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Antimicrobial effect of some Egyptian plants

Afaf A. Amin, Atef H. El-Sayed, Mervat A. Foad and Osama.A.Rashwan

NATIONAL NUTRITION INSTITUTE

Abstract

Twenty eight crude water extracts of four Egyptian plants: Hibiscus sabdariffa (Karkade) , Tamarindus indica (Tamarind) , Glycyrrhiza glabra (Liquorice) and Ceratonia siliqua (Carob) , were tested for their antibacterial activities against 10 common pathogenic bacteria,Bacillus cereus, Shigella flexenary and Escherichia coli showed sensitivity to the 28 extracts. Shigella sp. showed sensitivity to 24 extracts while Pseudomonas sp. and Salmonella typhimurium were sensitive to 23 extracts. Salmonella paratyphi and Aerobacter aerogenes showed sensitivity to 22 extracts, Serratia marcesence and Aeromonas hydrophila showed sensitivity to 20 extracts. Karkade exhibited marked antibacterial activity against all the 10 organisms. E. coli and Shigella flexenary had no observed sensitivity to Liquorice extracts. Aerobacter aerogenes was the only one which had no observed sensitivity to Tamarind extracts. All the extracts of Carob were effective against only two pathogens:Bacillus cereus and Shigella flexenary. The results support the traditional uses of extracts of these plants for the management of bacterial infections and for the development of antibacterial agents for the preservation of foods.

Introduction:

Herbal medicine depends on the action of non-essential nutrients and the phytochemical ***(Walker, 2006).***Liquorice has been used as medicine in China for centuries. It may be taken by mouth to treat stomach conditions such as ulcers and respiratory conditions as bronchitis and it may be anti-infective and anti-inflammatory ***(Frus-Moller et al., 2002).***Two compounds from Liquorice inhibit the growth of Streptococcus mutans , the primary bacteria responsible for causing cavities . In fact, Liquorice roots antimicrobial activity was seen in a number of experiments (Sahelian, R., 2005).

Polyphenols in Carob possess antibacterial and anti-inflammatory effects. Substantial in vitro and animal studies support the beneficial effects of polyphenols in many gastrointestinal diseases ***(Dryden et al., 2006). Evans et al. (2002), Ganamoni et al.(2003) , Khan et al. (2003) , Matu and Staden (2003) and Ram et al. (2004)*** detected antibacterial substances in some plant

extracts and their effects on some pathogenic microorganisms as Salmonella typhi Staphylococcus aureus , Pseudomonas spp. and Bacillus spp. in vitro.

Nowadays, there is an increasing trend throughout the world to go back to nature. Egypt is not far from what is happening in the whole world. The writings on the papyrus and temple walls showed that Ancient Egyptians were aware of the usefulness of many plants and used some of them for the same purposes as they are used today ***(C.A.P.A.,2000).***In Ancient Egypt, spices and condiments were used as embalming materials, as sweet scents in religious ceremonies and to mask the putrid flavor of meat in hot climates lacking refrigeration ***(I.C.M.S.F., 1980).*** Also different medical preparations containing aromatic plants extracts are now produced at mass production scale and a few plants have an antimicrobial effect at the concentrations used in food and thus serve as preservatives.

Bepoliver-Bevere (1986) mentioned that the plant constitutes with antibacterial action are tentatively grouped according to their main chemical groups: phenols, quinines, acids, alkaloids, flavonoides, terpenoids and proteolytic enzymes.***Alian and Eid (1983)*** reported that Tamarind beverage had a bacteriostatic effect at concentration as low as 0.75% on Klebsiella pneumoniae , 1% on E. coli , 4% on Bacillus subtilis and S.typhi giving inhibition zones of 10m.m. . Inhibition depended on concentration of Tamarind and on the tested organisms. Ethanol extract from Tamarind was the most effective inhibitor against all tested organisms, while chloroform extract had the least inhibitory effect.

Ray and ***Majumdar (1976)*** reported that ethanol 95% extract of fruit of Tamarind on agar plate was active on Bacillus subtilis , E. coli , Salmonella typhosa , Staphylococcus aureus and Vibrio cholera. On the other hand , ***Ross et al. (1980)*** found that acetone extract of Tamarind fruit on agar plate was active on S, typhimurium , while ethanol 70% extract was active on B. cereus , B. megaterium , E. coli , Pseudomonas aeruginosa , S. typhimurium , Staph. albus and Staph. Aureus

Neidhardt et al. (1990) mentioned that, the wall of a typical Gram- negative cell consists a single, gigantic and sac like molecule of murein , of one or few layers thick while the wall of a Gram-positive cell , e.g. B.subtilis or Staphylococci , is made up of many layers of murein plus teicholic acids . These variations between the cell wall structure of Gram-negative and –positive bacteria may act as a limiting factor in controlling the level of saponin interaction or penetration within these two bacterial groups.

Gamal (1992) showed that bacteria, in general was more resistant to the applied concentrations of saponin when compared with fungi, a fact which may be due to lack of sterols in bacteria. On the other hand, ***Leath et al. (1972)*** reported that saponins inhibit growth of some microorganisms which lack sterols in their cell membrane.

