* chewing sticks were cut into small pieces and ground to powder form in a ball mill. The powder was weighed into 10 gm portions and placed in a sterile screw capped bottle to which 100 mL of sterile deionized distilled water was added. The extract was allowed to soak for 48 h at 4°C before the mixture was centrifuged at 2000 rpm for 10 min (Al-Lafi and Abadneh, 1995). The supernatant was passed through a 0.45 mm membrane filler. The extract stored in sterile screw-capped vials in the refrigerator until needed.

3.3.1.2 Aqueous extracts (hot)

To prepare the aqueous extract, *Salvadora persica* chewing sticks were cut into small pieces and ground to powder form in a ball mill. The powder was weighed into 10 gm portions and placed in a sterile screw capped bottle to which 100 mL of sterile deionized distilled water was boiled added. The extract was allowed to soak for 48 h at

4°C before the mixture was centrifuged at 2000 rpm for 10 min (Al-Lafi and Abadneh, 1995). The supernatant was passed through a 0.45 mm membrane filler. The extract stored in sterile screw-capped vials in the refrigerator until needed.

3.3.2 Ethanolic extract

Preparation of alcoholic extract of miswak was carried out by taking 800 g of *Salvadora Persica* chewing sticks and cutting them with a sharp knife. The resulting pieces of *Salvadora persica* were ground to a powder with a commercially available food blender. 120 mL of 60% ethanol was added to 40 g of powder in a sterile well capped flask, left for 3 days at room temperature and then filtered using number 1 filter paper. The extract then evaporated in a rotary evaporator at 40°C until ethanol removing. The extract stored in sterile screw-capped vials in the refrigerator until needed (Al-Koubaisi, 2001; Darmani *et al*., 2003)

3.4 Bacteria used

3.4.1 Bacteria isolated from mouth

for several persons from Banghazi hospital (wamen,men)

Staphylococcus aureus, *Streptococcus mutans*, *Streptococcus faecalis* ,*Streptococcus pyogenis*, *Enterococcus faecalis* and *Staphylococcus mutans* **.**

3.4.2 Bacteria cause infection of children(UTI)

Bacteria were taken from the laboratory of microbiology in hospital children. *Escherichia coli* , *Pseudomonas aeruginosa* ,*klebsiella pnemoniae algala* , *Staphylococcus albus and Streptococcus pnemoniae.*

3.4.3 Bacteria urinary tract infection

Bacteria were taken from the laboratory of microbiology in Banghazi medical center *Pseudomonas aeruginosa , Escherichia coli , Acinetobacter baumannii urine, proteus mirabilis algala and Staphylococcus epidrimidis urine*.

3.4.4 Bacteria multi drag resistance (MDR)

Bacteria were taken from the laboratory of microbiology in Banghazi medical center M*icococcus lylae,* B*acillus stearothermophilus, Escherichia coli , klebsiella pnemoniae algala and Acinetobacter baumannii urine.*

3.4.5 Bacteria infection after the operation

Bacteria were taken from the laboratory of microbiology in Banghazi medical center *Enterobacter aeruginosa, Pseudomonas aeruginosa , Staphylococcus aureus, klebsiella pnemoniae and Acinetobacter baumannii urine.*

The organisms were isolated and identified by standard methods, and identification confirmed by using Biochimical tests such as Gram stain ,Urease test, Catalaes ensym,Indol test ,Coagulase test, Oxidase test and Hydrogen sulphid produition .

3.5 Antibacterial activities of *Salvadora persica*

3.5.1 Preparation of *Salvadora persica* samples for testing

In this study work diluted of powdered stems *Salvadora persica* and The diluted were used Aqueous extract, ethanolic extract The diluted Aqueous extract was prepared by using sterile distilled water from to obtain 30%(v/v), 50%(v/v) and 100%(v/v) concentrations . and The diluted Methanol extracts was prepared by using ethanolic extract 60%(v/v) from to obtain 30%(v/v), 50%(v/v) and 100%(v/v) concentrations .

3.5.2 Inoculum preparation for testing

The inoculum was prepared by suspending a few colonies of the bacteria in test tubes that contain 9 ml of sterile normal saline .the turbidity of the bacterial suspensions was matched visually against the turbidity equivalent to the barium chloride standard ,which has a similar broth culture appearance.(Chauhan ,2010)

3.5.3 Antibacterial activity assay by Disc diffusion

A modified agar diffusion method was used to determine antimicrobial activity. Nutrient agar was inoculated with a microbial cell suspension (200 µl in 20 ml of medium) and poured into sterile petri dishes. Sterile filter paper discs 6 mm in diameter were impregnated with 20 µl of each extract concentration (30, 50 and 100 mg/ml), which were prepared using the same solvents employed to dissolve the plant extracts, and then sterilized via pasteurization and membrane filtration (regarding the aqueous extract), and placed on the inoculated agar surface. Standard 6-mm paper discs containing in distilled water. After pre-incubation for 2 h in a refrigerator the plates were incubated overnight at 37 °C for 18-24 h.. At the end of the incubation period antimicrobial activity was evaluated by measuring the zones of inhibition.

3.6 Minimal Inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC is defined as the lowest concentration of ethanolic , aqueous extracts that is able to inhibit the growth of bacteria. Mueller Hinton broth was employed for the determination of MIC in serial dilution tests tube preparation. Serial dilutions of the tow extracts samples were made in test tubes that containd contained 1 mL of Mueller Hinton broth medium to give a final concentration of 30,50 and 100 mg/mL . 20 µl of the test organisms (10^8 CFU⁄mL) was dispensed into the tubes. Negative

control tube just contained 1 mL of tow extracts but no organisms. Positive control tubes contained only 1 mL broth medium and each of the organisms but no tow extracts . The tubes were incubated at 37°C for 24 h. After incubation, turbidity of each tube was visually inspected. Clear test tube indicated break point . From the tubes showing no visible sign of growth/turbidity in MIC determination , test microorganisms were inoculated onto sterile nutrient agar plates by streak plate method . The plates were then incubated at 37°C for 24 h. The least concentration that did not show growth of test organisms was considered as the MBC (Mackie and McCartney, 1996).

3.7 Effects of commercially antibiotics against isolated bacteial

The effect of ethanolic extract, aqueous extract on the tested bacteria was compared with the sensitivity of the same bacteria to five antibiotics (Ampiclin , Amoxycillin, Erythromycin, , Streptomycin and Sulphamycin). Muller Hinton agar plates were inoculated by rubbing sterile cotton swabs after immerse 100 µl bacterial suspensions on plates (over night cultures grown at 37°C on nutrient agar and adjusted to 0.5 McFarland in sterile saline) over the entire surface of the plate media ,the antibiotics discs were placed on the surface of the medium, (a distance of 2-4mm between each discs) and incubated at 37 °C for 24 hours .At the end of 24 hours, the diameters of the resulting zones of inhibition were measured and the average values were recorded. (Mayrhofer *et al* ., 2008)

3.8 Statistical analysis

Bonferroni test was used for statistical analysis to show if there any significant difference ($P \leq 0.05$).

CHAPTER Four

4.1 Results

From the various available methods for testing antibacterial activity , the Disc -plate diffusion method (measurable inhibition zones),was used to determine the potency of aqueous and methanol extracts on for several species of human pathogenic bacteria.

4.2 Evaluations of the antibacterial potential of th Aquatic extract(Col) of *S. persica* stems used .

4.2.1The effects Aquatic extract against isolated from mouth bacteria(women)

The results of the assays of antibacterial activity of the Aquatic extract sample with three concentrations (3%, 50% and100% v/v) used in this study on bacteria isolated from mouth the are shown in Tables (1) and Fig. (5).

The effect of Aquatic extract neat Aquatic extract (100%) concentration inhibited the growth of *Enterobacter faecalis* with a mean of inhibition zone equal to 13.67mm in diameter . And the results was observed at concentration of (50%) the diluted Aquatic extract prevented the growth this bacterium with a mean of inhibition zone

equal to 13.00mm in diameter. Lower effect was observed at concentration of (30%) reach to 12.33mm in diameter, Fig. (5).

The effect of Aquatic extract neat Aquatic extract (100%) concentration inhibited the growth of *Staphylococcus aureus* with a mean of inhibition zone equal to 15.00mm in diameter. And the results was observed at concentration of (50%) the diluted Aquatic extract prevented the growth this bacterium with a mean of inhibition zone equal to 13.00mm in diameter. Lower effect was observed at concentration of (30%) reach to 10.67mm in diameter, Fig. (5).

The effect of Aquatic extract neat Aquatic extract (100%) concentration inhibited the growth of *Staphylococcus mutans* with a mean of inhibition zone equal to 11.33 mm in diameter. And the results was observed at concentration of (50%) the diluted Aquatic extract prevented the growth this bacterium with a mean of inhibition zone equal to 10.33mm in diameter. Lower effect was observed at concentration of (30%) reach to 10.00mm in diameter, Fig. (5).

The effect of Aquatic extract neat Aquatic extract (100%) concentration prevented the growth of *Streptococcus pyogenis* with a mean of inhibition zone equal to 21.33 mm in diameter. While, the diluted Aquatic extract inhibited the bacterial growth with a mean of inhibition zone equal to 14.33mm in diameter at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 12.67mm in diameter, Fig. (5).

When data are statistically analysid by using Two Anova, it was showed that there were significant differences of potency between the four types of bacterias used (P = .000) . And there were significant differences between the concentrations used (P=.000). And was Significant differences between the concentrations 30% 50% = 0.161, 30% 50% = 0.000 and 50% 100% =0.005 .

Table 1. Antibacterial effect of Aquatic extract of *S. persica* stems against isolated from mouth bacteria(women)**.**

Bacterias	**Concentrations**		
	30%	**50%**	**100%**
	Mean of inhibition zone(mm)		
Enterobacter faecalis	12.33	13.00	13.67
Staphylococcus aureus	10.67	13.00	15.00
Staphylococcus mutans	10.00	10.33	11.33

Streptococcus pyogenis	12.67	14.33	21.33

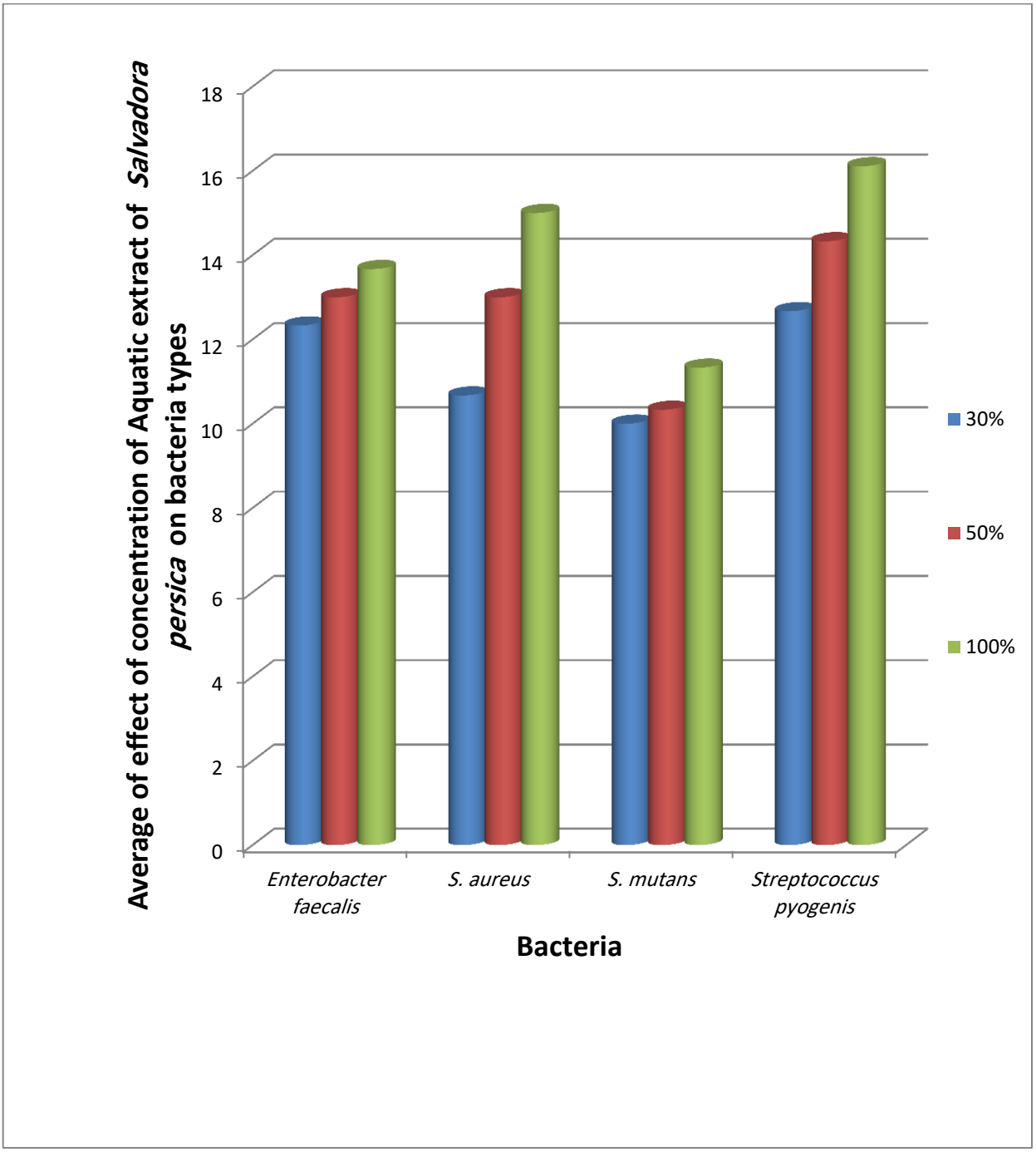


Fig. (5) Mean inhibition zone of Aqueous extract against bacteria used

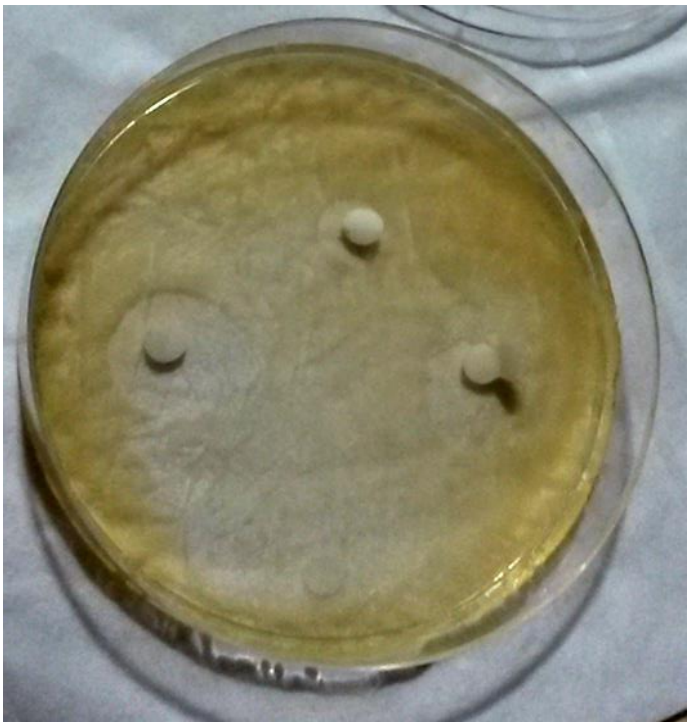


Fig. (6) The inhibition zone by using 30% cocentration Aquatic extract against bacteria *Staphylococcus mutans*

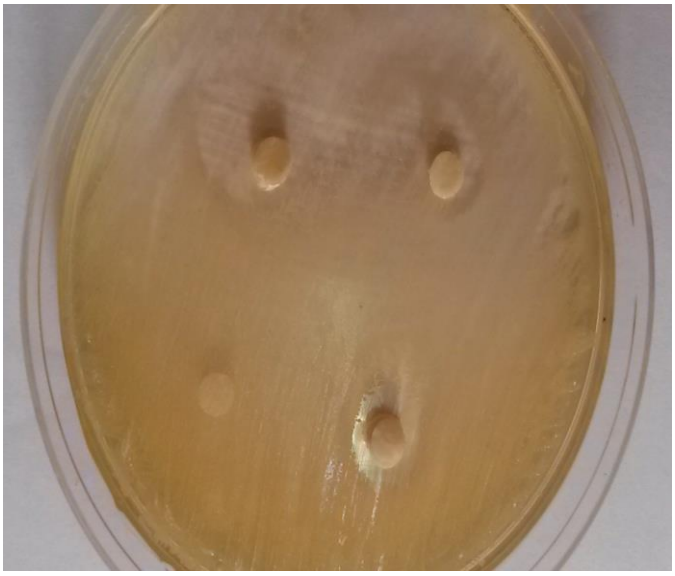


Fig. (7) The inhibition zone by using 50% concentration of Aquatic extract against bacteria *Streptococcus pyogenis*

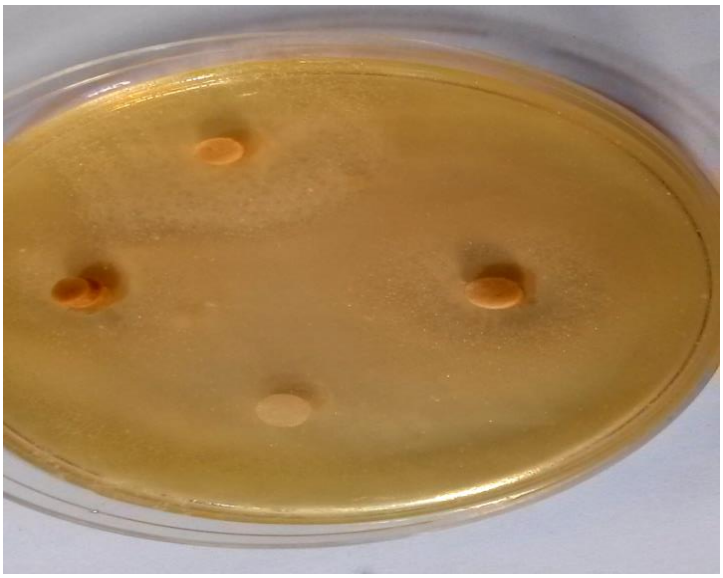


Fig. (8) The inhibition zone by using 100% cocentration of Aquatic extract on bacteria *Staphylococcus aureus* 100%

4.2.2 The effects of Aquatic extract against isolated from mouth bacteria (Men)

The results of the assays of antibacterial activity of the Aquatic extract sample with three concentrations (3%, 50% and100% v/v) used in this study on bacteria isolated from mout the are shown in Tables (2), Fig. (9) .

The effect of Aquatic extract neat Aquatic extract (100%) and(50%) Similar results was observed at concentration inhibited the growth of *Streptococcus pyogenis* with a mean of inhibition zone equal to 15.00 mm in diameter.and concentration (30%) reach to 12.00mm in diameter. Fig. (9).

The effect of Aquatic extract neat Aquatic extract (100%) concentration prevented the growth of *Staphylococcus aureus* with a mean of inhibition zone equal to 13.33 mm in diameter. While, the diluted Aquatic extract inhibited the bacterial growth with a mean of inhibition zone equal to 12.33mm in diameter at (50%) concentration to ,

and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 11.00mm in diameter, Fig. (9).

The effect of Aquatic extract neat Aquatic extract (100%) concentration inhibited the growth of *Streptococcus mutans* with a mean of inhibition zone equal to 12.67mm in diameter While, the diluted Aquatic extract inhibited the bacterial growth with a mean of inhibition zone equal to 11.67mm in diameter. at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 10.67mm in diameter, Fig. (9).

The effect of Aquatic extract neat Aquatic extract (100%) concentration inhibited the growth of *Streptococcus facalis* with a mean of inhibition zone equal to 14.00mm in diameter While, the diluted Aquatic extract inhibited the bacterial growth with a mean of inhibition zone equal to 11.33mm in diameter. at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 11.00mm in diameter, Fig. (9).

When data are statistically analysid by using Two Anova, it was showed that there were no significant differences of potency between the four types of bacterias used (P = .0.26) . And there were significant differences between the concentrations used (P=.002) .and was Significant differences between the concentrations 30% 50% = 0.041, 30% 50% = 0 .001 and 50% 100% =0.008,

Table 2. Antibacterial effect of Aquatic extracts of *S. persica* stems against isolated from mouth bacteria (men)

Bacterias	**Concentrations**		
	30%	**50%**	**100%**
	Mean of inhibition zone(mm)		
Streptococcus pyogenis	12.00	15.00	15.00
Staphylococcus aureus	11.00	12.33	13.33

Streptococcus mutans	10.67	11.67	12.67
Streptococcus facalis	11.00	11.33	14.00

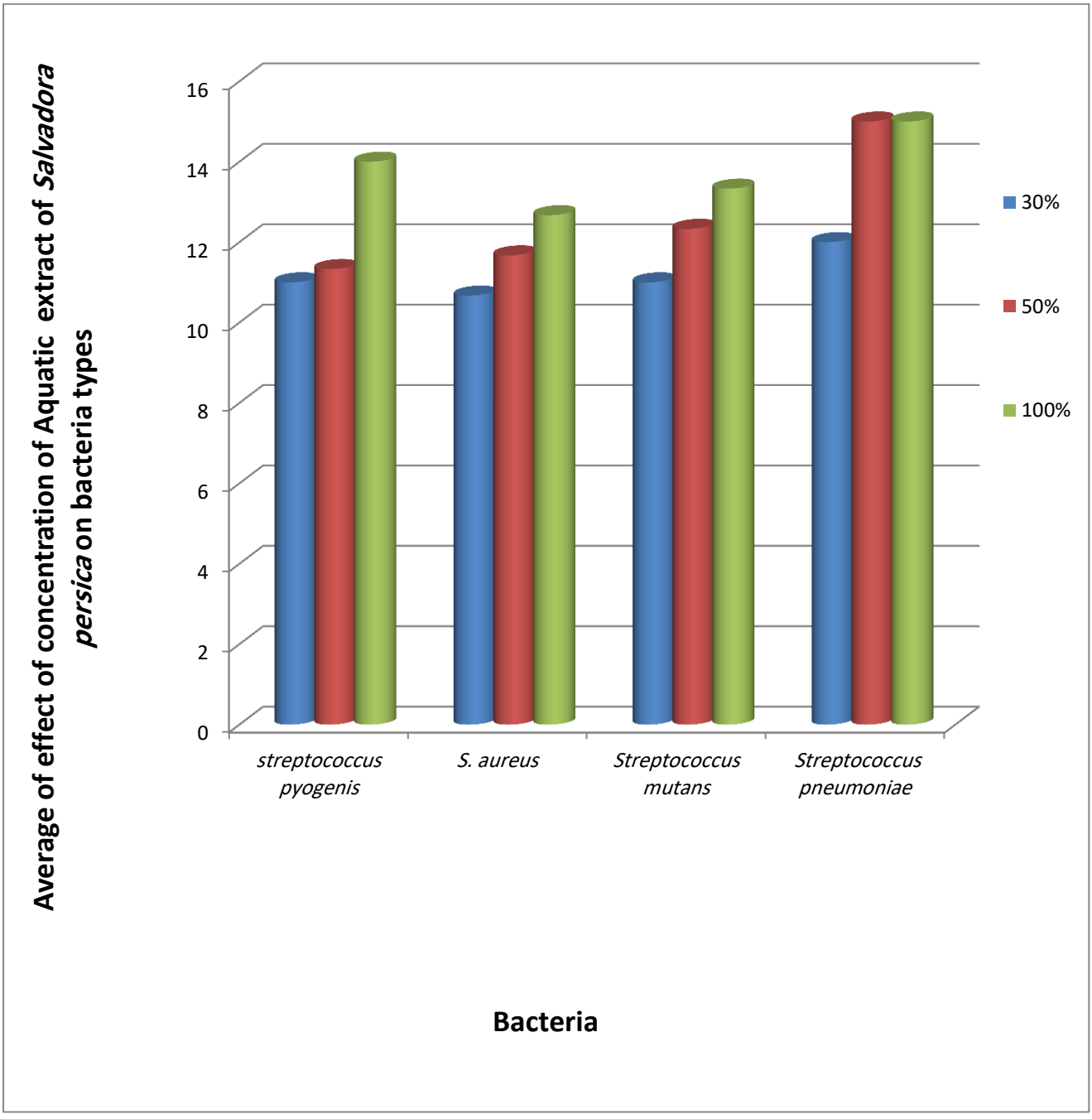


Fig. (9) Mean inhibition zone of Aquatic extract against bacteria .