Haraguchi et al. (1998) examined the antimicrobial activity of retrochalcones , licochalcone A , B , C and D and echinatin isolated from the roots of Glycerrhiza inflate " a source of Liquorice" and showed that they inhibited the growth of Gram-positive bacteria . ***Abo El-Azm (1999)*** showed that cool aqueous extract of Liquorice inhibited the growth of S.aureus , B.cereus , B. subtilis , Proteus mirabilis , Salmonella typhimurium and E. coli . He also mentioned that the higher the concentration, the more inhibition zone for all organisms. The two major constituents of Liquorice are glycyrrhizin and flavonoids . According to test tube studies, glycyrrhizin has anti-inflammatory actions and may inhibit the breakdown of the cortisol produced by the body. Liquorice may also have antiviral, antioxidants and work to protect liver cells. In the test tubes, the flavonoides have been shown to kill Helicobacter pylori, the bacteria that cause most ulcers and stomach inflammation ***(Josephs et al., 2001, Beil et al., 1995, Amer and Metwalli, 2000.***

Leob et al. (1989) reported that the main constituents of Carob are sugars and tannins. Carob tannins have an astringent effect in the gastrointestinal tract making them useful for treating diarrhea. They may also bind to (and thereby inactivate) toxins and inhibit growth of bacteria.The sugar makes Carob gummy and able to act as thickener to absorb water, another action that may help decrease diarrhea. ***Oboh and Elusiyan (2004)*** reported that the antimicrobial effect of the unfortified roselle extract was low against the entire organisms.

Imbabi et al. (1992) mentioned that Tamarind seed extracts have recently been strong antimicrobial action against E. coli and may be due to tamarindineol (5-hydroxy-2-oxo-hexa-3, 5-dienol). Antimicrobial properties of plants are desirable tools in the control of undesirable microorganisms especially in the treatment of infections and in food spoilage ***(Aboaba et al. 2006).***

This study is aimed to determine the rate of susceptibility of 10 organisms to the aqueous extracts of the tested 4 Egyptian plants for use as therapeutic agents.

Material and Methods

The antibacterial effect of aqueous extract of Karkade"Hibiscus sabdariffa", Tamarind"Tamarindus indica", Liquorice"Glycyrrhiza glabra"and Carob"Ceratonia siliqua" were tested against 10 selected organisms : Aeromonas hydrophila , Bacillus cereus , Aerobacter aerogenes , Escherichia coli , Pseudomonas sp. , Salmonella typhimurium , Salmonella paratyphi , Serratia marcesence , Shigella flexenary and Shigella sp. The investigated plants were obtained from Faculty of Pharmacy Farm, Giza, Cairo University.The tested microorganisms were kindly provided from Faculty of Agriculture, Cairo University and National Institute of Diabetes and Endocrine Glands, on nutrient agar slants at 4degree c. Each organism was transferred to tryptic soy broth with 0.6% yeast extract (Difco) and grown for 24 h. at 30 degree c. before use.

Preparation of plant extracts:

1a - A 100 g. of dried plant added to 1 L boiled distilled water in a sterile flask.

1b -- The content was filtered and the extract used in the experiments.

2 - Repeat (1a), the flask was left for 10 min., and then filtered.

3 - In another flask add 1 L cold distilled water on the same amount of plant, then filtered and the extract used in the experiments.

4 - As in (3) but left for 6 h.

5 - As in (3) but left for 12 h.

6 - As in (3) but left for 18 h.

7 - As in (3) but left for 24 h.

then filtered after each time and the extracts used in the experiments.

Antimicrobial activity:

It was tested using agar diffusion assay ***(Abou Zeid and Shehata , 1969, Aboaba et al. 2006)*** o.2 ml of a 24h broth culture of pathogens were aseptically introduced and evenly spread using bent sterile glass rod on the surface of sterile Mueller-Hinton agar plates. Three wells of 6.0mm in diameter were aseptically punched on each agar plate using a sterile cork bore. Fixed volume (0.1ml) of the plant extracts was carefully placed in each well. The plates were incubated at 37 degree c. for 24 h. The zone of inhibition of each well was obtained by measuring the underside of the plate in two planes with a ruler calibrated in millimeter. The control was placed with 0.1ml of distilled water and incubated.

Results and Discussion

Twenty eight aqueous extracts of four Egyptian plants were tested for their antibacterial activity against 10 pathogenic bacteria. 10% concentrate extracts of various plants were used for testing antibacterial potential. The four plants were found to have inhibitory effect against bacteria. Tables 1-4 show that water extracts of Hibiscus sabdariffa , Tamarindus indica , Glycyrrhiza glabra and Ceratonia siliqua exhibited antibacterial activity.

Ceratonia siliqua (Carob) extracts are the highest effective against Bacillus cereus (Table 4 and Fig. 4) specially the cold aqueous extract, left for 18 h. then filtered, (diameter of the inhibition zone was 42mm.). Also all the different aqueous extracts of this plant inhibit the growth of Shigella flexenary(mean inhibition zone was 17.8mm.) . All the Carob extracts had no activity against Serratia marcesence and Aeromonas hydrophila .***Kivcak et al. (2002)*** found that the ethanol extract of Carob inhibited the growth of five of the 10 microorganisms but had no effect on the growth of E. coli, Staphylococcus aureus , Enterococcus faecalis or Pseudomonas aeruginosa .

Tables 1and 3 showed the effect of Liquorice on growth of all the tested organisms. The Seven extracts of each plant were able to produce clear zones on all the ten strains used indicating bactericidal activity. S. typhimurium was the most sensitive of the boiling water extract of Liquorice , the agar diffusion test results showed the presence of a wide zone of inhibition -23mm.of S. typhimurium , 15mm. of S. paratyphi- surrounding the agar wells indicating antisalmonellic activity , followed by Pseudomonas sp.(20 mm. in diameter) . Cold water extract of Liquorice inhibited the growth of B. cereus , the diameter size of inhibition zone was 23 mm.(as show in Fig.1 ,Table 1) .