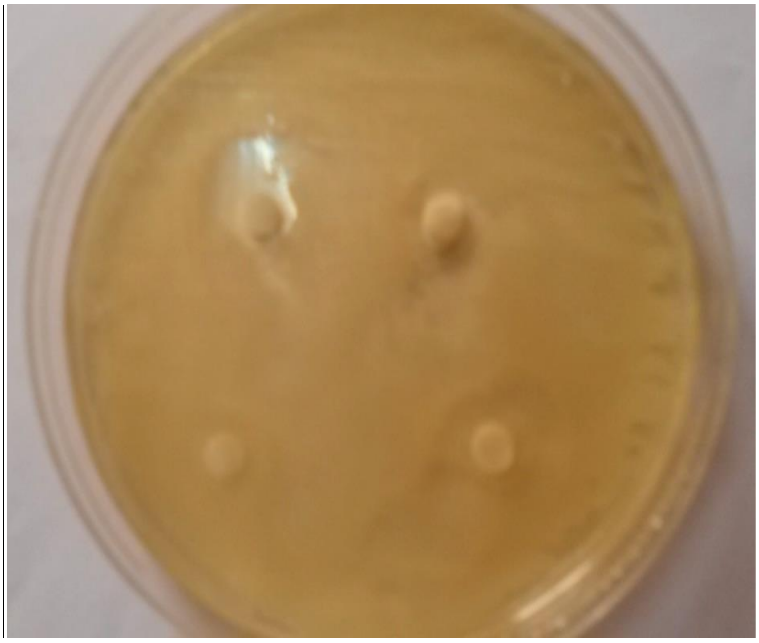


Fig. (10) The inhibition zone by using 30%cocentration of Aquatic extract against bacteria *Staphylococcus aureus*

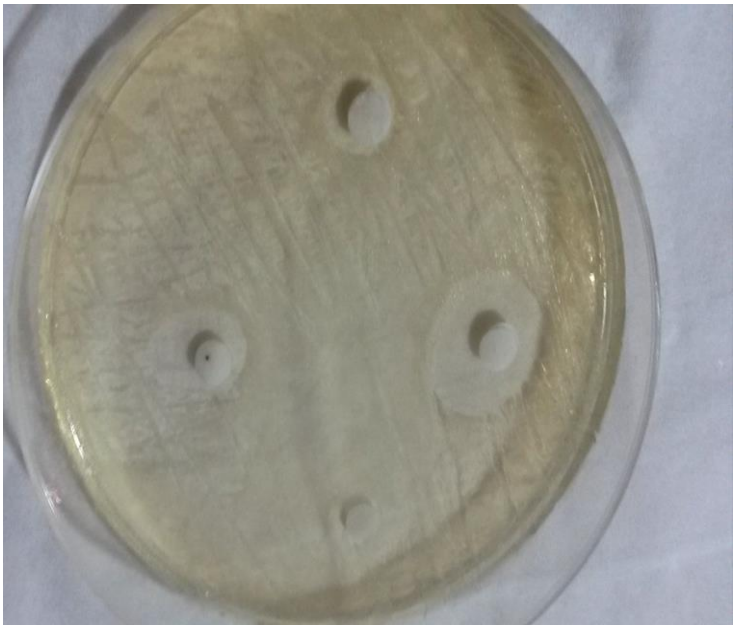


Fig. (11) The inhibition zone by using50% concentration of Aquatic extract against bacteria *Streptococcus pyogenis*

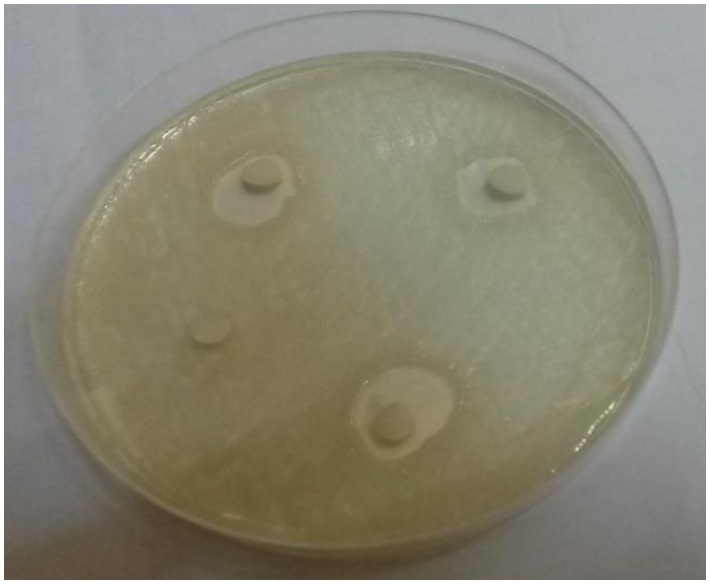


Fig. (12) The inhibition zone by using 100% concentration of Aquatic extract against bacteria *Streptococcus facalis*

4.2.3 The effects of Aquatic extract against cause infection of children(UTI) bacteria

The results of the assays of antibacterial activity of the Aquatic extract sample with three concentrations (3%, 50% and100% v/v) used in this study on bacteria cause infection of chiidren the are shown in Tables (3), Fig. (13).

The effect of Aquatic extract neat Aquatic extract (100%) concentration inhibited the growth of *Pseudomoens aeruginosa* with a mean of inhibition zone equal to12.33mm in diameter . And concentration of (50%) , the diluted Aquatic extract prevented the growth this bacterium with a mean of inhibition zone equal to 11.67mm in diamete. And Lower effect was observed at concentration of(30%) the mean of inhibition zone was equal to 11.33mm in diameter, Fig. (13).

The effect of Aquatic extract neat Aquatic extract (100%) concentration prevented the growth of *Staphylococcus albus* with a mean of inhibition zone equal to 17.00 mm in diameter. While, the diluted Aquatic extract inhibited the bacterial growth with a mean of inhibition zone equal to 14.33mm in diameter at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 14.00mm in diameter, Fig. (13).

The effect of Aquatic extract neat Aquatic extract (100%) concentration prevented the growth of *Klebsiella pneumonia* with a mean of inhibition zone equal to 14.67 mm in diameter. While, the diluted Aquatic extract inhibited the bacterial growth with a mean of inhibition zone equal to 14.33mm in diameter at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 9.67mm in diameter, Fig. (13).

The effect of Aquatic extract neat Aquatic extract (100%) concentration inhibited the growth of *Escherichia coli* with a mean of inhibition zone equal to 14.00mm in

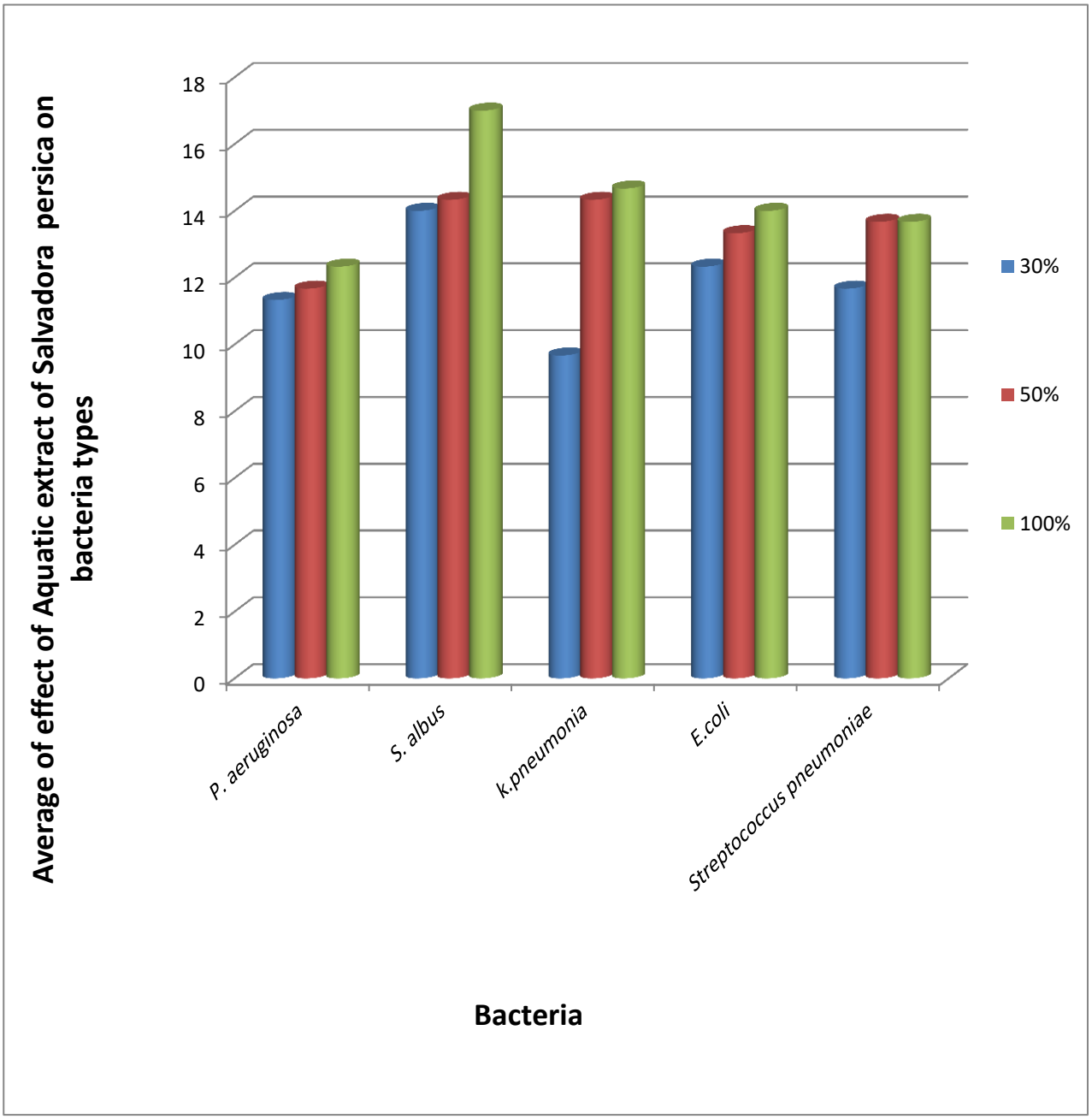
diameter While, the diluted Aquatic inhibited the bacterial growth with a mean of inhibition zone equal to 13.33mm in diameter. at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 12.33mm in diameter, Fig. (13).

The effect of Aquatic extract neat Aquatic extract (100%) and(50%) Similar results was observed at concentration inhibited the growth of *Streptococcus pneumonia* with a mean of inhibition zone equal to 13.67 mm in diameter.and concentration (30%) reach to 11.67mm in diameter. Fig. (13)

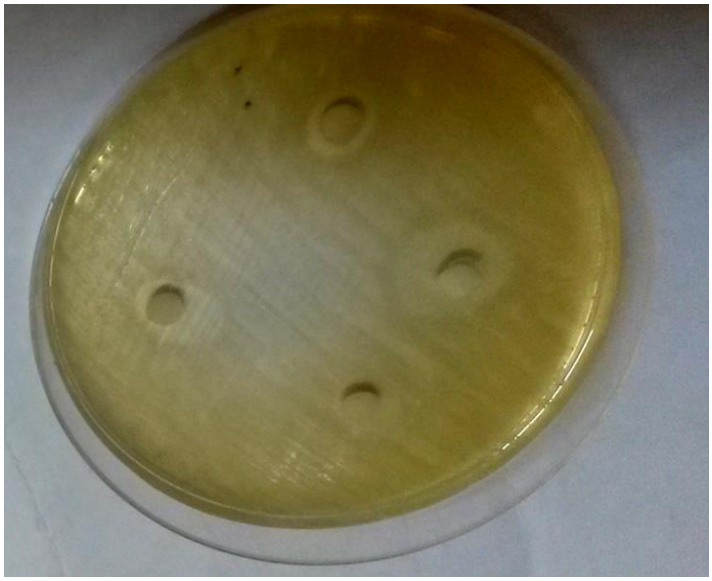
When data are statistically analysid by using Two Anova, it was showed that there were significant differences of potency between the four types of bacterias use (P = .277) . And there were significant differences between the concentrations used (P=.095) and was mean Significant differences between the concentrations 30% 50% = ----, 30% 100% = ----, ----50% 100% = and

Table 3.Antibacterial effect of Aquatic extracts of *S. persica* stems against cause infection of children(UTI) bacteria

Bacterias	Concentrations		
	30%	**50%**	**100%**
	Mean of inhibition zone(mm)		
Pseudomoens aeruginosa	11.33	11.67	12.33
Staphylococcus albus	14.00	14.33	17.00
Klebsilla pneumonia	9.67	14.33	14.67
Escherichia Coli	12.33	13.33	14.00
Streptococcus pneumonia	11.67	13.67	13.67



Fig(13) Mean inhibition zone of Aquatic extract against bacteria used.



Fig(14) The inhibition zone by using 30%concentration of Aquatic extract against bacteria *Escherichia Coli*

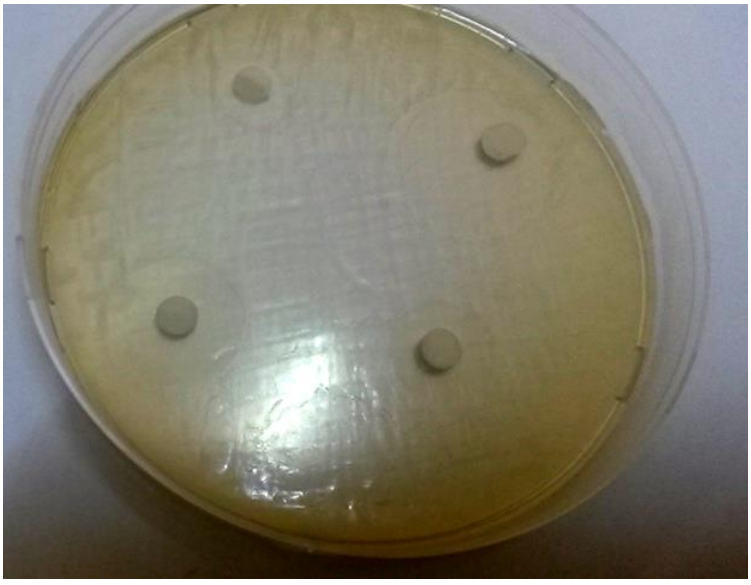


Fig .(14) The inhibition zone by using50% cocentration of Aquatic extract against bacteria *Streptococcus pneumonia*

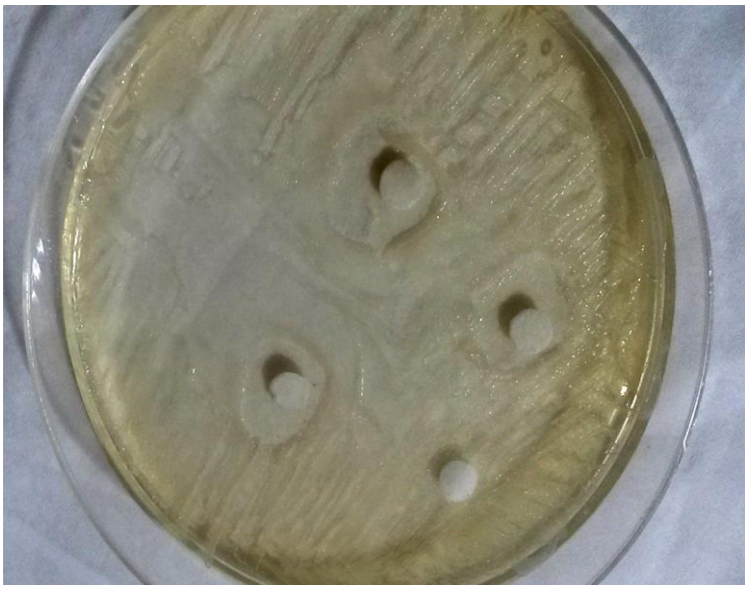


Fig. (15) The inhibition zone by using 100% concentration of Aquatic extract against bacteria *Klebsilla pneumonia*

4.2.4 The effects of Aquatic extract against urinary tract infection bacteria

The results of the assays of antibacterial activity of the Aquatic extract sample with three concentrations (3%, 50% and100% v/v) used in this study on bacteria urinary tract infection the are shown in Tables (4)and Fig. (16).

The effect of Aquatic extract neat Aquatic extract (100%) concentration inhibited the growth of *Pseudomoens aeruginosa* with a mean of inhibition zone equal to15.67mm in diameter . And concentration of (50%) , the diluted Aquatic extract prevented the growth this bacterium with a mean of inhibition zone equal to 12.00mm in diamete. And Lower effect was observed at concentration of(30%) the mean of inhibition zone was equal to 10.33mm in diameter, Fig. (16).

The effect of Aquatic extract neat Aquatic extract (100%) and(50%) Similar results was observed at concentration inhibited the growth of *Staphyococcus epidirmidis* with a mean of inhibition zone equal to 13.33 mm in diameter.and concentration (30%) reach to 10.33mm in diameter. Fig. (16).

The effect of Aquatic extract neat Aquatic extract (100%) concentration inhibited the growth of *Escherichia coli* with a mean of inhibition zone equal to 12.33mm in diameter While, the diluted Aquatic extract inhibited the bacterial growth with a mean of inhibition zone equal to 10.67mm in diameter. at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 9.67mm in diameter, Fig. (16).

The effect of Aquatic extract neat Aquatic extract (100%) concentration prevented the growth of *Acientobacter baumannii* with a mean of inhibition zone equal to 15.33 mm in diameter. While, the diluted Aquatic extract inhibited the bacterial growth with a mean of inhibition zone equal to 13.33mm in diameter at (50%) concentration to and

the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 12.67mm in diameter, Fig. (16).

The effect of Aquatic extract neat Aquatic extract (100%) concentration inhibited the growth of *Proteus mirabilis* with a mean of inhibition zone equal to 17.67mm in diameter . And the results was observed at concentration of (50%) the diluted Aquatic extract prevented the growth this bacterium with a mean of inhibition zone equal to 11.00mm in diameter. Lower effect was observed at concentration of (30%) reach to 10.67mm in diameter, Fig. (16).

When data are statistically analysid by using Two Anova, it was showed that there were significant differences of potency between the five types of bacterias used (P = .026) . And there were significant differences between the concentrations used (P=.002) .and was mean Significant differences between the concentrations 30% 50% = 0 .043, 30% 100% = 0.000 and 50% 100% =0.000 .

Table 4. Antibacterial effect of Aquatic extracts of *S. persica* stems against urinary tract infection bacteria.