Liquorice is one of the most extensively used and scientifically investigated herbal medicines. Its traditional uses include the treatment of peptic ulcers, asthma, pharyngitis, malaria, abdominal pain, insomnia and infections. Abo El-***Azm(1999)*** showed that aqueous extract of Liquorice inhibited the growth of S. typhimurium , Staphylococcus aureus , B. cereus and E. coli.The majority of its anti-microbial effects are due to its isoflavanoid components. Chemical investigations have revealed the presence of a wide variety of bioactive phenolic constituents in liquorice; these have attracted attention as a potential source of activity against all tested organisms specially S. typhimurum and B.cereus ***(Tsukiyama, et al. , 2002)***.

The aqueous extract of Hibiscus sabdariffa, as in ***Owulade et al. (2004),*** inhibited effectively the growth of all tested bacterial pathogens, caused inhibition zones measuring 10 - 40 mm. Boiling water extract of this plant appeared to be the most effective on B. cereus (diameter of the inhibition

zone was 40 mm.), results from the antimicrobial screening tests are shown in Table (3). Moderate antimicrobial activity was shown by Karkade extracts on Shigella flexenary (mean diameters of the inhibition zones was 20.29mm.), Pseudomonas sp.(19,71mm.) , Shigella sp.(18.71mm.) and on E. coli(17.86mm.) .Extracts of the roots were effective against bacteria that may cause diarrhea as Escherichia coli, Salmonella typi, and Vibrio cholera thus supporting the traditional uses for this purpose ***(Ajagbonna et al. 2001, Moshi et al., 2006).***

The in vitro inhibitory effect of Karkade on the growth of Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter baumannii was ***studied by Liu et al.(2005)*** . The data from inhibition zone values showed that roselle calyx (Karkade) extract inhibited effectively the growth of all test bacterial pathogens as in our results.The antibacterial effect of this plant may be due to saponins and tartaric acid. Oboh and ***Elusiyan (2004)*** mentioned that fortification of roselle extract with pineapple juice and lemon grass greatly enhanced the inhibition of the growth of Bacillus sp., Pseudomonas aeruginosa , Lactobacillus sp. and Corynebacterium sp.

Burkholderia pseudomallei cause melioidosis, a life-threatening infection common among paddy cultivators in Southeast Asian countries. No plant materials have been investigated for its activity against Burkholderia pseudomallei . Therefore, a preliminary study was carried out using disc diffusion. Only methanol leaf extracts of Tamarindus indica exhibited anti-B. pseudomallei activity ***(Muthu et al. , 2005)*** . They found that the leaves of T. indica could be an important source of new antimicrobial agent against Burkholderia pseudomallei .

In our study cold water (left 12, 18, 24h. then filtered) extracts of Tamarindus indica , as in Table(2),Fig 2 , inhibit the growth of Pseudomonas sp.(the inhibition zone was 20 mm. for each extract) . Also Hibiscus sabdariffa and Glycyrrhiza glabra succeed to show zones of inhibition of the organism (Tables1 and 3). Boiling water extract of Tamarindus indica was the most effective on B. cereus (Table 2). Tamarindus indica extracts have antimicrobial activities against all the ten bacteria except its cold water extract on Aeromonas hydrophila , Aerobacter aerogenes , S. paratyphi and Serratia marcesence .Imbabi et al. (1992) mentioned that the antibacterial effect of Tamarind may be due to tamarindineol.

Only one extract (boiling water extract) out of 7 derived from Carob showed an antimicrobial activity against S. paratyphi (Table 4).To evaluate the effects of herbal extracts derived from plants commonly prescribed by traditional practitioners for the treatment of typhoid fever (Nkuo-Akenji et

al., 2001),the 4 plants were tested for antibacterial activity against Salmonella paratyphi and S. typhimurium. S. paratyphi was most sensitive to boiling water , left for 10 min., then filtered , extract of Glycyrrhiza glabra(Liquorice) and Hibiscus sabdariffa (Karkade) , also to cold water , left for 6 h and 24 h. , then fitered , extracts of Glycyrrhiza glabra . While S. typhimurium was most sensitive to boiling water extract of Glycyrrhiza glabra. Ceratonia siliqua (Carob) did not show effect on the two pathogens.

Of the 28 extracts, B. cereus , Shigella flexenary and E.coli were sensitive to all extracts with diameters of inhibition zones ranging from 10 – 42mm , as shown on Tables (6, 13 and 8) , respectively.Eight extracts showed no inhibition of growth of each: Aeromonas hydrophila and Serratia marcescens i.e. all extracts of Carob and one of Tamarind (Table 5, 12). Pseudomonas was sensitive to twenty three extracts (Table 9) and no effect with cold water extracts of Carob.Karkade produced the highest zones of inhibition (average of inhibition zone was 19.71mm.) against Pseudomonas.

S. typhimurium and S. paratyphi were not sensitive to any of the Carob extracts , except some low effects with few extracts only (Tables 10,11). We observed zones of inhibition, ranging from 10 – 24mm, for twenty four extracts against Shigella sp. (Table 14). Table (7) shows that the zones of inhibition of the bacteria Aerobacter aerogenes ranging from 11 – 20mm. Cold water extracts of Carob showed no inhibitory effects against Aerobacter aerogenes .

The most effective method of extraction which gave the highest zone of inhibition (more than or equal 15 mm.) against the tested bacteria are shown in tables and figures (5- 14)

A) Liquorice :

*E. coli and Shigella flexenary had no observed sensitivity to all extracts of this plant.

1-- Boiling water extract, is effective against

Salmonella typhimurum	Bacillus cereus	Pseudomonas sp.
Salmonella paratyphi	Aeromonas hydrophila	

2-- Boiling water extract, left for 10 min. then filtered, is effective against

Salmonella typhimurum	Bacillus cereus	Pseudomonas sp.
Salmonella paratyphi	Aeromonas hydrophila	Aerobacter aerogenes

3-- Cold water extract, is effective against

Bacillus cereus	Aerobacter aerogene	Shigella sp.