Bacterias	**Concentrations**		
	30%	**50%**	**100%**
	Mean of inhibition zone(mm)		
Psedumonas aeruginosa	10.33	12.00	15.67
Staphyococcs epidirmidis	10.33	13.33	13.33
Escherichia coli	9.67	10.67	12.33
Acientobacter baumannii	12.67	13.33	15.33
Proteus mirabilis	10.67	11.00	17.67

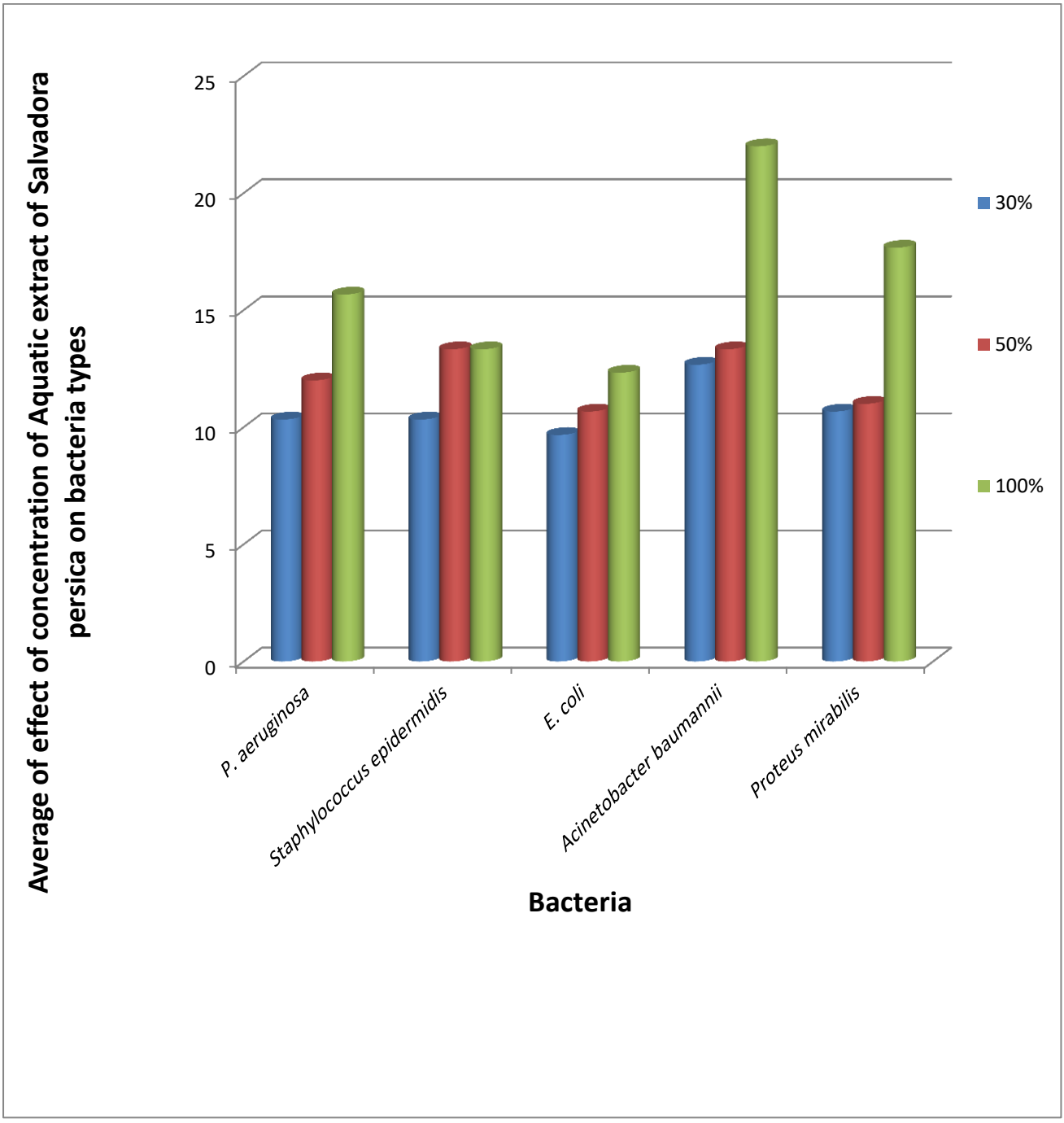


Fig. (16) Mean inhibition zone of Aquatic extract bacteria used.

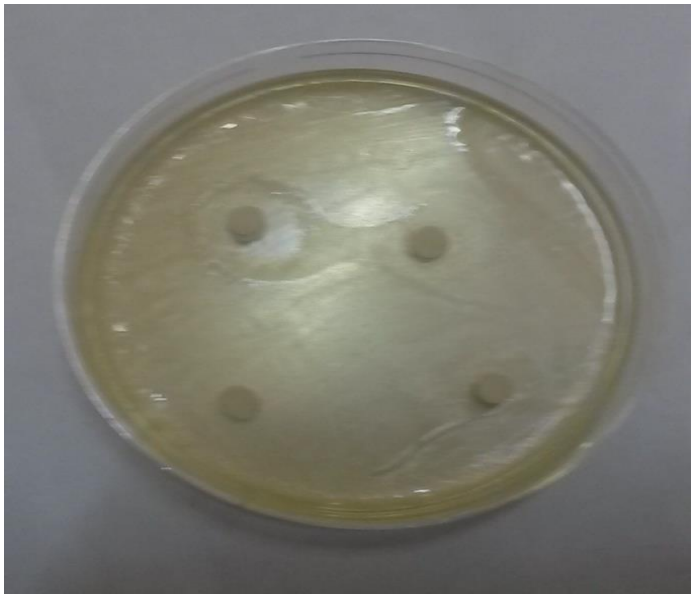


Fig. (17) The inhibition zone by using 30% concentration of Aquatic extract against bacteria *Proteus mirabilis*

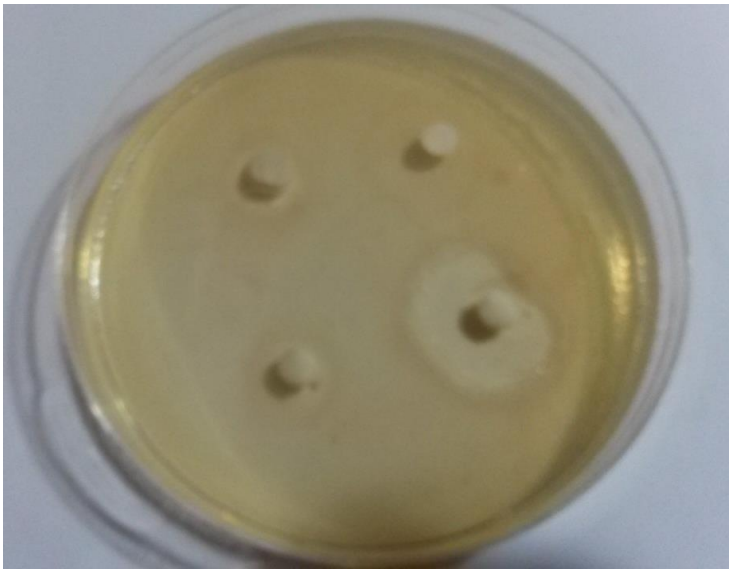


Fig. (18) The inhibition zone by using50% concentration of Aquatic extract against bacteria *Staphyococcus epidirmidis*

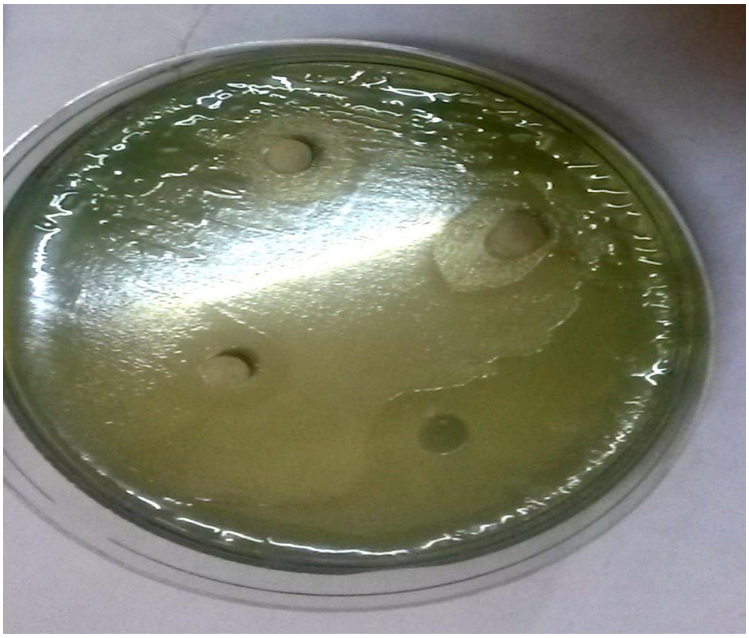


Fig. (19) The inhibition zone by using 100 % concentration of Aquatic extract against bacteria *Psedumonas aeruginosa*

4.2.5The effects of Aquatic extract against multi drag resistance bacteria (MDR)

The results of the assays of antibacterial activity of the Aquatic extract sample with three concentrations (3%, 50% and100% v/v) used in this study on bacteria multi drag resistance (MDR) the are shown in Tables (5) and Fig. (20).

The effect of Aquatic extract neat Aquatic extract (100%) concentration inhibited the growth of *Klebsiella Pneumonia* with a mean of inhibition zone equal to16.00mm in diameter . And concentration of (50%) , the diluted Aquatic extract prevented the growth this bacterium with a mean of inhibition zone equal to 15.67mm in diamete. And Lower effect was observed at concentration of(30%) the mean of inhibition zone was equal to 13.67mm in diameter, Fig. (20).

The effect of Aquatic extract neat Aquatic extract (100%) concentration inhibited the growth of *Escherichia coli* with a mean of inhibition zone equal to 15.67 mm in diameter. Similar results was observed at concentration (50%) and (30%) reach to 14.00mm in diameter. Fig. (20).

The effect of Aquatic extract neat Aquatic extract (100%) concentration prevented the growth of *Bacillus stearotherrmophilus* with a mean of inhibition zone equal to 20.67 mm in diameter. While, the diluted Aquatic extract inhibited the bacterial growth with a mean of inhibition zone equal to 15.00mm in diameter at (50%) concentration , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 14.00mm in diameter, Fig. (20).

The effect of Aquatic extract neat Aquatic extract (100%) concentration prevented the growth of *Acientobacter baumanniiwith* a mean of inhibition zone equal to 16.33 mm in diameter. While, the diluted Aquatic extract inhibited the bacterial growth with a mean of inhibition zone equal to 14.67mm in diameter at (50%) concentration to ,

and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 11.33mm in diameter, Fig. (20).

The effect of Aquatic extract neat Aquatic extract (100%) concentration inhibited the growth of *Micrococcus lylae* with a mean of inhibition zone equal to 17.67mm in diameter . While, the diluted Aquatic extract inhibited the bacterial growth with a mean of inhibition zone equal to 12.33mm in diameter at (50%) concentration , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 11.33mm in diameter, Fig. (20).

When data are statistically analysid by using Two Anova, it was showed that there were significant differences of potency between the five types of bacterias used (P = .429) . And there were significant differences between the concentrations used (P=.003) .and was mean Significant differences between the concentrations 30% 50% = 0.232, 30% 100% = 0.001 and 50% 100% = 0 .021

Table 5. Antibacterial effect of Aquatic extracts of *S. persica* stems agasint multi drag resistance bacteria (MDR) .

Bacterias	**Concentrations**		
	30%	**50%**	**100%**
	Mean of inhibition zone(mm)		
Klebsiella Pneumonia	13.67	15.67	16.00
Escherichia Coli	14.00	14.00	15.67
Bacillus stearotherrmophilus	14.00	15.00	20.67
Acinetobacter baumannii	11.33	14.67	16.33
Micrococcus lylae	11.33	12.33	17.67

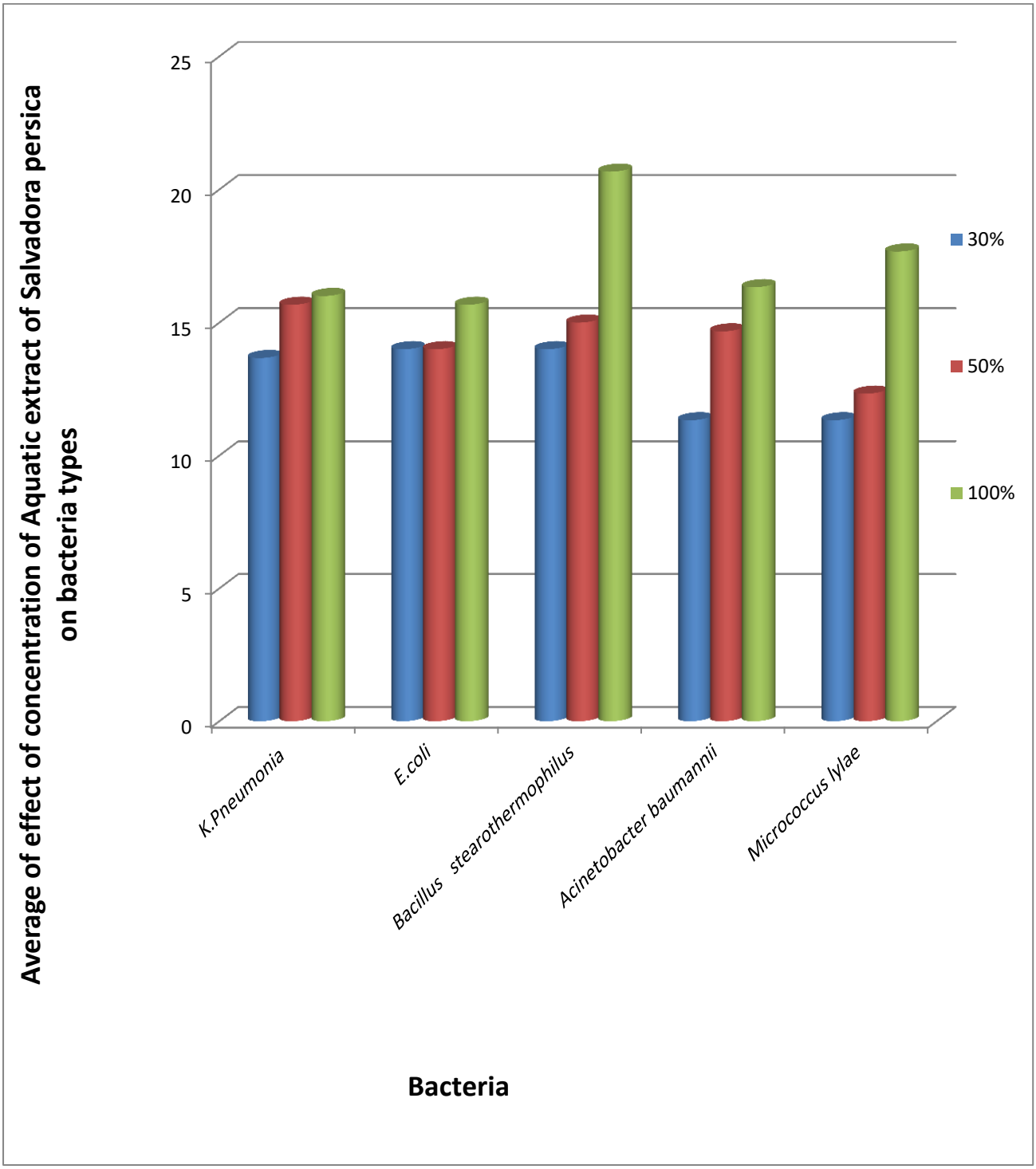


Fig. (20) Mean inhibition zone of Aquatic extract against bacteria used.

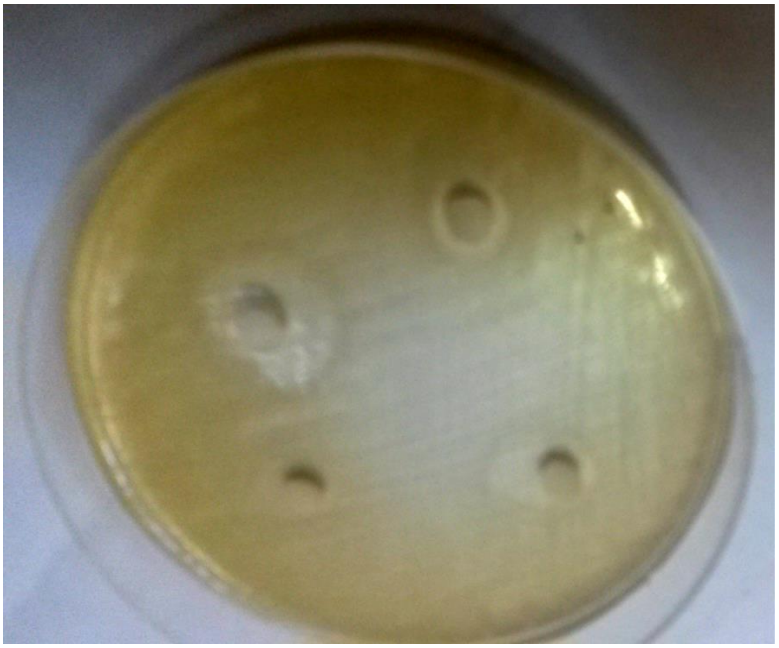


Fig. (21) The inhibition zone by using 30% cocentration of Aqueous extract against bacteria *Escherichia Coli*

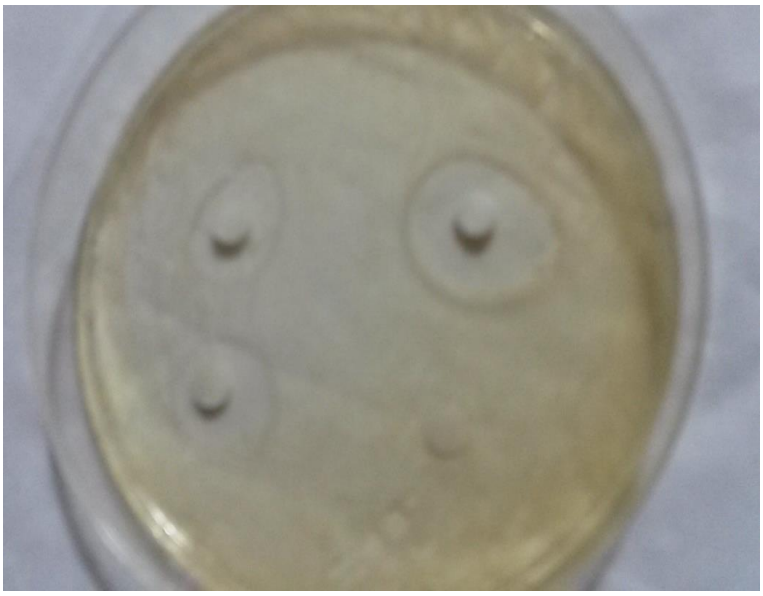


Fig. (22) The inhibition zone by using 50% cocentration of Aqueous extract against bacteria *Klebsiella Pneumonia*

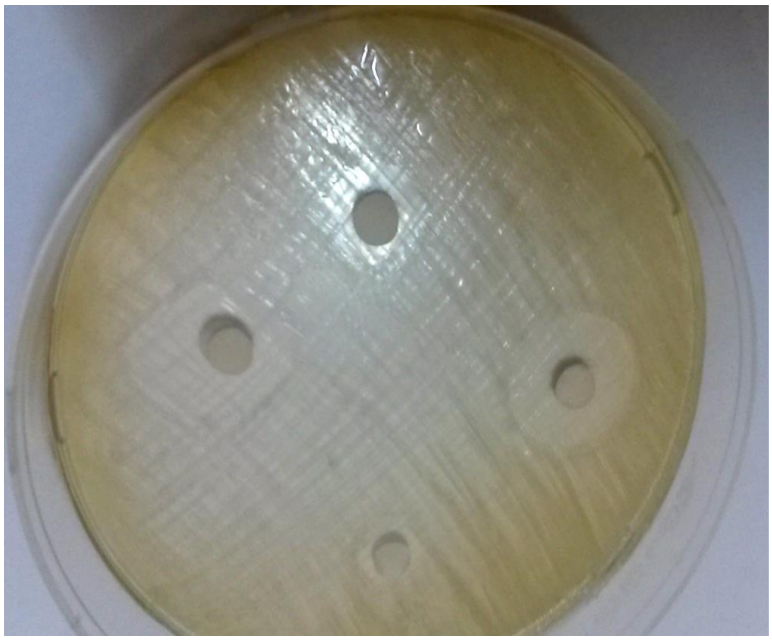


Fig. (23) The inhibition zone by using 100% cocentration of Aqueous extract against bacteria *Acinetobacter baumannii*

4.2.6The effects of Aquatic extract against caus infection after the operation bacteria.

The results of the assays of antibacterial activity of the Aquatic extract sample with three concentrations (30%, 50% and100% v/v) used in this study on bacteria caus infection after the operation the are shown in Tables (6) and Fig. (24).

The effect of Aquatic extract neat Aquatic extract (100%) concentration inhibited the growth of *Psedumoens aeruginosa* with a mean of inhibition zone equal to14.00mm in diameter . And concentration of (50%) , the diluted Aquatic extract prevented the growth this bacterium with a mean of inhibition zone equal to 13.67mm in diamete. And Lower effect was observed at concentration of(30%) the mean of inhibition zone was equal to 10.67mm in diameter, Fig. (24).

The effect of Aquatic extract neat Aquatic extract (100%) concentration inhibited the growth of *Enterobacter aeruginosa* with a mean of inhibition zone equal to 12.67mm in diameter While, the diluted Aquatic extract inhibited the bacterial growth with a mean of inhibition zone equal to 12.33mm in diameter. at (50%) concentration , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 11.33mm in diameter, Fig. (24).

The effect of Aquatic extract neat Aquatic extract (100%) concentration prevented the growth of *Staphylococcus aureus* with a mean of inhibition zone equal to 14.67 mm in diameter. While, the diluted Aquatic extract inhibited the bacterial growth with a mean of inhibition zone equal to 13.33mm in diameter at (50%) concentration , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 12.33mm in diameter, Fig. (24).

The effect of Aquatic extract neat Aquatic extract (100%) and (50%) concentration inhibited the growth of *Klebsiella pneumonia* with a Similar results was observed at mean of inhibition zone equal to 14.00mm in diameter And Lower effect was observed at concentration of(30%) the mean of inhibition zone was equal to 13.33mm in diameter . Fig. (24)

The effect of Aquatic extract neat Aquatic extract (100%) concentration prevented the growth of *Acientobacter baumannii with* a mean of inhibition zone equal to 17.00 mm in diameter. While, the diluted Aquatic extract inhibited the bacterial growth with a mean of inhibition zone equal to 14.33mm in diameter at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 12.67mm in diameter, Fig. (24).

When data are statistically analysid by using Two Anova, it was showed that there were non significant differences of potency between the five types of bacterias used. And there were non significant differences between the concentrations used

30% 50% = ----, 30% 100% = ----and 50 % 100% = ---- .

Table 6. Antibacterial effect of Aquatic extracts of *S. persica* stems against caus infection after the operation bacteria.