4-- Cold water extract, left for 6h.then filtered is effective against

Salmonella typhimurum Shigella sp. Salmonella paratyphi
Serratia marcesence Aerobacter aerogenes

5-- Cold water extract, left for 12h.then filtered is effective against

Salmonella typhimurum Pseudomonas sp. Aerobacter aerogenes Shigella sp.
Salmonella paratyphi Serratia marcesence
Aeromonas hydrophila
Bacillus cereus

6-- Cold water extract, left for 18h.then filtered is effective against

Salmonella typhimurum Shigella sp. Aerobacter aerogenes Bacillus cereus
Salmonella paratyphi Serratia marcesence

7-- Cold water extract, left for 24h.then filtered is effective against

Salmonella typhimurum Shigella sp. Salmonella paratyphi
Aerobacter aerogenes Bacillus cereus

B) Tamarind:

*Aerobacter aerogenes had no observed sensitivity to the extracts of this plant.

1-- Boiling water extract, is effective against

Salmonella typhimurum Pseudomonas sp. Bacillus cereus
Shigella flexenary

2-- Boiling water extract, left for 10 min. then filtered, is effective against

As in 1-- without Salmonella typhimurum

3-- Cold water extract, is effective against

Shigella sp. Only

4-- Cold water extract, left for 6h.then filtered, is effective against

Pseudomonas sp. Bacillus cereus Shigella flexenary

5-- Cold water extract, left for 12h.then filtered, is effective against

Pseudomonas sp.	Shigella sp.	Shigella flexenary
Bacillus cereus	E. coli	Aeromonas hydrophila
Serratia marcesence		

6-- Cold water extract, left for 18h.then filtered, is effective against

Pseudomonas sp.	Bacillus cereus	Shigella sp.

7-- Cold water extract, left for 24h.then filtered, is effective against

Pseudomonas sp.	Salmonella typhimurum	S. paratyphi
Bacillus cereus	Shigella sp.	Shigella flexenary

C) Karkade :

*The 10 tested bacteria were inhibited .

*All the 7 extracts of this plant were effective on the growth of:-

Pseudomonas sp.	Shigella flexenary
Bacillus cereus	Shigella sp.

Beside, the extracts are also effective against other bacteria as follows:

1-- Boiling water extract:

S. paratyphi	Aerobacter aerogenes	Serratia marcesence

2-- Boiling water extract, left for 10 min. then filtered:

S. paratyphi	E. coli
Aeromonas hydrophi	Serratia marcesence

3-- Cold water extract:

S. paratyphi	E. coli

4-- Cold water extract, left for 6h.then filtered:

S. paratyphi	Serratia marcesence	E. coli

5-- Cold water extract, left for 12h.then filtered:

E. coli	Aeromonas hydrophila
Serratia marcesenc	Aerobacter aerogenes

6-- Cold water extract, left for 18h.then filtered:

Salmonella typhimurum E. coli

7-- Cold water extract, left for 24h.then filtered:

Salmonella typhimurum Serratia marcesence E. coli

S. paratyphi Aerobacter aerogenes

D) Carob:

*All the extracts of this plant were effective against only these two pathogens :

Bacillus cereus Shigella flexenary

The most effective extracts which inhibit (the diameter of inhibition zone more than or equal 15 mm.) the tested bacteria as shown in tables and figures (5- 14) were as follows :

Aeromonas hydrophila:

Liquorice

- Boiling water extract, filtered directly.

Liquorice or Karkade

- Boiling water extract, left for 10 min., then filtered.

Liquorice , Tamarind or Karkade

- Cold water extract, left for 12h. , then filtered

Bacillus cereus :

Liquorice, Tamarind , Karkade or Carob

- Boiling water extract (or) Boiling water extract, left for 10 min., then filtered .

Liquorice

- Cold water extract (or) Cold water extract, left for 12h. or 18h. or 24h. , then filtered.

Tamarind, Karkade and Carob

- Cold water extract, left for 6h. or 12h. or 18h. or 24h. , then filtered.

Aerobacter aerogenes

Liquorice

- Boiling water extract, left for 10 min., then filtered
- Cold water extract, left for 6h. or 12h. or 18h. or 24h. , then filtered.

Karkade

- Boiling water extract, filtered directly.
- Cold water extract, left for 12h. or 24h. , then filtered.

E. coli

Tamarind

- Cold water extract, left for 12h. , then filtered

Karkade

- Boiling water extract, left for 10 min., then filtered.
- Cold water extract (or) Cold water extract, left for 6h. or 12h. or 18h. or 24h. , then filtered

Pseudomonas sp.

Liquorice, Tamarind , Karkade

- Boiling water extract (or) boiling water extract, left for 10 min., then filtered.

Liquorice

- Cold water extract, left for 12h. , then filtered

Tamarind or Karkade

- Cold water extract, left for 6h. or 12h. or 18h. or 24h. , then filtered

Karkade

- Cold water extract, filtered directly.

Salmonella typhimurum

Liquorice

- Boiling water extract (or) boiling water extract, left for 10 min., then filtered
- Cold water extract, left for 6h. or 12h. or 18h. or 24h. , then filtered

Tamarind

- Boiling water extract, then filtered
- Cold water extract, left for 24h. , then filtered

Karkade

- Cold water extract, left for 18h. or 24h. , then filtered

S. paratyphi

Liquorice and Karkade

- Boiling water extract (or) boiling water extract, left for 10 min., then filtered.

Liquorice

- Cold water extract, left for 6h. or 12h. or 18h. or 24h. , then filtered

Tamarind

- Cold water extract, left for 24h. , then filtered

Karkade

- Cold water extract (or) Cold water extract, left for 6h. or 24h. , then filtered

Serratia marcesence

Liquorice

- Cold water extract, left for 6h. or 12h. or 18h. , then filtered

Tamarind

- Cold water extract, left for 12h. , then filtered

Karkade

- Boiling water extract (or) boiling water extract, left for 10 min., then filtered
- Cold water extract, left for 6h. or 12h. or 24h. , then filtered

Shigella flexenary

Tamarind, Karkade and Carob

- Boiling water extract (or) boiling water extract, left for 10 min., then filtered

Tamarind

- Cold water extract, left for 6h. or 12h. , then filtered

Karkade and Carob

- Cold water extract (or) Cold water extract, left for 6h. or 12h. or 18h. or 24h. , then filtered

Shigella sp.

Liquorice , Tamarind or Karkade

- Cold water extract, filtered directly.

Liquorice or Karkade

- Cold water extract, left for 6h. or 12h. or 18h. or 24h. , then filtered

Tamarind

- Cold water extract, left for 12h. or 18h. or 24h. , then filtered

Karkade

- Boiling water extract (or) boiling water extract, left for 10 min., then filtered

Antimicrobial properties of plants are desirable tools in the control of undesirable microorganisms especially in the treatment of infections and in food spoilage **(Aboaba et al. 2006)**

It is necessary to fractionate the most active bactericidal extracts to better assess the activity of their components. Some of the plant extracts tested in this work may contain compounds with selective action against certain bacteria, this may account for the traditional use as medicinal plants **(Ates and Erdogrul , 2003 , Moses et al. 2006).**

In conclusion, the antimicrobial activity observed on the tested extracts may provide useful data for the utilization of antimicrobial principles of these aqueous extracts.

Table (1): Antibacterial activity (as inhibition zone) of LIQUORICE extracts on the tested organisms

The different methods used	A. hydr.	B. cer.	Ae. aer.	E. coli	Ps. sp.	S. typhim.	S. para.	Se. mar.	Sh. flex.	Sh. sp.
Add boiling water	16	21	14	10	20	23	15	13	10	12
Add boiling water, left for10 min.	16	22	15	10	19	20	20	13	10	12
Add cold water	10	23	20	10	11	14	14	11	10	15
Add cold water, left for 6 h.	13	15	16	10	14	16	20	16	11	15
Add cold water, left for 12 h.	16	15	16	10	15	17	15	16	13	15
Add cold water, left for 18 h.	10	15	16	10	13	20	15	16	14	15
Add cold water, left for 24 h.	10	16	20	10	13	17	20	12	10	18

Zone of Inhibition = or > 10mm was considered sensitive

Table (2): Antibacterial activity (as inhibition zone) of TAMARIND extracts on the tested organisms

The different methods used	A. hydr.	B. cer.	Ae. aer.	E. coli	Ps. spp.	S. typhim.	S. para.	Se. mar.	Sh. flex.	Sh. spp.
Add boiling water	13	21	11	12	19	17	12	12	15	11
Add boiling water, left for 10 min.	13	17	11	12	19	12	10	13	18	12
Add cold water	0	14	0	10	13	12	0	0	11	15
Add cold water, left for 6 h.	13	17	12	14	17	12	10	14	16	13
Add cold water, left for12 h.	15	19	12	18	20	12	10	16	16	16
Add cold water, left for 18 h.	13	19	12	12	20	13	11	16	12	15
Add cold water, left for24 h.	14	19	13	14	20	15	15	18	14	14

Table (3) Antibacterial activity (as inhibition zone) of KARKADE extracts on the tested organisms

The different methods used	A. hydr.	B. cer.	Ae. aer.	E. coli	Ps. Spp.	S. typhim.	S. para.	Se. mar.	Sh. flex.	Sh. spp.
Add boiling water	14	40	15	10	21	14	19	16	18	21
Add boiling water, left for 10 min.	15	39	14	20	18	14	20	12	19	24
Add cold water	11	20	11	17	19	12	16	15	18	20
Add cold water, left for 6 h.	10	21	12	19	20	13	15	15	22	19
Add cold water, left for 12 h.	15	36	17	18	19	13	12	15	28	15
Add cold water, left for 18 h.	14	35	12	21	21	15	13	13	17	17
Add cold water, left for 24 h.	13	36	15	20	20	15	15	15	20	15

Table (4): Antibacterial activity (as inhibition zone) of CAROB extracts on the tested organisms

The different methods used	A. hydr.	B. cer.	Ae. aer.	E. coli	Ps. Spp.	S. typhim.	S. para.	Se. mar.	Sh. flex.	Sh. spp.
Add boiling water	0	35	14	10	11	0	12	0	16	13
Add boiling water, left for 10 min.	0	35	14	10	12	10	0	0	17	11
Add cold water	0	21	0	10	0	0	0	0	17	0
Add cold water, left for 6 h.	0	35	0	10	0	10	0	0	20	10
Add cold water, left for12 h.	0	40	0	10	0	0	0	0	19	0
Add cold water, left for 18 h.	0	42	0	10	0	0	0	0	19	0
Add cold water, left for24 h.	0	21	0	10	0	0	0	0	17	0

Results are reported as inhibition zones (IZ;mm.)