Bacterias	**Concentrations**		
	30%	**50%**	**100%**
	Mean of inhibition zone(mm)		
Psedumoens aeruginosa	10.67	13.67	14.00
Enterobacter aeruginosa	11.33	12.33	12.67
Staphylococcus aureus	12.33	13.33	14.67
Klebsiella pneumonia	13.33	14.00	14.00
Acinetobacter baumannii	12.67	14.33	17.00

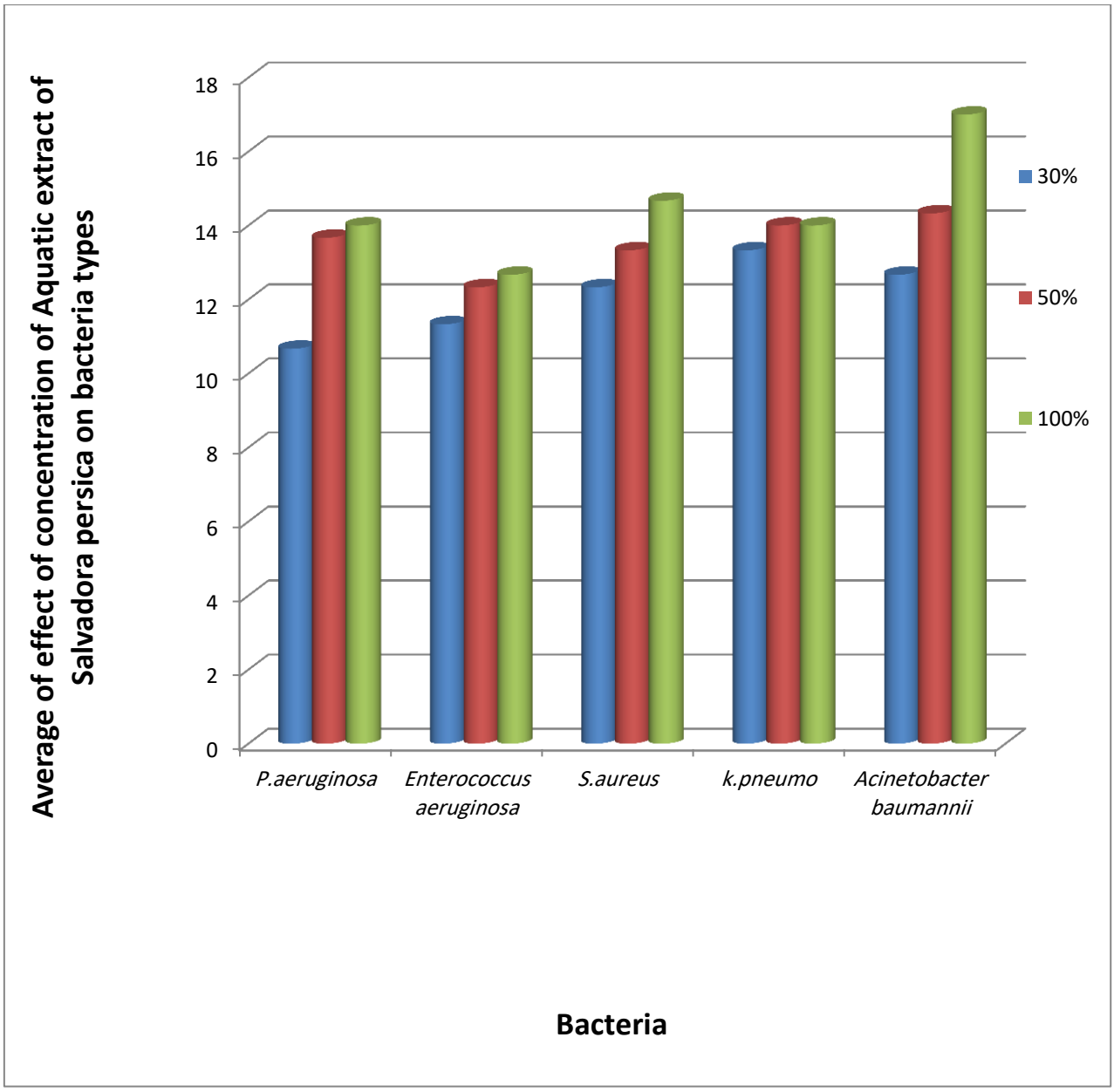


Fig. (24) Mean inhibition zone of Aquatic extract against bacteria used.

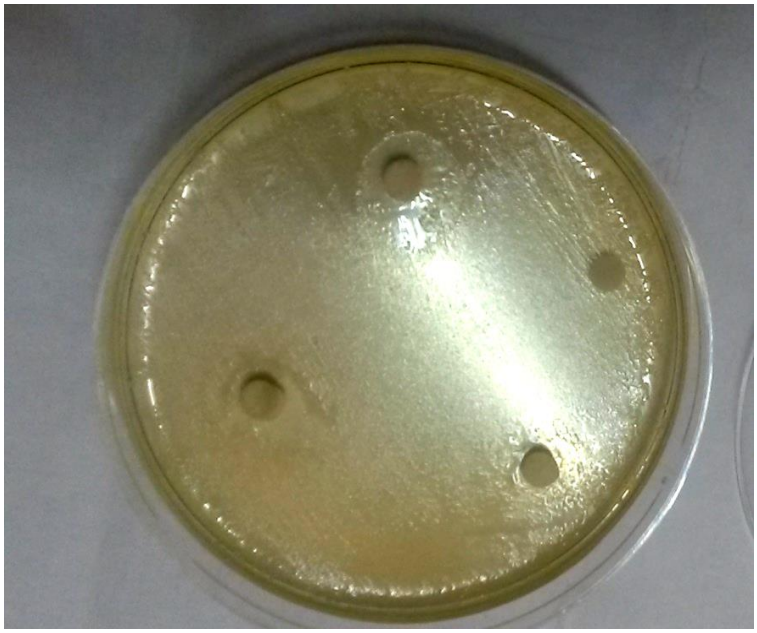


Fig. (25) The inhibition zone by using 30% concentration of Aquatic extract against bacteria *Enterobacter aeruginosa* 30%

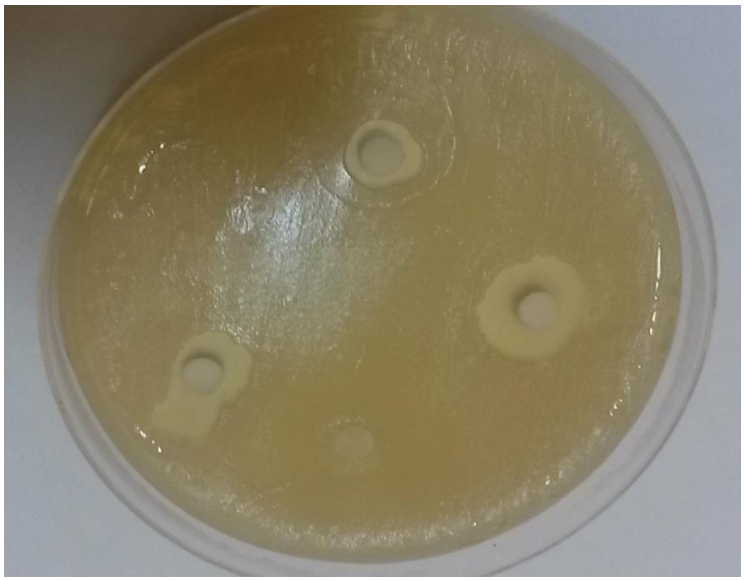


Fig.(26) The inhibition zone by using 50% concentration of Aquatic extract against bacteria *Acinetobacter baumannii*

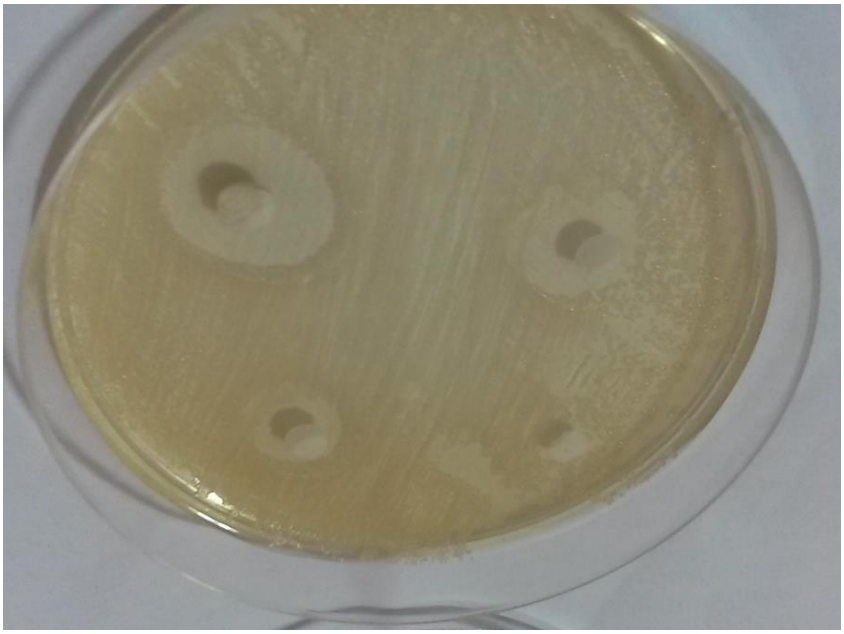


Fig.(27) The inhibition zone by using 100% concentration of Aquatic extract againt bacteria *Staphylococcus aureus*

4.3 Evaluations of the antibacterial potential of the Aquatic extract(Hot) of *S. persica* stems used .

The results of the assays of antibacterial activity of the Aquatic extract (Hot) in all bacteria was negative there is no inhibition of abstract .

4.4 Evaluations of the antibacterial potential of the Ethanolic extract of *S. persica* stems used .

4.4.1 The effects of Ethanolic extract against isolated from mouth bacteria (women)

The results of the assays of antibacterial activity of the ethanolic extract sample with three concentrations (3%, 50% and100% v/v) used in this study on bacteria isolated from mout are shown in Tables (7) and Fig. (28).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration inhibited the growth of *Enterobacter faecalis* with a mean of inhibition zone equal to 17.33mm in diameter . And the results was observed at concentration of (50%) the diluted Ethanolic extract prevented the growth this bacterium with a mean of inhibition zone equal to 16.33mm in diameter. Lower effect was observed at concentration of (30%) reach to 14.67mm in diameter, Fig. (28).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration inhibited the growth of *Staphylococcus aureus* with a mean of inhibition zone equal to 17.00mm in diameter. Similar results was observed at concentration (50%) and (30%) reach to 15.00mm in diameter. Fig. (28).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration inhibited the growth of *Staphylococcus mutans* with a mean of inhibition zone equal to 19.00 mm in diameter. Similar results was observed at concentration (50%) and (30%) reach to 17.67mm in diameter. Fig. (28).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration prevented the growth of *Streptococcus pyogenis* with a mean of inhibition zone equal to 20.00 mm in diameter. While, the diluted Ethanolic extract inhibited the bacterial growth with a mean of inhibition zone equal to 19.00mm in diameter at (50%)

concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 12.67mm in diameter, Fig. (28).

When data are statistically analysid by using Two Anova, it was showed that there were significant differences of potency between the four types of bacterias used (P = 0.155) . And there were significant differences between the concentrations used (P=.008) .and was mean Significant differences between the concentrations 30% 50% =0 .052, 30% 100%=0.002 and 50% 100% = 0.186 .

Table 7. Antibacterial effect of ethanolic extract of *S. persica* stems against isolated from mouth bacteria(woman)

Bacterias	**Concentrations**		
	30%	**50%**	**100%**
	Mean of inhibition zone(mm)		
Enterobacter faecalis	14.67	16.33	17.33
Staphylococcus aureus	15.00	15.00	17.00
Staphylococcus mutans	17.67	17.67	19.00
Streptococcus pyogenis	12.67	19.00	20.00

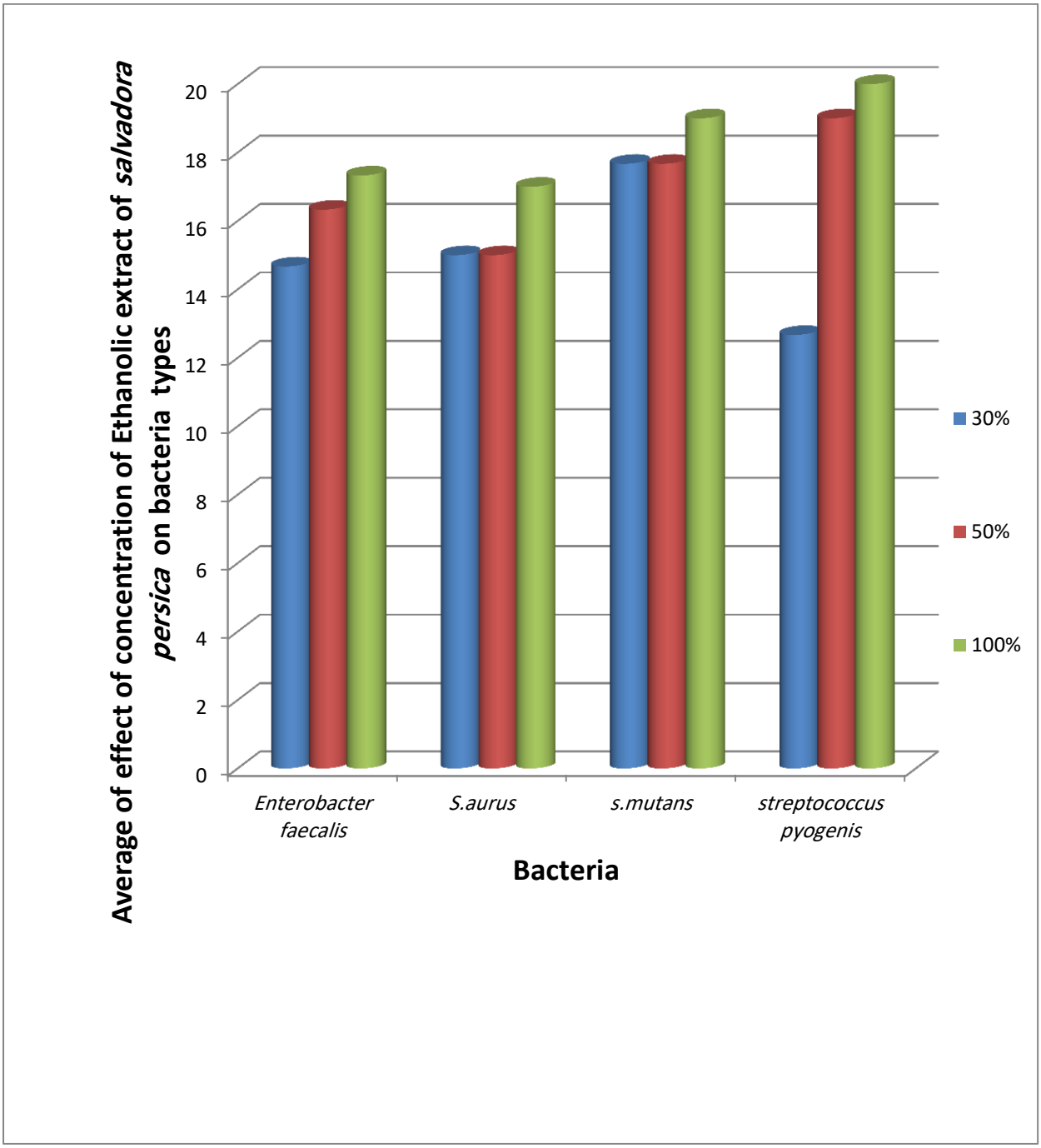


Fig. (28) Mean inhibition zone of Ethanolic extract against bacteria used

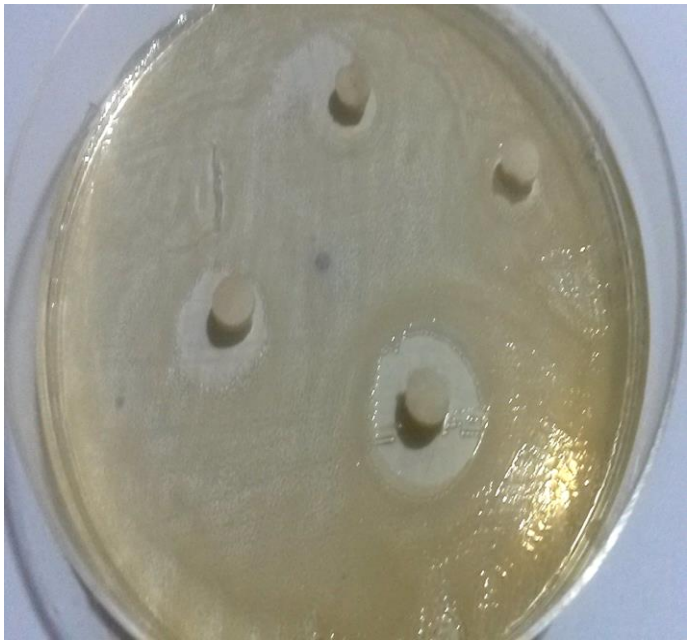


Fig. (29) The inhibition zone by using 30% cocentration of Ethanolic extract against bacteria *Staphylococcus aureus*

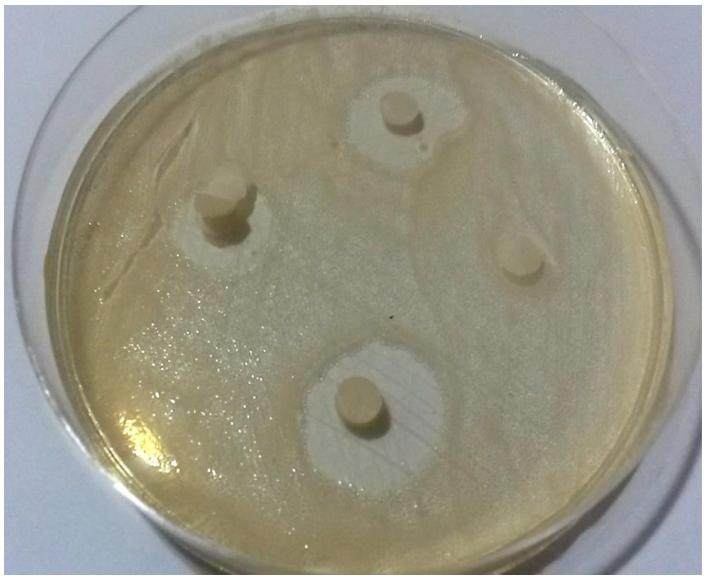


Fig. (30) The inhibition zone by using 50% cocentration of Ethanolic extract against bacteria *Streptococcus pyogenis*

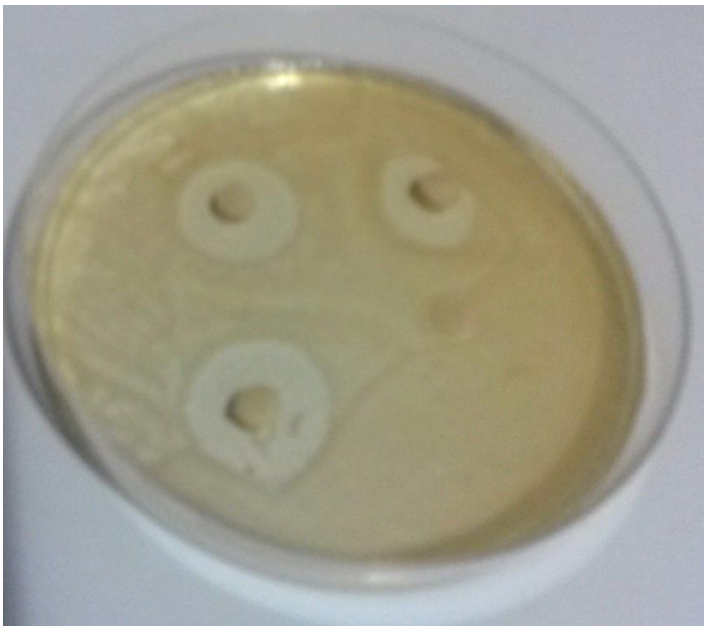


Fig. (31) The inhibition zone by using 100% cocentration of Ethanolic extract against bacteria *Staphylococcus mutans*

4.4.2 The effects of Ethanolic extract against isolated from mouth bacteria (men)

The results of the assays of antibacterial activity of the ethanolic extract sample with three concentrations (3%, 50% and100% v/v) used in this study on bacteria isolated from mout the are shown in Tables (8), Fig. (32).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration inhibited the growth of *Streptococcus pyogenis* with a mean of inhibition zone equal to 17.00mm in diameter . And the results at concentration of (50%) , the diluted Ethanolic extract prevented the growth this bacterium with a mean of inhibition zone equal to 16.33mm in diameter. Lower effect was observed at concentration of(30%) reach to 15.67mm in diameter, Fig. (32).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration prevented the growth of *Staphylococcus aureus* with a mean of inhibition zone equal to 18.00 mm in diameter. While, the diluted Ethanolic extract inhibited the bacterial growth with a mean of inhibition zone equal to 15.67mm in diameter at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 15.00mm in diameter, Fig. (32).

The effect of Ethanolic extract neat Ethanolic extract (100%) and(50%) Similar results was observed at concentration inhibited the growth of *Streptococcus mutans* with a mean of inhibition zone equal to 16.33 mm in diameter.and concentration (30%) reach to 15.67mm in diameter. Fig. (32).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration inhibited the growth of *Streptococcus facalis* with a mean of inhibition zone equal to 19.33mm in diameter While, the diluted Ethanolic extract inhibited the bacterial growth with a mean of inhibition zone equal to 16.00mm in diameter. at (50%)

concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 15.33mm in diameter, Fig. (32).

When data are statistically analysid by using Two Anova it was showed that there were no significant differences of potency between the four types of bacterias used (P = .838) . And there were significant differences between the concentrations used (P=.027) .and was mean Significant differences between the concentrations 30% 50% = .411, 30% 100%=.008 and 50% 100% = .058 .

Table 8 Antibacterial effect ethanolic extracts of *S. persica* stems against isolated from mouth bacteria(men)

Bacterias	**Concentrations**		
	30%	**50%**	**100%**
	Mean of inhibition zone(mm)		
Streptococcus pyogenis	15.67	16.33	17.00
Staphylococcus aureus	15.00	15.67	18.00
Streptococcus mutans	15.67	16.33	16.33
Streptococcus facalis	15.33	16.00	19.33

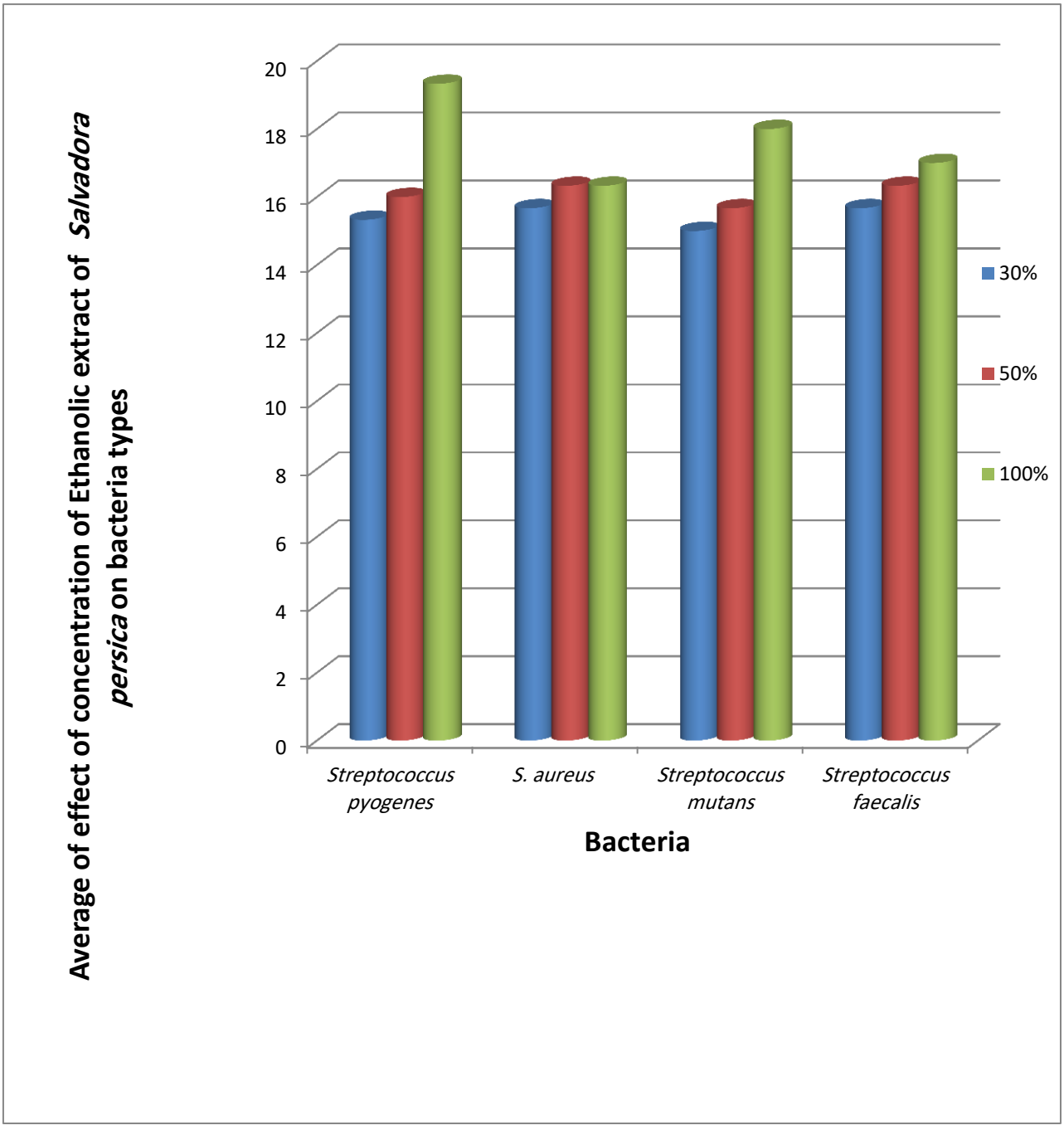


Fig. (32) Mean inhibition zone of Ethanolic extract against bacteria used

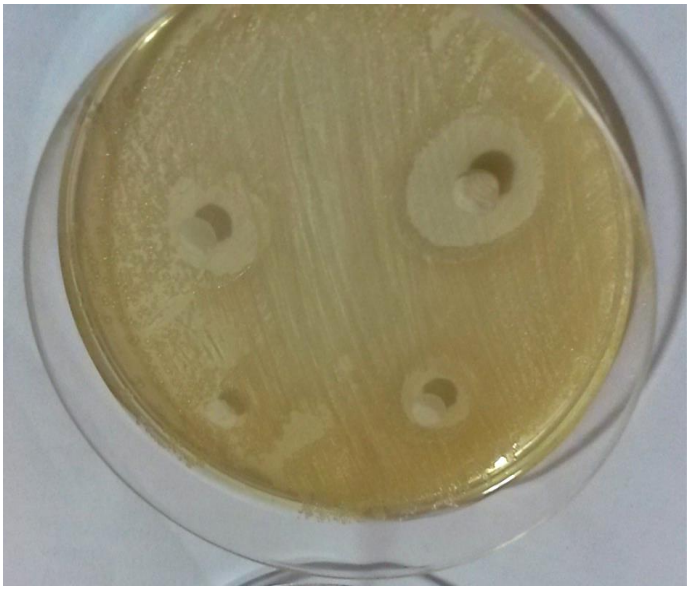


Fig. (33) The inhibition zone by using 30% cocentration of Ethanolic extract against bacteria *Streptococcus mutans*

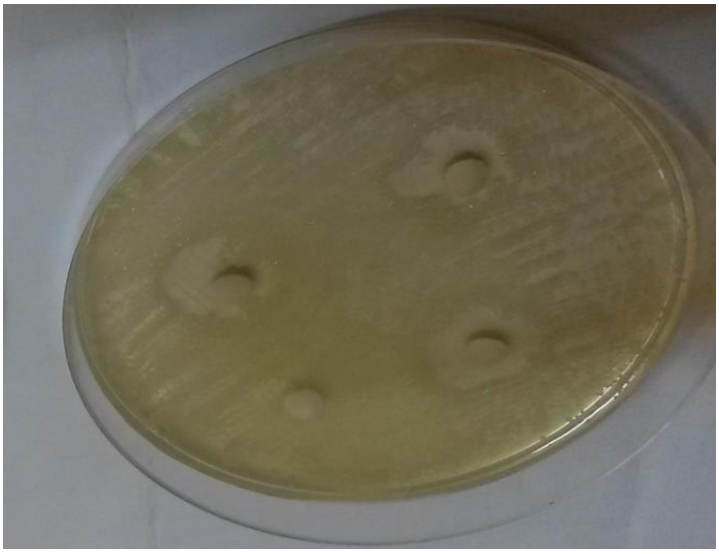


Fig. (34) The inhibition zone by using50% cocentration of Ethanolic extract against bacteria *Streptococcus facalis*

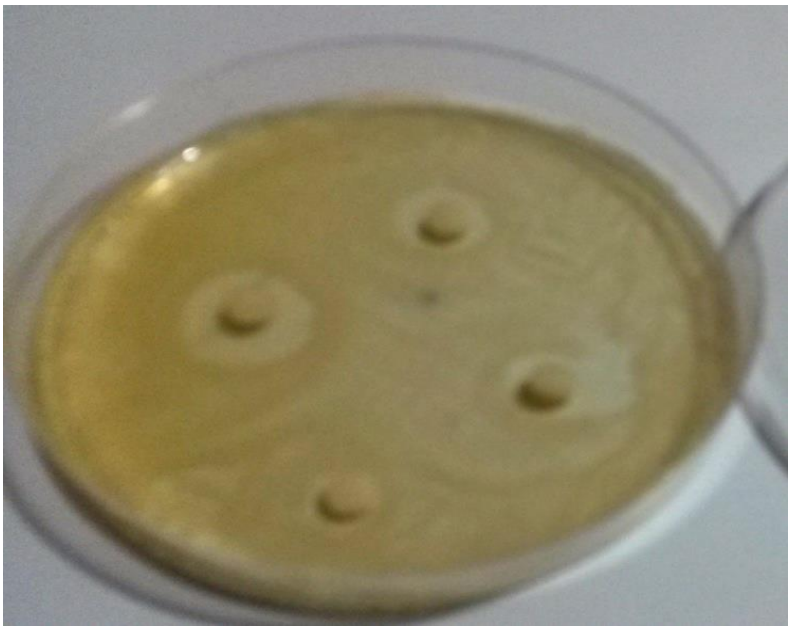


Fig. (35) The inhibition zone by using 100% concentration of Ethanolic extract agains bacteria *Streptococcus pyogenes*

4.4.3 The effects Ethanolic extract against cause infection of children (UTI) bacteria

The results of the assays of antibacterial activity of the ethanolic extract sample with three concentrations (3%, 50% and100% v/v) used in this study on bacteria cause infection of chiidren the are shown in Tables (9), Fig. (36).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration inhibited the growth of *Pseudomoens aeruginosa* with a mean of inhibition zone equal to16.67 mm in diameter . And concentration of (50%) , the diluted Ethanolic extract prevented the growth this bacterium with a mean of inhibition zone equal to 16.00mm in diamete. And Lower effect was observed at concentration of(30%) the mean of inhibition zone was equal to 15.33mm in diameter, Fig. (36).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration prevented the growth of *Staphylococcus albus* with a mean of inhibition zone equal to 25.00 mm in diameter. While, the diluted Ethanolic extract inhibited the bacterial growth with a mean of inhibition zone equal to 24.33mm in diameter at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 16.33mm in diameter, Fig. (36).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration prevented the growth of *Klebsiella pneumonia* with a mean of inhibition zone equal to 16.67 mm in diameter. While, the diluted Ethanolic extract inhibited the bacterial growth with a mean of inhibition zone equal to 15.33mm in diameter at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 15.00mm in diameter, Fig. (36).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration inhibited the growth of *Escherichia coli* with a mean of inhibition zone equal to 18.00mm in diameter While, the diluted Ethanolic extract inhibited the bacterial growth with a mean of inhibition zone equal to 14.67mm in diameter. at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 12.00mm in diameter, Fig. (36).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration inhibited the growth of *Streptococcus pneumonia* with a mean of inhibition zone equal to 21.00mm in diameter . And the results was observed at concentration of (50%) the diluted Ethanolic extract prevented the growth this bacterium with a mean of inhibition zone equal to 20.67mm in diameter. Lower effect was observed at concentration of (30%) reach to 16.67mm in diameter, Fig. (36).

When data are statistically analysid by using Two Anova, it was showed that there were significant differences of potency between the five types of bacterias used (P = .002) . And there were significant differences between the concentrations used (P=.009) .and was mean Significant differences between the concentrations 30% 50% = 0.029, 30% 100% = 0.003 and 50% 100% = 0.362 .

Table 9.Antibacterial effect of ethanolic extracts of *S. persica* stems
Against cause infection of children (UTI) bacteria

Bacterias	**Concentrations**		
	30%	**50%**	**100%**
	Mean of inhibition zone(mm)		
Pseudomoens aeruginosa	15.33	16.00	16.67
Staphylococcus albus	16.33	24.33	25.00
Klebsiella pneumonia	15.00	15.33	16.67
Escherichia coli	12.00	14.67	18.00
Streptococcus pneumonia	16.67	20.67	21.00

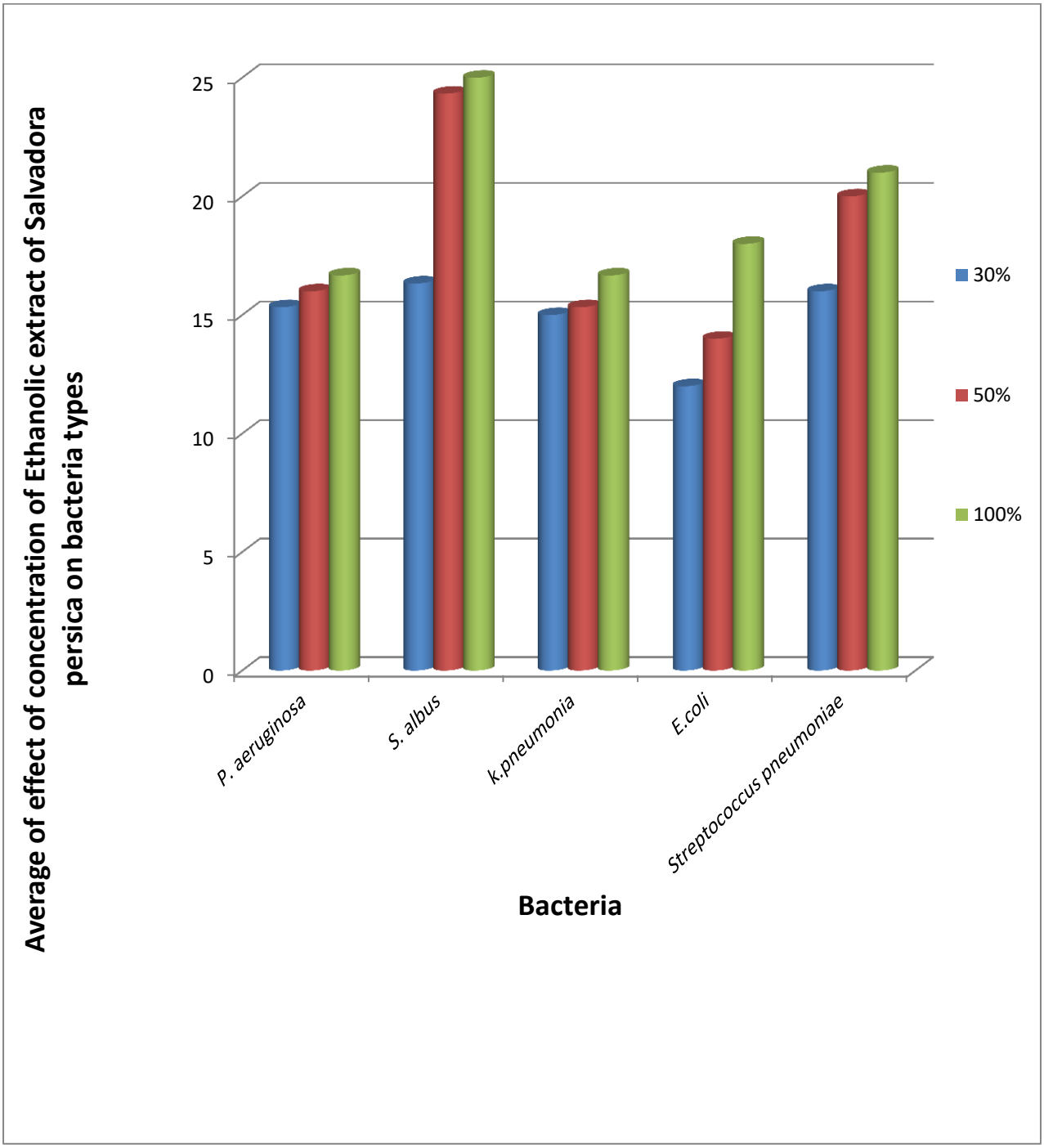

Average of effect of concentration of Ethanolic extract of Salvadora persica on bacteria types
25
20
15
10
5
0
30%
50%
100%
P. aeruginosa
S. albus
k.pneumonia
E.coli
Streptococcus pneumoniae
Bacteria

Fig.(36) Mean inhibition zone of Ethanolic extract against bacteria used

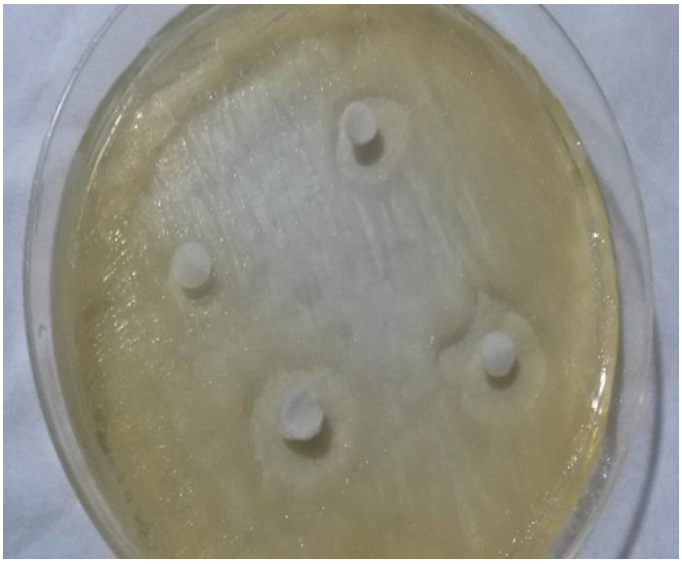


Fig. (37) The inhibition zone by using 30%cocentration of Ethanolic extract against bacteria *Klebsiella pneumonia*

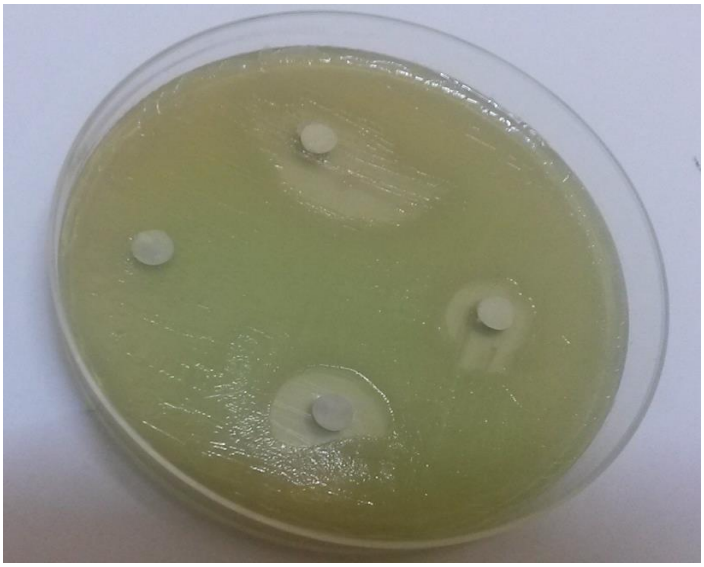


Fig. (38) The inhibition zone by using 50% cocentration of Ethanolic extract against bacteria *Pseudomoens aeruginosa*

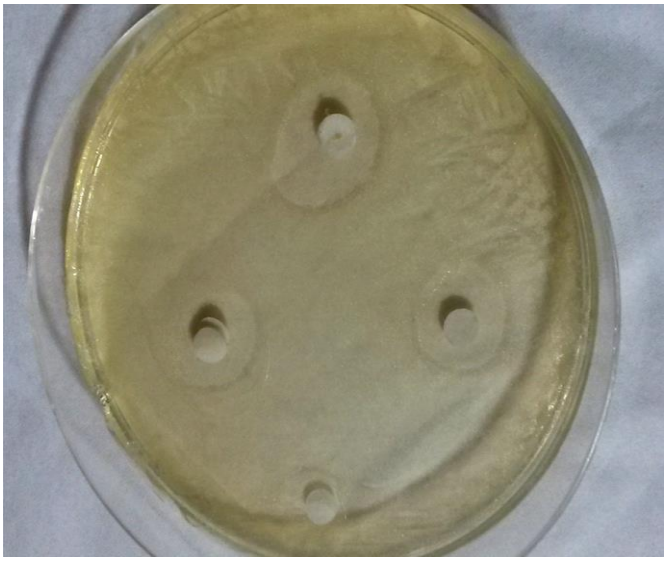


Fig. (39) The inhibition zone by using 100% cocentration of Ethanolic extract against bacteria *Escherichia coli*

4.4.4The effects Ethanolic extract against urinary tract infection bacteria

The results of the assays of antibacterial activity of the ethanolic extract sample with three concentrations (3%, 50% and100% v/v) used in this study on bacteria urinary tract infection the are shown in Tables (10), Fig. (40).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration inhibited the growth of *Pseudomoens aeruginosa* with a mean of inhibition zone equal to20.00mm in diameter . And concentration of (50%) , the diluted Ethanolic extract prevented the growth this bacterium with a mean of inhibition zone equal to 19.33mm in diamete. And Lower effect was observed at concentration of(30%) the mean of inhibition zone was equal to 17.33mm in diameter, Fig. (40).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration prevented the growth of *Staphyococcus epidirmidis* with a mean of inhibition zone equal to 22.00 mm in diameter. While, the diluted Ethanolic extract inhibited the bacterial growth with a mean of inhibition zone equal to 20.67mm in diameter at (50%) concentration to , and the effect at a concentration of (30%) the mean of inhibition zone was equal to 20.33mm in diameter, Fig. (40).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration inhibited the growth of *Escherichia coli* with a mean of inhibition zone equal to 25.33mm in diameter While, the diluted Ethanolic extract inhibited the bacterial growth with a mean of inhibition zone equal to 21.33mm in diameter. at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 20.67mm in diameter, Fig. (40).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration prevented the growth of *Acientobacter baumanniiwith* a mean of inhibition zone equal to 22.00 mm in diameter. While, the diluted Ethanolic extract inhibited the bacterial growth with a mean of inhibition zone equal to 21.67mm in diameter at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 16.00mm in diameter, Fig. (40).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration inhibited the growth of *Proteus mirabilis* with a mean of inhibition zone equal to 20.67mm in diameter . And the results was observed at concentration of (50%) the diluted Ethanolic extract prevented the growth this bacterium with a mean of inhibition zone equal to 19.33mm in diameter. Lower effect was observed at concentration of (30%) reach to 13.67mm in diameter, Fig. (40).

When data are statistically analysid by using Two Anova, it was showed that there were significant differences of potency between the five types of bacterias used (P = .075) . And there were significant differences between the concentrations used (P=.007) .and was mean Significant differences between the concentrations 30% 50% = 0.039 , 30% 100% =0.002 and 50% 100%= 0.216 .