Table (5): Comparison between the effects of the investigated plants on Aeromonas hydrophila

The methods used	KARKADE	TAMARIND	LIQUORICE	CAROB
Add boiling water	14	13	16	0
Add boiling water ,left for 10 min.	15	13	16	0
Add cold water	11	0	10	0
Add cold water ,left for 6 h.	10	13	13	0
Add cold water ,left for 12 h.	15	15	16	0
Add cold water ,left for 18 h.	14	13	10	0
Add cold water ,left for 24 h.	13	14	10	0

Table(6) : Comparison between the effects of the investigated plants on BACILLUS CEREUS

The methods used	KARKADE	TAMARIND	LIQUORICE	CAROB
Add boiling water	40	21	21	35
Add boiling water ,left for 10 min.	39	17	22	35
Add cold water	20	14	23	21
Add cold water ,left for 6 h.	21	17	13	35
Add cold water ,left for 12 h.	36	19	15	40
Add cold water ,left for 18 h.	35	19	15	42
Add cold water ,left for 24 h.	36	19	16	21

Table (7): Comparison between the effects of the investigated plants on AEROBACTER AEROGENES

The methods used	KARKADE	TAMARIND	LIQUORICE	CAROB
Add boiling water	15	11	14	14
Add boiling water ,left for 10 min.	14	11	15	14
Add cold water	11	0	20	0
Add cold water ,left for 6 h.	12	12	16	0
Add cold water ,left for 12 h.	17	12	16	0
Add cold water ,left for 18 h.	12	12	16	0
Add cold water ,left for 24 h.	15	13	20	0

Table (8): Comparison between the effects of the investigated plants on E. COLI

The methods used	KARKADE	TAMARIND	LIQUORICE	CAROB
Add boiling water	10	12	10	10
Add boiling water ,left for 10 min.	20	12	10	10
Add cold water	17	10	10	10
Add cold water ,left for 6 h.	19	14	10	10
Add cold water ,left for 12 h.	18	18	10	10
Add cold water ,left for 18 h.	21	12	10	10
Add cold water ,left for 24 h.	20	14	10	10

Table (9): Comparison between the effects of the investigated plants on Pseudomonas Sp.

The methods used	KARKADE	TAMARIND	LIQUORICE	CAROB
Add boiling water	21	19	20	11
Add boiling water ,left for 10 min.	18	19	19	12
Add cold water	19	13	11	0
Add cold water ,left for 6 h.	20	17	14	0
Add cold water ,left for 12 h.	19	20	15	0
Add cold water ,left for 18 h.	21	20	13	0
Add cold water ,left for 24 h.	20	20	13	0

Table (10): Comparison between the effects of the investigated plants on Salmonella Typhimurium

The methods used	KARKADE	TAMARIND	LIQUORICE	CAROB
Add boiling water	14	17	23	0
Add boiling water ,left for 10 min.	14	12	20	10
Add cold water	12	12	14	0
Add cold water ,left for 6 h.	13	12	16	10
Add cold water ,left for 12 h.	13	12	17	0
Add cold water ,left for 18 h.	15	13	20	0
Add cold water ,left for 24 h.	15	15	17	0

Table (11): Comparison between the effects of the investigated plants on Salmonella Paratyphi

The methods used	KARKADE	TAMARIND	LIQUORICE	CAROB
Add boiling water	19	12	15	0
Add boiling water ,left for 10 min.	20	10	20	10
Add cold water	16	0	14	0
Add cold water ,left for 6 h.	15	10	20	10
Add cold water ,left for 12 h.	12	10	15	0
Add cold water ,left for 18 h.	13	11	15	0
Add cold water left for 24 h.	15	15	20	0

Table (12): Comparison between the effects of the investigated plants on Serratia Marcescens

The methods used	KARKADE	TAMARIND	LIQUORICE	CAROB
Add boiling water	15	0	11	0
Add boiling water ,left for 10 min.	16	13	13	0
Add cold water	12	13	13	0
Add cold water ,left for 6 h.	15	13	16	0
Add cold water ,left for 12 h.	15	15	16	0
Add cold water ,left for 18 h.	13	13	16	0
Add cold water ,left for 24 h.	15	14	12	0

Table (13): Comparison between the effects of the investigated plants on Shigella Flexenary

The methods used	KARKADE	TAMARIND	LIQUORICE	CAROB
Add boiling water	18	15	10	16
Add boiling water ,left for 10 min.	19	18	10	17
Add cold water	18	11	10	17
Add cold water ,left for 6 h.	22	16	11	20
Add cold water ,left for 12 h.	28	16	13	19
Add cold water ,left for 18 h.	17	12	14	19
Add cold water ,left for 24 h.	20	14	10	17

Table (14) : Comparison between the effects of the investigated plants on Shigella Sp.

The methods used	KARKADE	TAMARIND	LIQUORICE	CAROB
Add boiling water	21	11	12	13
Add boiling water ,left for 10 min.	24	12	12	11
Add cold water	20	15	15	0
Add cold water ,left for 6 h.	19	13	15	10
Add cold water ,left for 12 h.	15	16	15	0
Add cold water ,left for 18 h.	17	15	15	0
Add cold water ,left for 24 h.	15	15	18	0

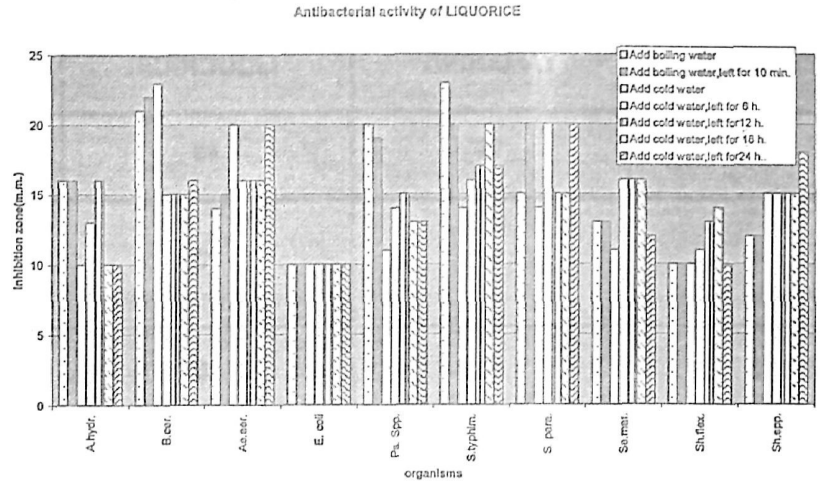

Figure 1

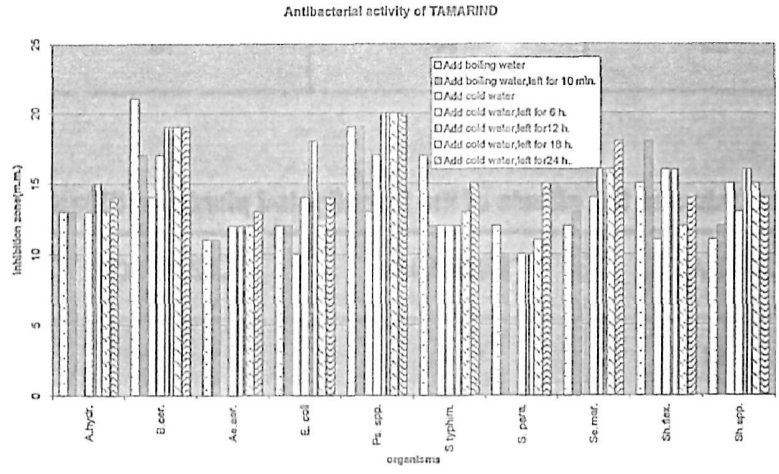

Figure 2

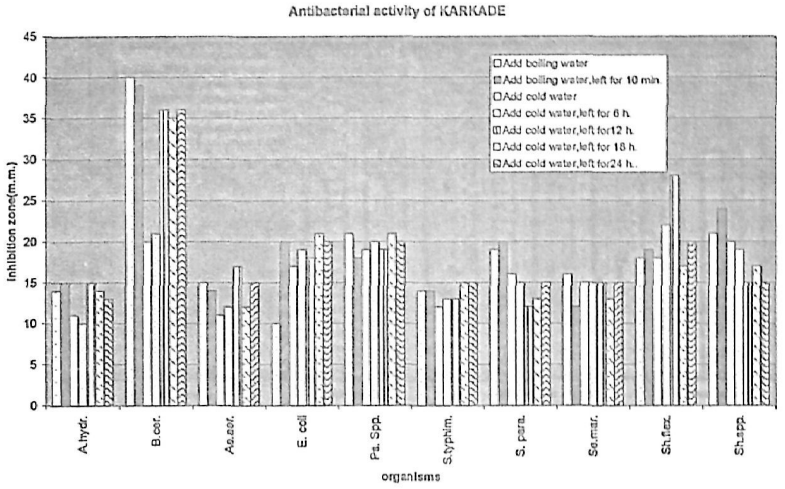

Figure 3

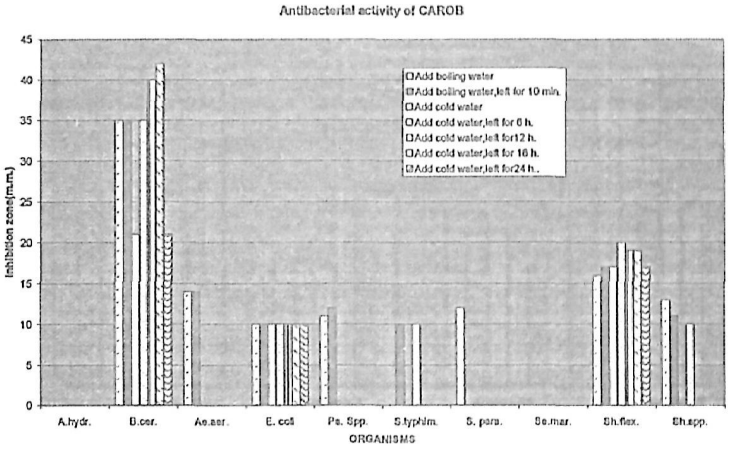

Figure 4

FIG 5 : Effects of the plants on AEROMONAS HYDROPHILA

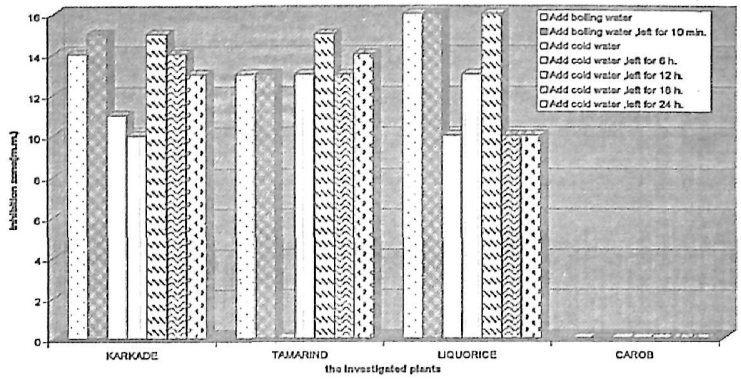

Figure 5

FIG 6 : The effects of investigated plants on BACILLUS CEREUS

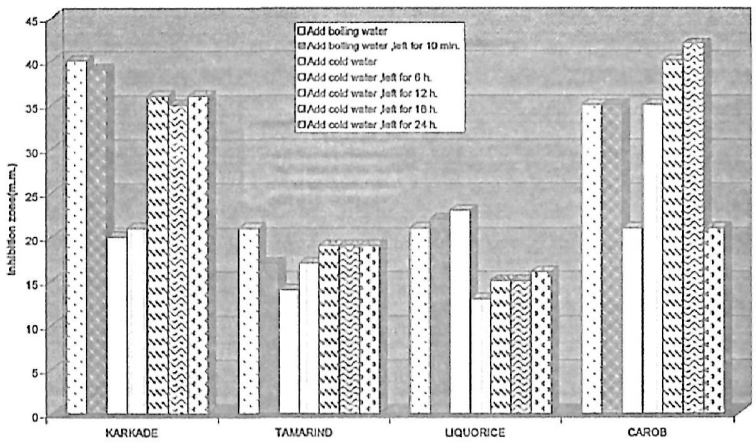

Figure 6