Table 10.Antibacterial effect of ethanolic extracts of *S. persica* stems against urinary tract infection bacteria

Bacterias	**Concentrations**		
	30%	**50%**	**100%**
	Mean of inhibition zone(mm		
Psedumonas aeruginosa	17.33	19.33	20.00
Staphyococcus epidirmidis	20.33	20.67	22.00
Escherichia coli	20.67	21.33	25.33
Acientobacter baumannii	16.00	21.67	22.00
Proteus mirabilis	13.67	19.33	20.67

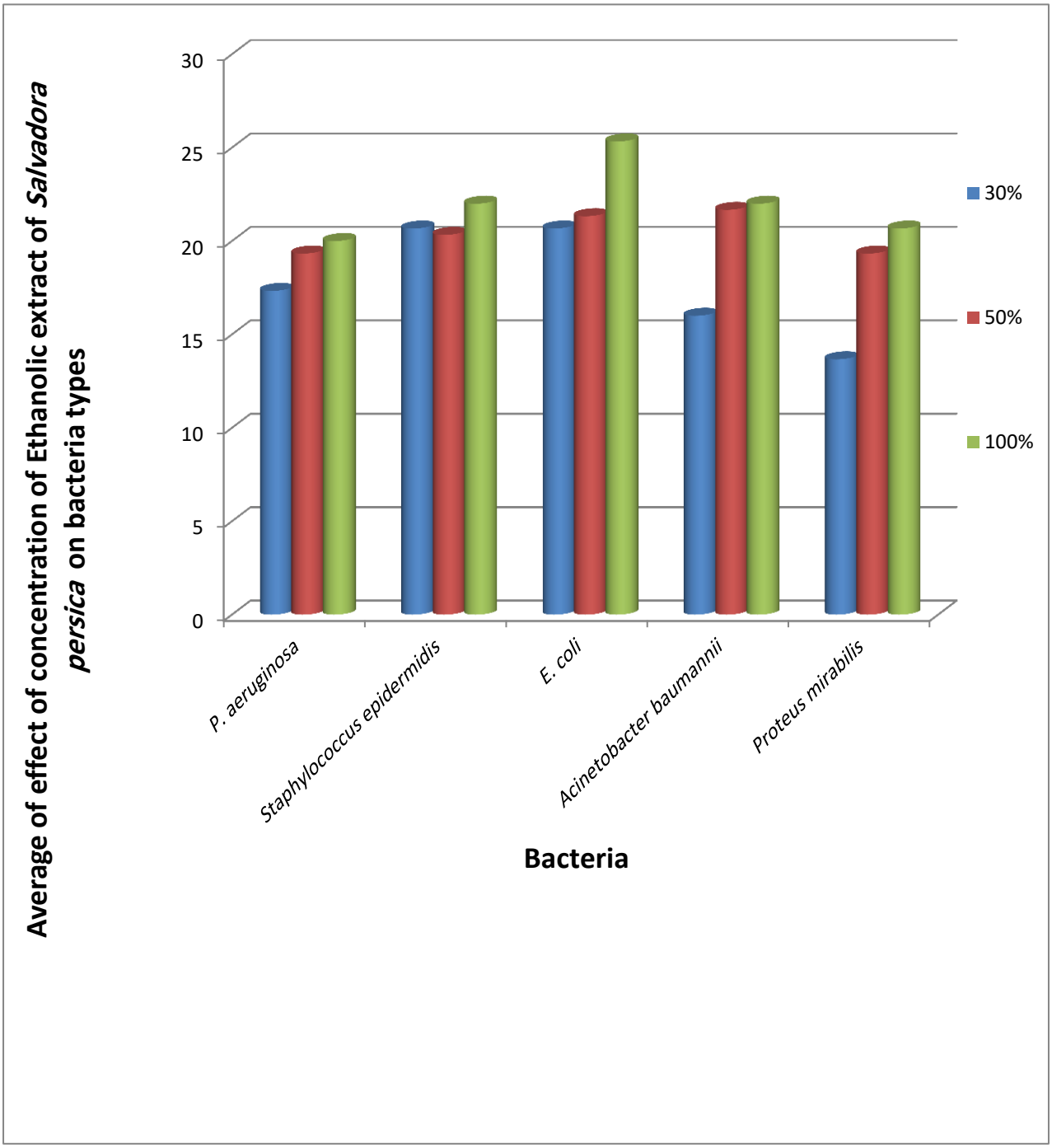


Fig. (40) Mean inhibition zone of Ethanolic extract against bacteria used .

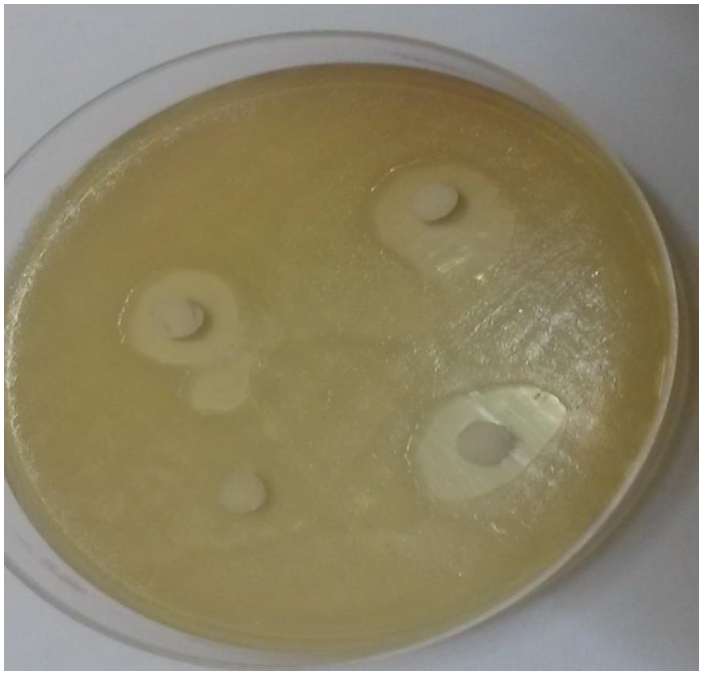


Fig. (41) The inhibition zone by using 30% cocentration of Ethanolic extract against bacteria *Acientobacter baumannii*

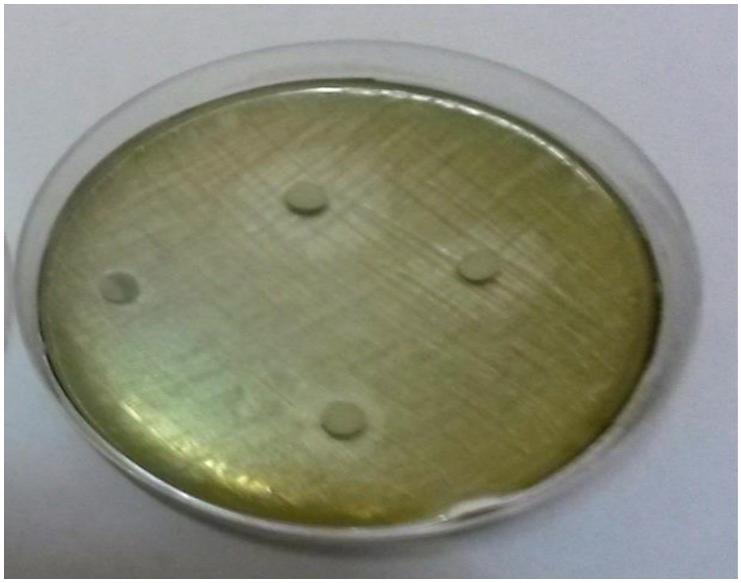


Fig. (42) The inhibition zone by using 50% cocentration of Ethanolic extract against bacteria *Psedumonas aeruginosa*

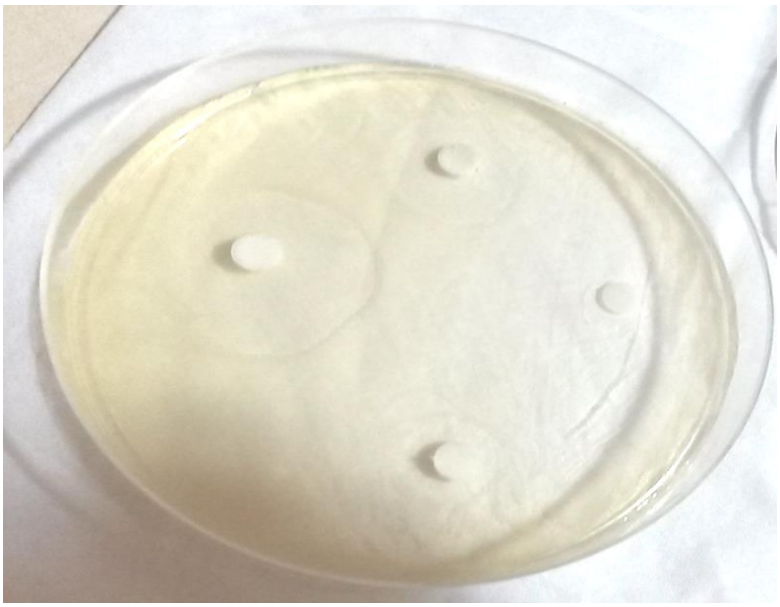


Fig. (43) The inhibition zone by using 100% cocentration of Ethanolic extract against bacteria *Escherichia coli*

4.4.5 The effects Ethanolic extract against multi drag resistance bacteria (MDR)

The results of the assays of antibacterial activity of the ethanolic extract sample with three concentrations (3%, 50% and100% v/v) used in this study on bacteria multi drag resistance (MDR) the are shown in Tables (11), Fig. (44).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration inhibited the growth of *Klebsiella Pneumonia* with a mean of inhibition zone equal to18.67mm in diameter . And concentration of (50%) , the diluted Ethanolic extract prevented the growth this bacterium with a mean of inhibition zone equal to 18.33mm in diamete. And Lower effect was observed at concentration of(30%) the mean of inhibition zone was equal to 14.00mm in diameter, Fig. (44).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration inhibited the growth of *Escherichia coli* with a mean of inhibition zone equal to 19.00mm in diameter While, the diluted Ethanolic extract inhibited the bacterial growth with a mean of inhibition zone equal to 18.33mm in diameter. at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 18.00mm in diameter, Fig. (44).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration prevented the growth of *Bacillus stearotherrmophilus* with a mean of inhibition zone equal to 23.00 mm in diameter. While, the diluted Ethanolic extract inhibited the bacterial growth with a mean of inhibition zone equal to 20.67mm in diameter at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 15.67mm in diameter, Fig. (44).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration prevented the growth of *Acientobacter baumanniiwith* a mean of inhibition zone equal

to 19.67 mm in diameter. While, the diluted honey inhibited the bacterial growth with a mean of inhibition zone equal to 18.67mm in diameter at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 17.00mm in diameter, Fig. (44).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration inhibited the growth of *Micrococcus lylae* with a mean of inhibition zone equal to 20.67mm in diameter . Similar results was observed at concentration (50%) and (30%) reach to 20.00mm in diameter. Fig. (44).

When data are statistically analysid by using Two Anova, it was showed that there were significant differences of potency between the five types of bacterias used (P = .008) . And there were significant differences between the concentrations used (P=.000) . and was Anova mean Significant differences between the concentrations 30% 50% = 0.002 , 30% 100%= 0.000 and 100% 50%= 0.150 .

Table 11.Antibacterial effect ethanolic extracts of *S. persica* stems against multi drag resistance (MDR) bacteria

Bacterias	**Concentrations**		
	30%	**50%**	**100%**
	Mean of inhibition zone(mm)		
Klebsiella Pneumonia	14.00	18.33	18.67
Escherichia Coli	18.00	18.33	19.00
Bacillus stearotherrmophilus	15.67	20.67	23.00
Acinetobacter baumannii	17.00	18.67	19.67
Micrococcus lylae	20.00	20.00	20.67

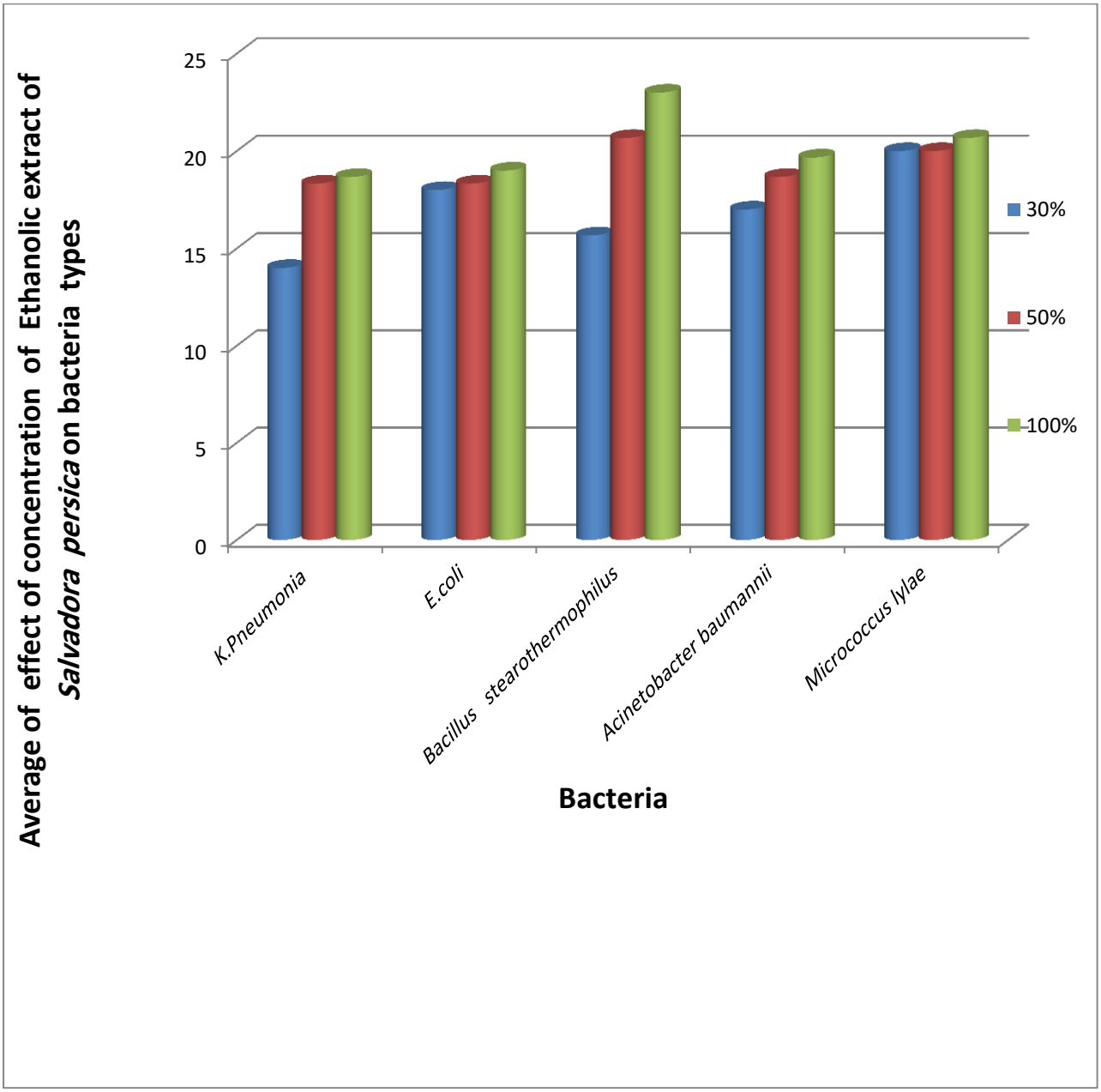


Fig. (44) Mean inhibition zone of Ethanolic extract against bacteria used .

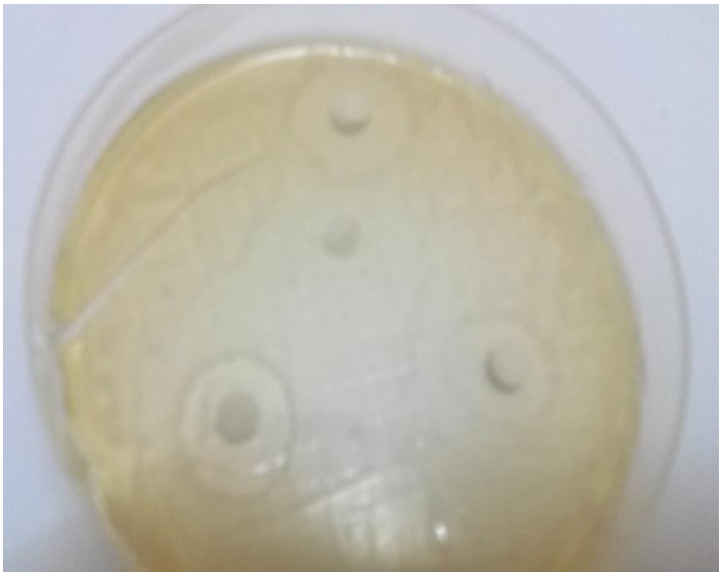


Fig. (45) The inhibition zone by using 30%cocentration of Ethanolic extract against bacteria *Escherichia Coli*

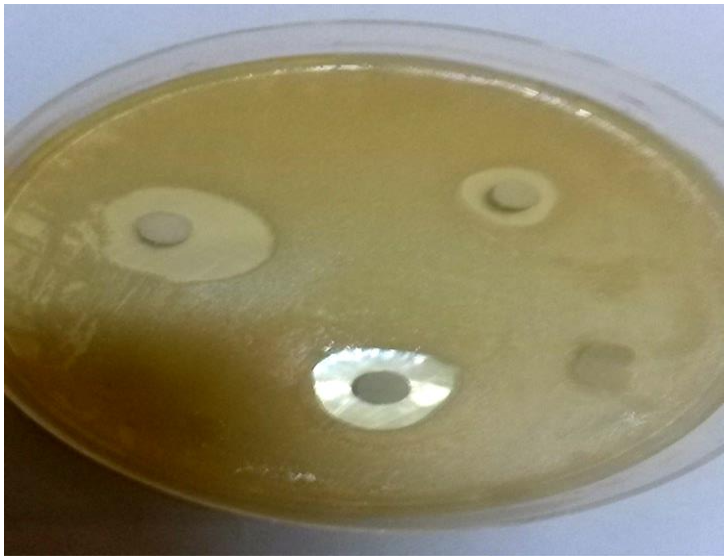


Fig. (46) The inhibition zone by using 50% concentration of Ethanolic extract against bacteria *Acinetobacter baumannii*

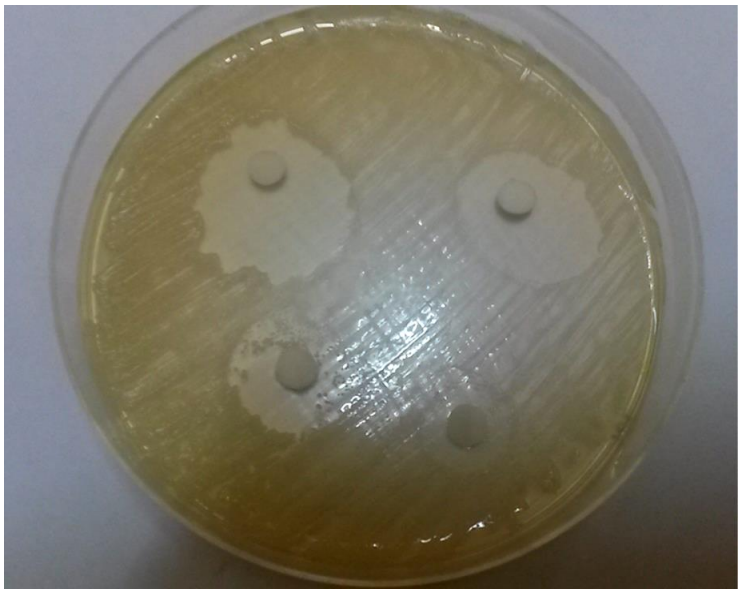


Fig. (47) The inhibition zone by using 100% concentration of Ethanolic extract against bacteria *Micrococcus lyla*

4.4.6The effects Ethanolic extract against caus infection after the operation bacteria

The results of the assays of antibacterial activity of the ethanolic extract sample with three concentrations (30%, 50% and100% v/v) used in this study on bacteria caus infection after the operation the are shown in Tables (12), Fig. (48).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration inhibited the growth of *Psedumoens aeruginosa* with a mean of inhibition zone equal to21.00mm in diameter . And concentration of (50%) , the diluted Ethanolic extract prevented the growth this bacterium with a mean of inhibition zone equal to 19.00mm in diamete. And Lower effect was observed at concentration of(30%) the mean of inhibition zone was equal to 16.33mm in diameter, Fig. (48).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration inhibited the growth of *Enterobacter aeruginosa* with a mean of inhibition zone equal to 23.00mm in diameter While, the diluted Ethanolic extract inhibited the bacterial growth with a mean of inhibition zone equal to 18.33mm in diameter. at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 16.33mm in diameter, Fig. (48).

The effect of Ethanolic extract neat Ethanolic extract (100%),(50%) and (30%) concentration inhibited the growth of *Staphylococcus aureus* with a Similar results was observed at mean of inhibition zone equal to 15.00mm in diameter. Fig. (48).

The effect of Ethanolic extract neat Ethanolic extract (100%) concentration prevented the growth of *Klebsiella pneumonia* with a mean of inhibition zone equal to 18.33 mm in diameter. While, the diluted Ethanolic extract inhibited the bacterial

growth with a mean of inhibition zone equal to 16.33mm in diameter at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 15.67mm in diameter, Fig. (48).

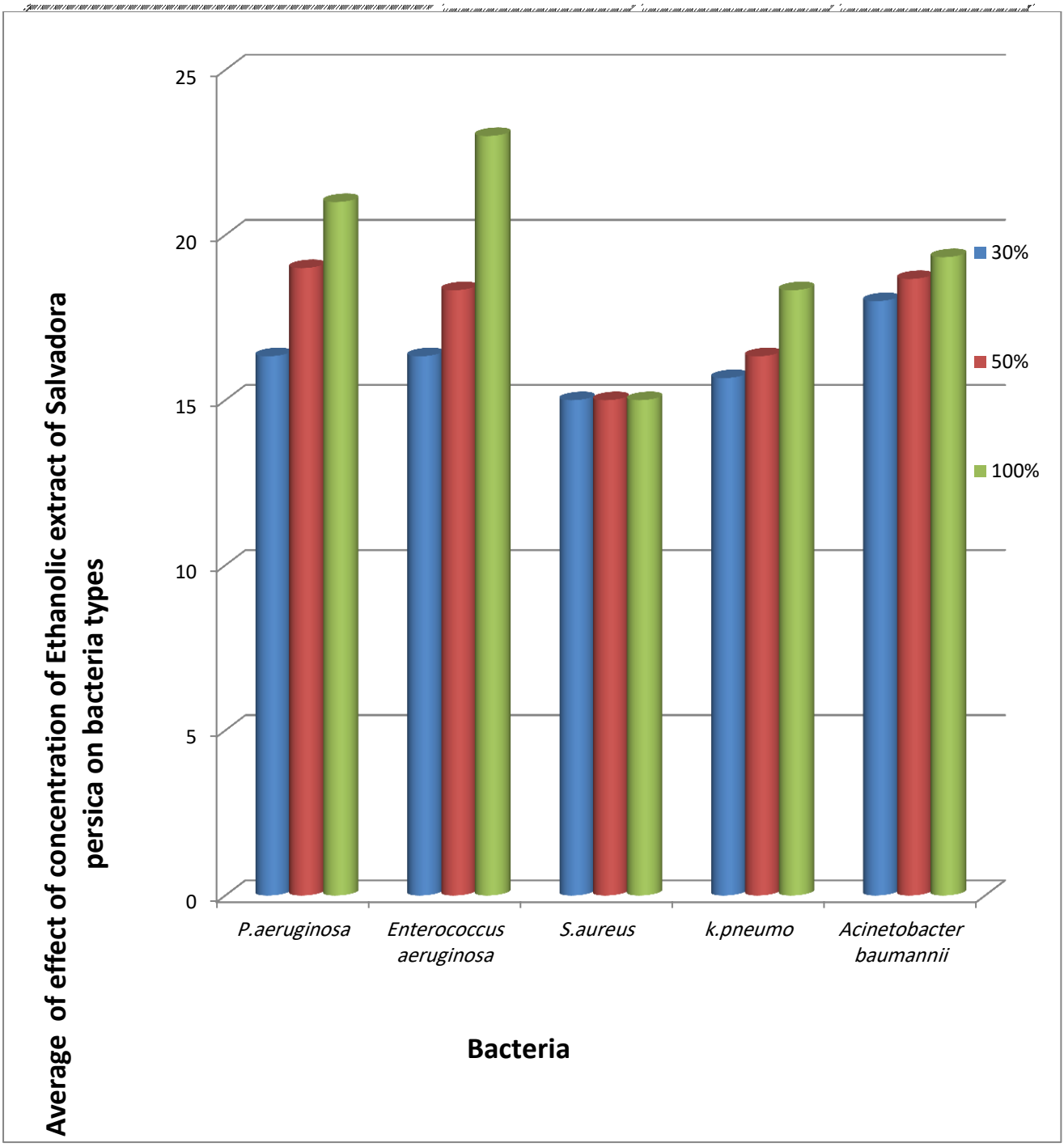
The effect of Ethanolic extract neat Ethanolic extract (100%) concentration prevented the growth of *Acientobacter baumannii with* a mean of inhibition zone equal to 19.33 mm in diameter. While, the diluted Ethanolic extract inhibited the bacterial growth with a mean of inhibition zone equal to 18.67mm in diameter at (50%) concentration to , and the effect was less at a concentration of (30%) the mean of inhibition zone was equal to 18.00mm in diameter, Fig. (48).

When data are statistically analysid by using Two Anova, it was showed that there were significant differences of potency between the five types of bacterias used (P = .062) . And there were significant differences between the concentrations used (P=.070). And was mean Significant differences between the concentrations 30% 50% = ---, 30% 100%= --- and 50% 100% = ----.

Table 12.Antibacterial effect ethanolic extracts of *S. persica* stems against caus infection after the operation bacteria

Bacterias	**Concentrations**		
	30%	**50%**	**100%**
	Mean of inhibition zone(mm)		
Psedumoens aeruginosa	16.33	19.00	21.00
Enterobacter aeruginosa	16.33	18.33	23.00
Staphylococcus aureus	15.00	15.00	15.00
Klebsiella pneumonia	15.67	16.33	18.33

Acinetobacter baumannii	18.00	18.67	19.33



Fig(48) Mean inhibition zone of Ethanolic extract against bacteria used .

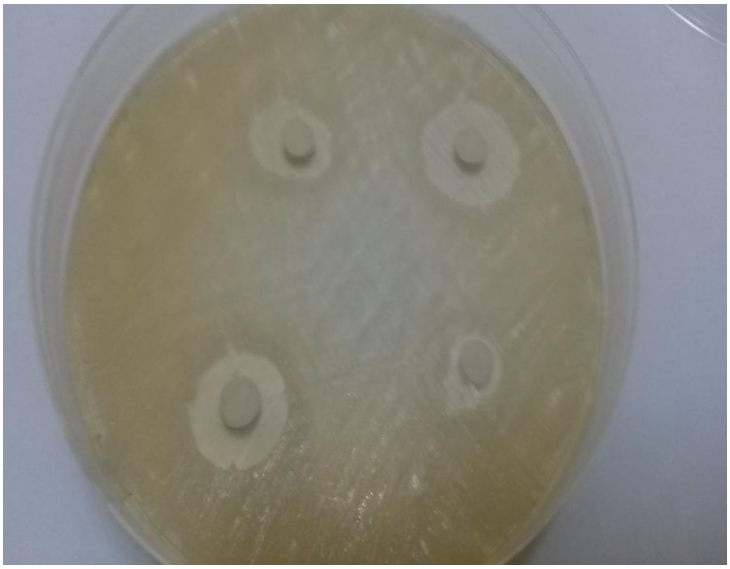


Fig. (49) The inhibition zone by using 30%concentration of Ethanolic extract against bacteria *Enterobacter aeruginosa*

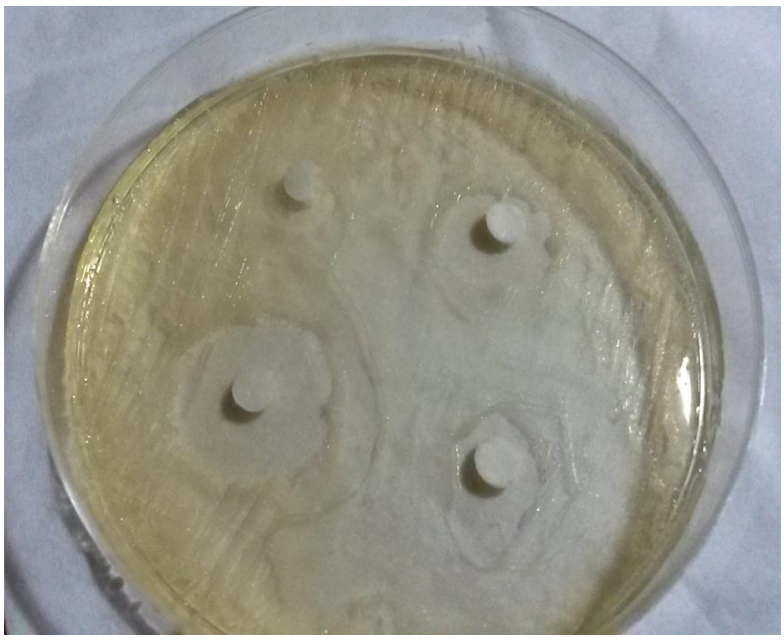


Fig. (50) The inhibition zone by using 50% concentration of Ethanolic extract against bacteria *Klebsiella pneumonia*

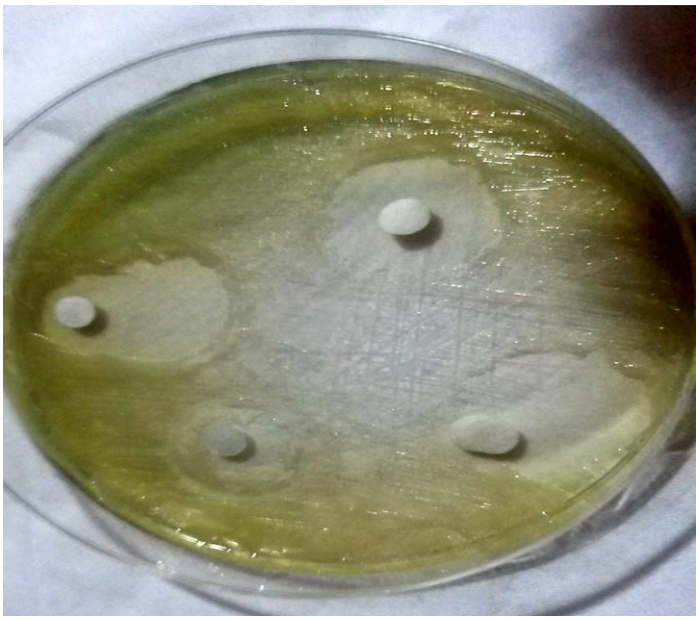


Fig. (51) The inhibition zone by using 100%cocentration of Ethanolic extract against bacteria *Psedumoens aeruginosa*

4.5 Effect of antibacterial against bacteria used .

The antibiotics that were used in this study for the comparison with the Ethanolic Aquatic and extracts gave different effects on the bacteria (Table 13,14 and15)**,** *(Fig. 52,57and 62)***.** While some of them sensitive all antibiotic used the and other resistant

Table (13) Effect of Antibiotic against Bacteria used

Isoiation	Antibiotics / Bacteria	Amoxyc-ilin	Erythro mycin	Strptom ycin	Sulpham ycin	Ampcilin
1	***Staphylococcus aureus***	**R**	**I**	**I**	**R**	**R**
2	***Staphylococcus aureus***	**R**	**I**	**R**	**R**	**S**
3	***Staphylococcus aureus***	**R**	**R**	**S**	**R**	**R**
1	***Streptococcus pyogenis***	**R**	**R**	**R**	**R**	**R**
1	***Staphylococcus mutans***	**R**	**R**	**R**	**R**	**R**
2	***Staphylococcus mutans***	**R**	**I**	**R**	**S**	**R**

1	***Streptococcus faecalis***	**R**	**R**	**R**	**S**	**R**
1	***Staphylococcus albus***	**R**	**S**	**S**	**S**	**R**
1	***Streptococcus pneumonia***	**R**	**R**	**R**	**R**	**R**
1	***Staphylococcus epidermids***	**S**	**I**	**I**	**S**	**R**

R= Resistant

I= Intermediate

S= Susceptibility

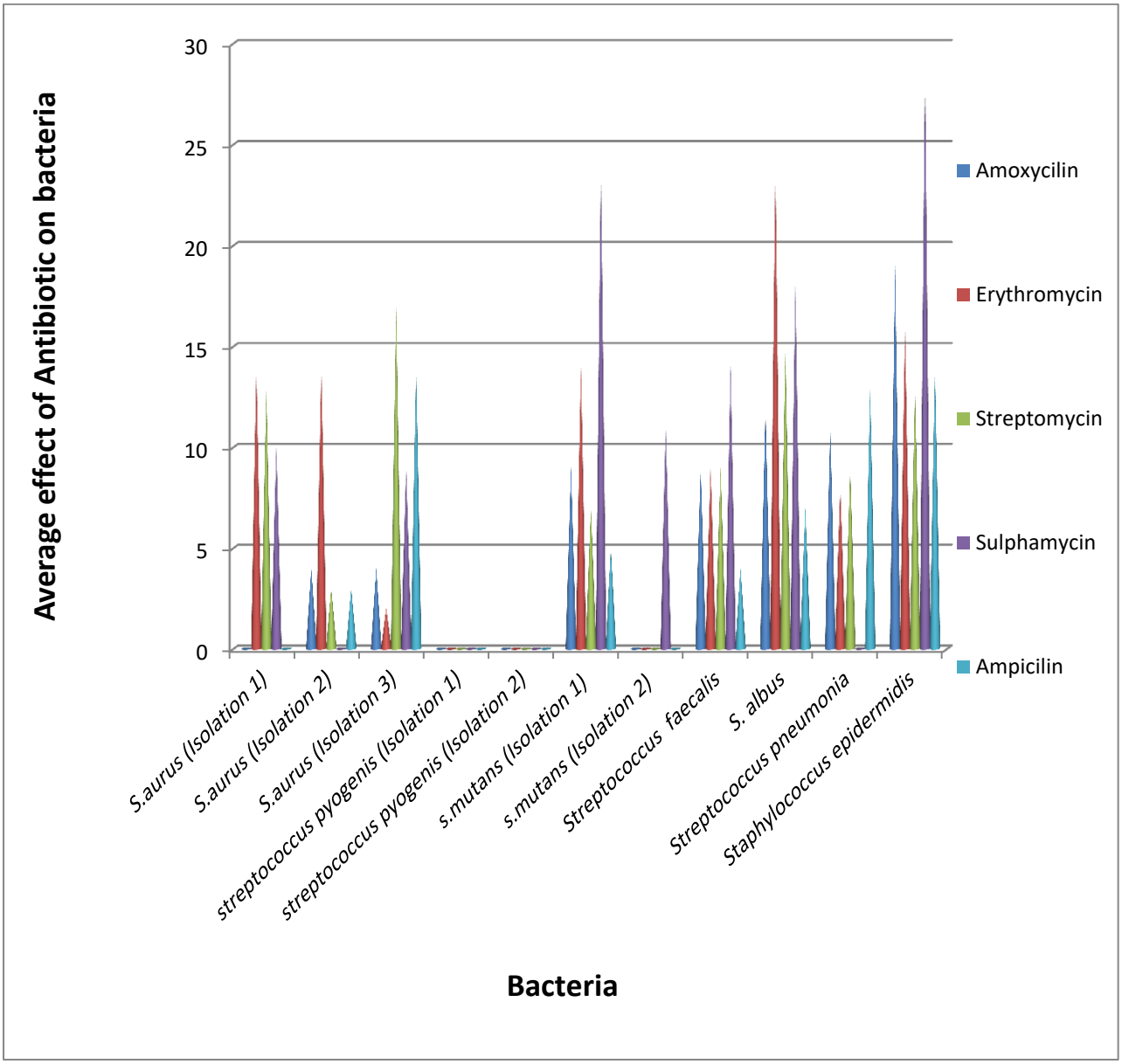


***Fig*. (52) Mean inhibition zone of Antibiotic against bacteria used.**

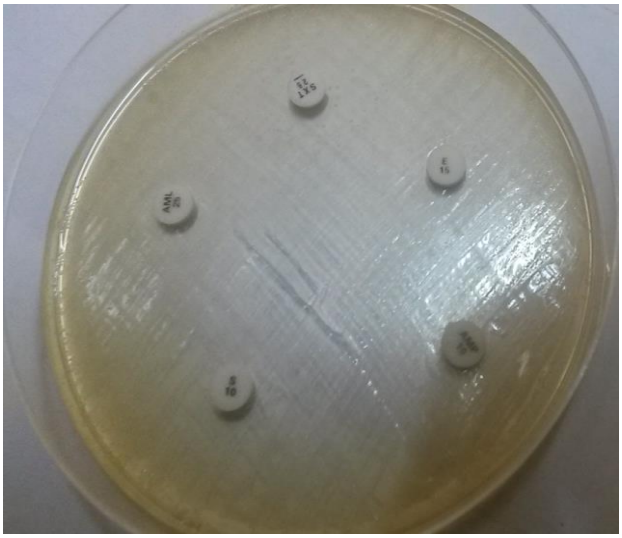


Fig. (53) The inhibition zone by effect of antibiotic *Streptococcus pyogenis*(Mouth)

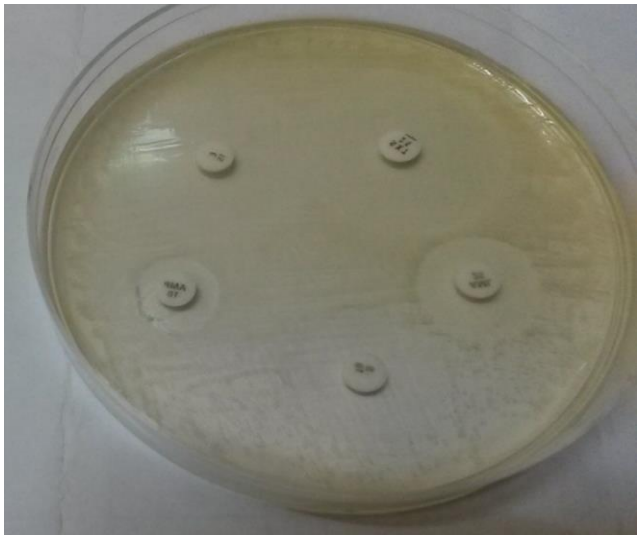


Fig. (54) The inhibition zone by effect of antibiotic *Staphylococcus aureus*(Mouth)

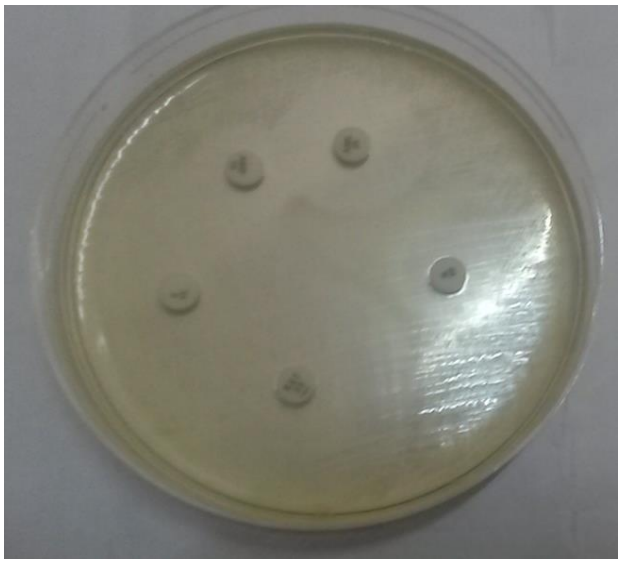


Fig. (55) The inhibition zone by effect of antibiotic *Streptococcus epidermids* (UTI)

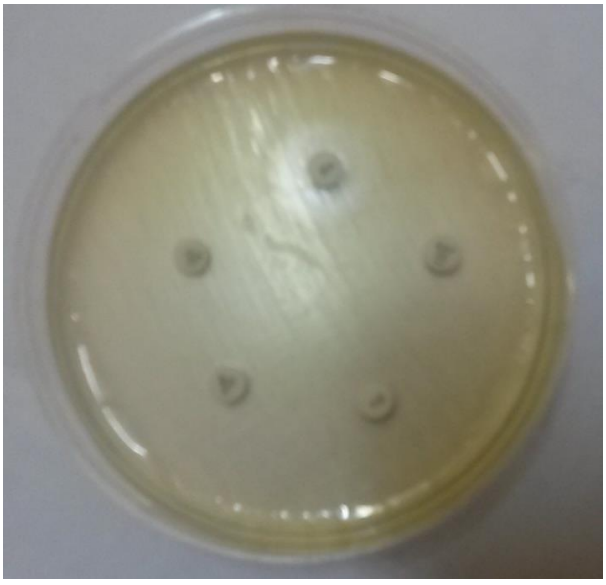


Fig. (56) The inhibition zone by effect of antibiotic *Streptococcus mutans*(mouth)

Table (14) Effect of Antibiotic against Bacteria used.

Isoiation	Antibiotics / Bacteria	Amoxyc-ilin	Erythromycin	Strptomycin	Sulphamycin	Ampcilin
1	*Klebsiella pneumonia*	R	R	R	I	R
2	*Klebsiella pneumonia*	R	R	R	R	R
3	*Klebsiella pneumonia*	R	R	R	R	R
1	*Psedomonas aeruginosa*	R	R	R	R	R
2	*Psedomonas aeruginosa*	R	R	R	R	R
3	*Psedomonas aeruginosa*	R	R	R	R	R
1	*Bacillus stearothermophilus*	R	R	R	R	R
1	*Micrococcus laylai*	R	R	R	R	R

R= Resistant

I= Intermediate

S= Susceptibility

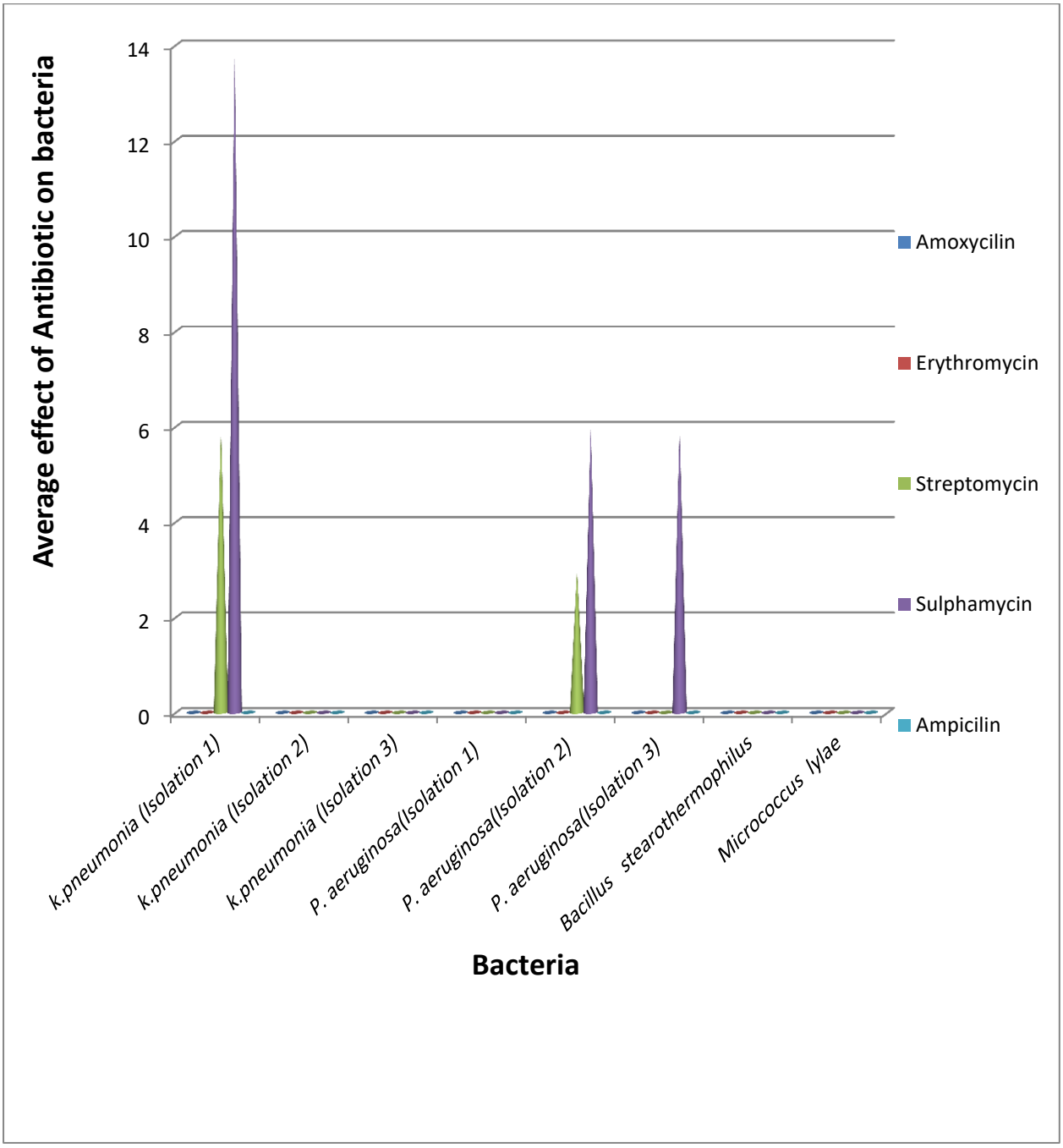


***Fig*. (57) Mean inhibition zone of Antibiotic against bacteria used.**

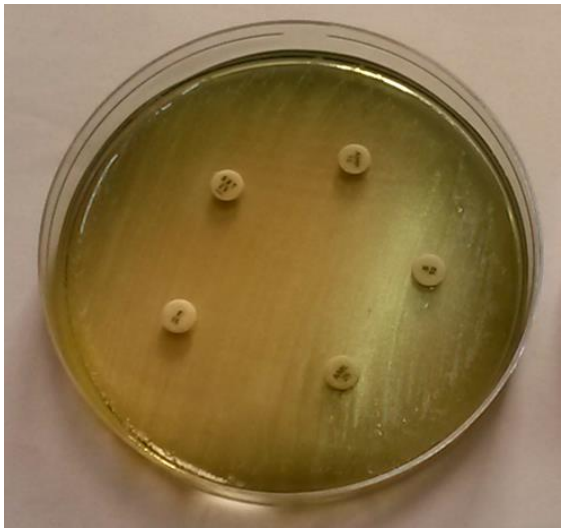


Fig. (58) The inhibition zone by effect of antibiotic *Psedomonas aeruginosa*(UTI)

Fig. (59) The inhibition zone by effect of antibiotic *Klebsiella pneumonia* (UTI children)

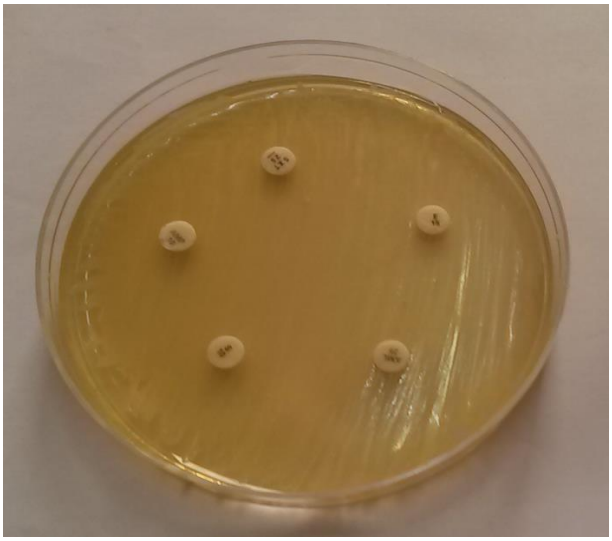


Fig. (60) The inhibition zone by effect of antibiotic *Micrococcus laylai* (MDR)

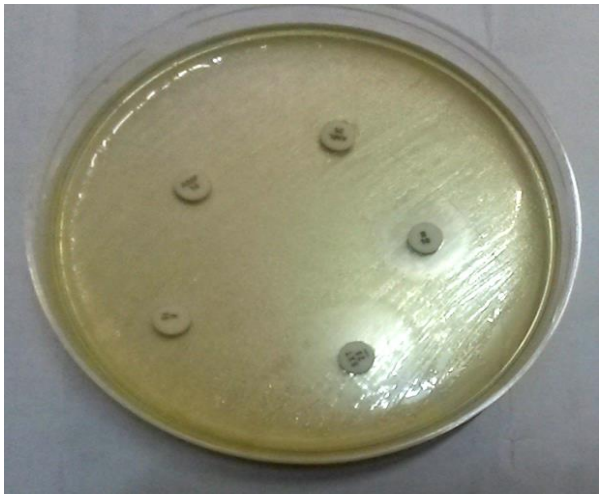


Fig. (61) The inhibition zone by effect of antibiotic *Klebsiella pneumonia*(UTI children)

Table (15) Effect of Antibiotic against Bacteria used.

Isoiation	Antibiotics / Bacteria	Amoxyc-ilin	Erythro mycin	Strptom ycin	Sulpham ycin	Ampcilin
1	*Enterobacter faecalis*	R	R	R	S	R
1	*Enterobacter aeruginosa*	R	S	R	R	R
1	*Escherichia coli*	R	R	R	R	R
2	*Escherichia coli*	R	R	R	R	R
3	*Escherichia coli*	R	R	R	R	R
1	*Proteus mirabilis*	R	R	R	I	R
1	*Acinetobacter baumannii*	R	R	R	R	R
2	*Acinetobacter baumannii*	R	R	R	R	R
3	*Acinetobacter baumannii*	R	R	R	R	R

R= Resistant

I= Intermediate

S= Susceptibility

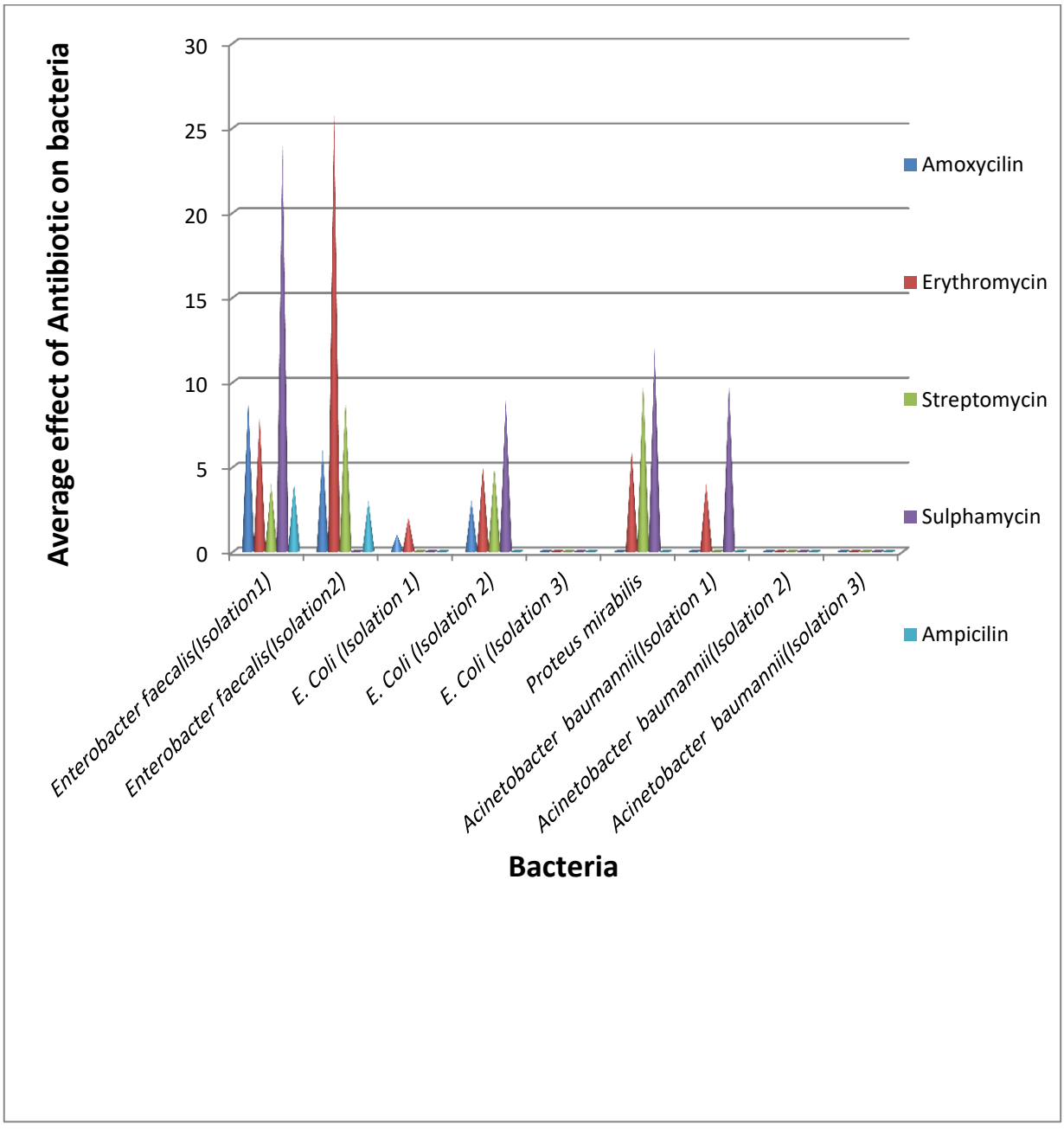


Fig. (62) Mean inhibition zone of Antibiotic against bacteria used.

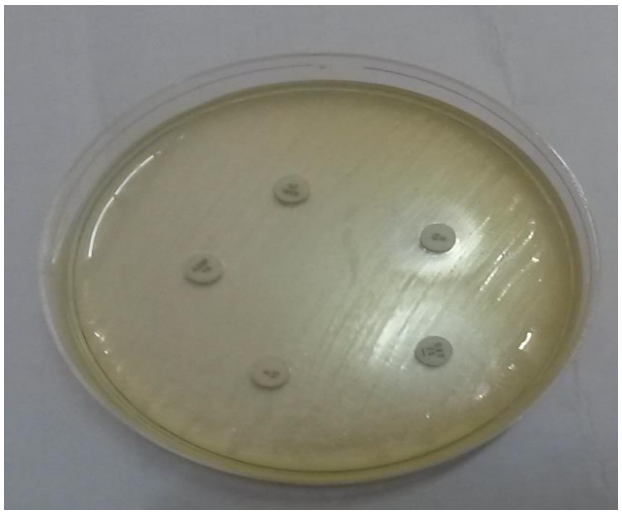


Fig. (63) The inhibition zone by effect of antibiotic *Proteus mirabilis* (UTI)

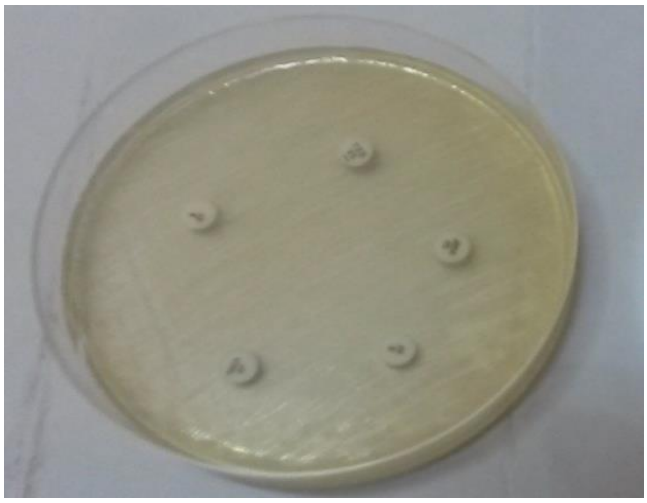


Fig. (64) The inhibition zone by effect of antibiotic *Escherichia coli*(MDR)

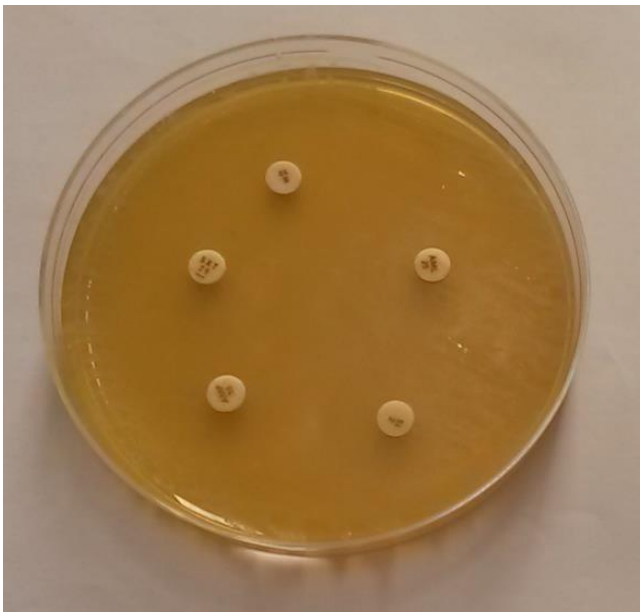


Fig. (65) The inhibition zone by effect of antibiotic *Acinetobacter baumannii*(MDR)

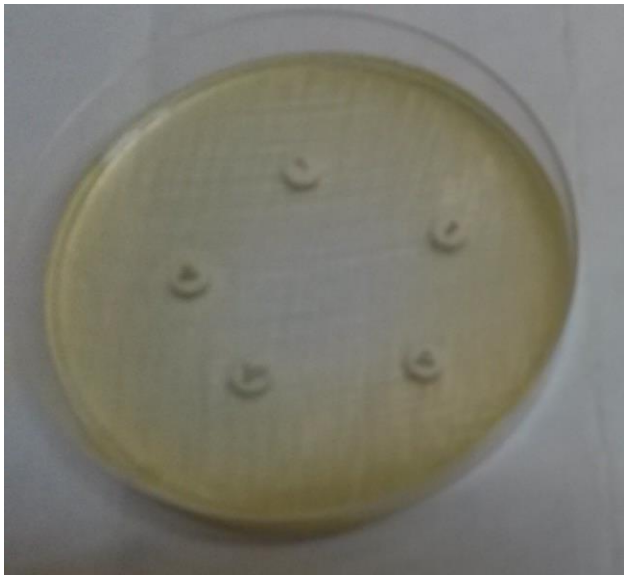


Fig. (66) The inhibition zone by effect of antibiotic *Escherichia coli* (UTI children)

4.6 Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

The Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) for two extracts Ethanolic, Aquatic were assessed on bacterial strains for control and comparison purposes and was the result obtained showed in Table (16).

Table (16) Minimum inhibitory and bactericidal concentrations of Ethanolic extract of and Aquatic extract of propolis against bacteria.

		Antimicrobial activity of S. persica % (V/V)	

Isolation	Bacterial strains with inoculums density of 10^8 CFU/m	Ethanolic extract		Aquatic extract	
		MIC	MBC	MIC	MBC
Mouth	***Staphylococcus aureus***	30	50	30	50
After operation	***Staphylococcus aureus***	50	100	30	50
Mouth	***Streptococcus pyogenis***	30	50	30	50
Mouth	***Staphylococcus mutans***	50	100	30	50
Mouth	***Staphylococcus mutans***	30	50	30	50
Mouth	***Streptococcus faecalis***	30	50	30	50
UTI children	***Staphylococcus albus***	30	50	30	50
UTI children	***Streptococcus Pneumonia***	30	50	30	50

UTI	***Staphylococcus epidermids***	30	50	30	50
UTI children	***Klebsiella pneumonia***	30	50	30	50
After operation	***Klebsiella pneumonia***	30	50	30	50
MDR	***Klebsiella pneumonia***	30	50	30	50
UTI children	***Psedomonas aeruginosa***	30	50	30	50
UTI	***Psedomonas aeruginosa***	30	50	30	50
After operation	***Psedomonas aeruginosa***	30	50	30	50
MDR	***Bacillus stearothermophilus***	30	50	30	50
MDR	***Micrococcus laylai***	50	100	30	50
Mouth	***Enterobacter faecalis***	30	50	30	50

After operation	***Enterobacter aeruginosa***	30	50	30	50
MDR	***Escherichia coli***	30	50	50	100
UTI	***Escherichia coli***	30	50	30	50
UTI children	***Escherichia coli***	30	50	30	50
UTI	***Proteus mirabilis***	30	50	30	50
MDR	***Acinetobacter baumannii***	30	50	30	50
After operation	***Acinetobacter baumannii***	30	50	30	50
UTI	***Acinetobacter baumannii***	30	50	30	50

Chapter Five

Discussion

Miswak is a common name for *S. persica*, which is commonly used in Saudi Arabia and the Arab world. Miswak wicks clean between the teeth and do not break, regardless of the amount of pressure applied, as they are flexible and strong. The small wicks bend to the appropriate shape to clear plaque and left over food in between teeth and do not damage the gums.

Urged us Holy Mohammed Prophet, (peace be upon him), where he said { you Siwaak it cleanses the mouth and pleases the Allah}.

The WHO recommended the use of miswak in 1986 and in 2000 an international consensus report on oral hygiene concluded that further research was needed to document.

This study indicated that ethanolic and aquatic extracts(col) had promising effect against all oral bacteria ; *Streptococcus faecalis ,Streptococcus mutans*, *Streptococcus pyogenis Enterobacter faecalis ,Staphylococcus aureus ,Staphylococcus mutans* reported that *S. persica* extracts were effective against Eight types bacteria isolated from mouth both extract used low extract concentrations.
as observed during present study, were in agreement with the findings of earlier researchers (Hussein, 1992; Saleh et al., 2006). The results of the current study are in agreement with the earlier investigations where *S. persica* exhibited significant antimicrobial activity against bacteria collected from teeth with inflamed gums and necrotic pulps (Al-Sabawi et al., 2007).

Our results were further supported by other reports using the same disc diffusion and micro well assay method and established water extract of *S. persica* to be effective against *S. pyrogenis, S. faecalis, P. aeruginosa,* (Suffredini et al., 2004; Al-Sabawi et al., 2007; The results of the current study clearly demonstrated that aqueous and alcoholic extracts of *Salvodora persica* could inhibit the growth of several pathogenic bacteria, however, the effectiveness varied against the different tested microorganisms. Study results in agreement with (Almas *et al*., 2005; Darmani *et al*., 2006) who examined the effects of miswak extracts on the growth of microorganisms including *Streptococcus mutans*. The result showed inhibition in growth of *Streptococcus mutans*. AL-Bayati and Sulaiman (2008) investigated the aqueous and methanol extracts of *Salvadora persica* for its antimicrobial activities against seven isolated oral.

This study found that Aquatic extract (col) had a good effect against *Streptococcus pyrogenis, Streptococcus mutans, Streptococcus pyrogenis, Streptococcus faecalis, Streptococcus mutans* and *Enterobacter faecalis* with mean of inhibition zone at the highest concentration100%. Agreement with results observed by AL-Bayati and Sulaiman (2007). Too Similar results observed by Sarmad Ghazi Mohammed;(2013) found that Aquatic extract (col) had a good effect against *Streptococcus mutans, Staphylococcus aureus* and *Escherichia coli*.

This study phouaed that ethanolic extract had a good effect against *Streptococcus pyrogenis* with mean of inhibition zone at the highest concentration100% similar effect was observed against *Streptococcus mutans*. However too effect was found against *Enterobacter faecalis*. Agreement with results observed by AL-Bayati and Sulaiman (2007).

This study phouaed that ethanolic extract, Aquatic extract(col) had a good effect against *Staphylococcus aureus* with mean of inhibition zone at the highest all concentration100%,50% and 30%. Agreement with the study Amas(2001) researcher who did not find any effect of this extract bacteria *Staphylococcus aureus.*

The results of the assays of antibacterial activity of the Aquatic extract (Hot) in all bacteria was negative there is no inhibition of abstract It may have been affected due to the enzymes active substances due to the boiling of water . I Agreement with the researcher El.Desoakey ,(2015) of the Aquatic extract (Hot) who did not find any effect against bacteria. I Difference wiht El.Desoukey;(2014) of Aquatic extract (Hot) found effect against bacteria.

Note this study Aquatic extract(cold) and ethanolic extract given effect against *Psedomonas aeruginosa*. I Difference with the study. Al-bayati and sulaiman.,(2008) The Agreement with results were *Psedomonas aeruginosa*. of aquatic extract only given effect ;but alcohol extract has not effect against *Psedomonas aeruginosa*. And Sarmad Ghazi Mohammed;(2013) The get results were *Psedomonas aeruginosa*. of aquatic extract not given effect ;but alcohol extract has effect against *Psedomonas aeruginosa*.

This study aqueous extract (cold) effective against *Psedomonas aerogenes* , *Klebsiella pneumonia and Streptococcus epidermis* Agreement with with El.Desoukey;(2014) the aqueous extract is the most effective against Ps. *aerogenes* followed by *K. pneumonia and S.epidermis* cold aqueous extract showed significant antibacterial activity against, .While the Alcohol extract did not show any significant antibacterial *S.pyogens , and E. coli*. Difference wiht this study ethanolic extract

showed significant antibacterial activity against *Streptococcus pyogens* and *Escherichia coli*.

Both this study antimicrobial assays indicated that the ethanolic extract of S. persica was more efficient than the aquatic extract in all oral bacteria, and The rest of the other bacteria. Agreed with Howaida F.AbdElrahman.,(2002).Too Agreement with Sarmad Ghazi Mohammed;(2013). and Disagreses with the study. al-bayati, sulaiman(2008) The results were of aquatic extract The best alcohol extract.

We note that the concentration of 100% most effective concentrations of 50% and 30% and these results are consistent with previous results, which found that the greater the concentration was more effect.Eid et,al ;(1990),Al.bayat and D.sulaiman;(2008).

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It could be concluded that miswak and powdered miswak are excellent oral hygiene agents, and their use should be promoted based on scientific knowledge of their benefits and proper use. Because it is widely available in this part of the world and is inexpensive, miswak chewing sticks can be a great help in developing countries with financial constraints and limited oral health care facilities.

Studies confirmed that the plant meswak instrumental in reducing the proportion of bacteria in saliva and gums as containing anti-bacterial groups include(CL2 , SO4 , SCN , NO3) To inhibition the growth of many kinds of bacteria Darout and Skaug (2004).

S. persica is known to contain several biologically active chemical constituents such as volatile oils, flavonoids, alkaloids, steroids, terpenoids, saponins, and carbohydrates (Abdillahi et al., 2010; Garboui et al.,2009; Kamil et al., 1999).

There is a continued interest in identifying efficient aquatic extract agents that could be used daily without side effects (Prabuseenivasan *et Al*., 2006). Folk medicine is a potential source of medicaments and has recently become a focus of dental research (Fouad *et al*., 2000; Walid and Fouad, 2000 and 2001; Fouad and Wassan ;2001) Chewing sticks or Miswaks are still popular compared with the use of modern tooth brushes, the type of chewing sticks used in the Middle East is derived from the plant S. persica (Almas, 2001).

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