FIG 7 : Effects of the plants on AEROBACTER AEROGENES

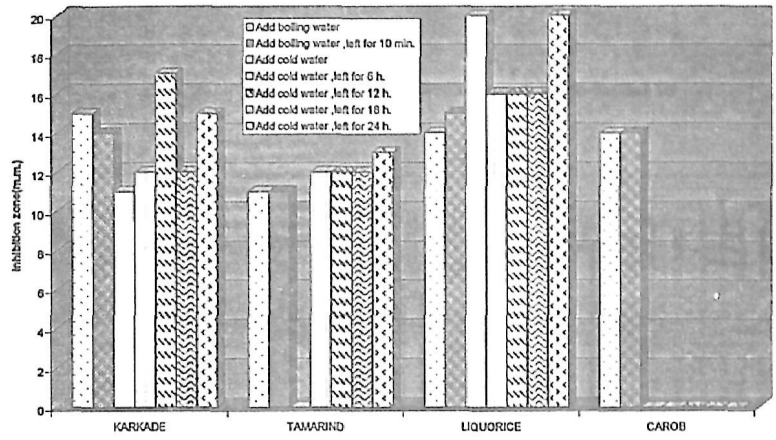

Figure 7

FIG 9 : The effects of the investigated plants on E. COLI

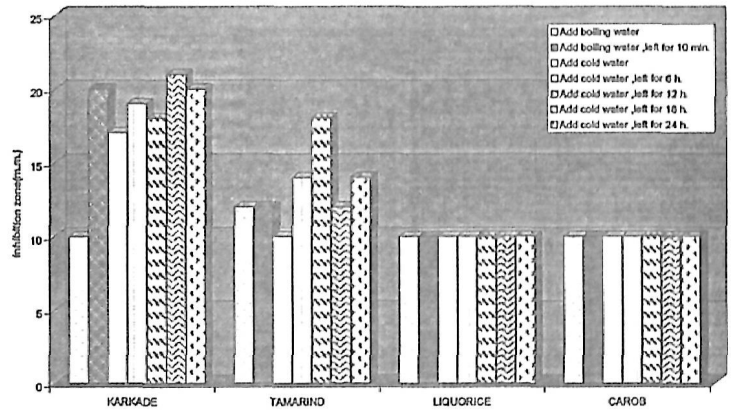

Figure 8

FIG 8 : Effects of the 4 plants on PSEUDOMONAS sp.

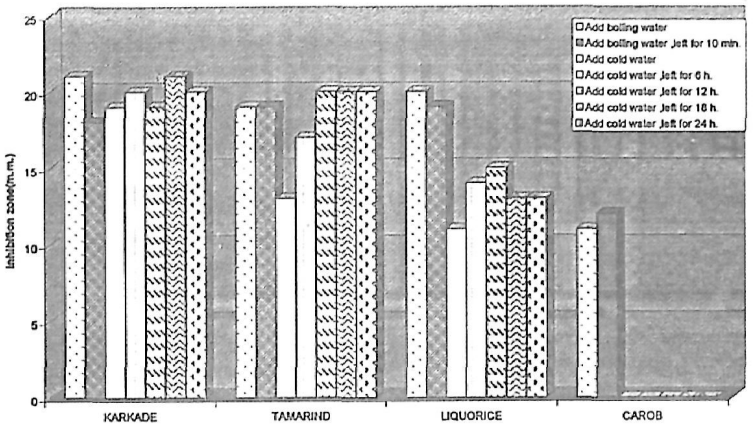

Figure 9

FIG 10 : Effects of the 4 plants on SALMONELLA TYPHIMURUM

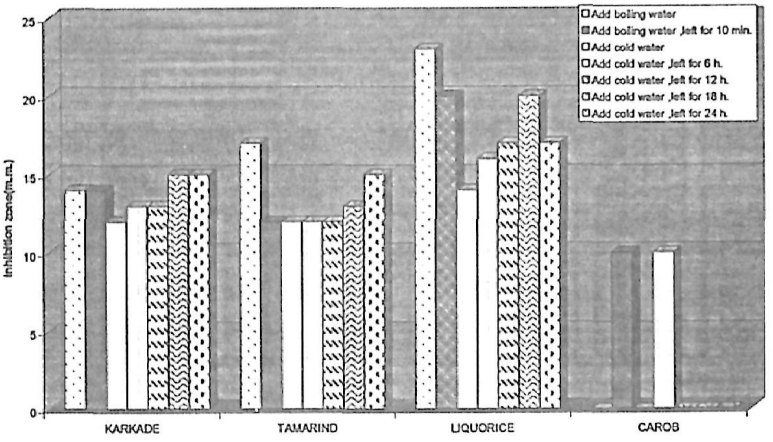

Figure 10

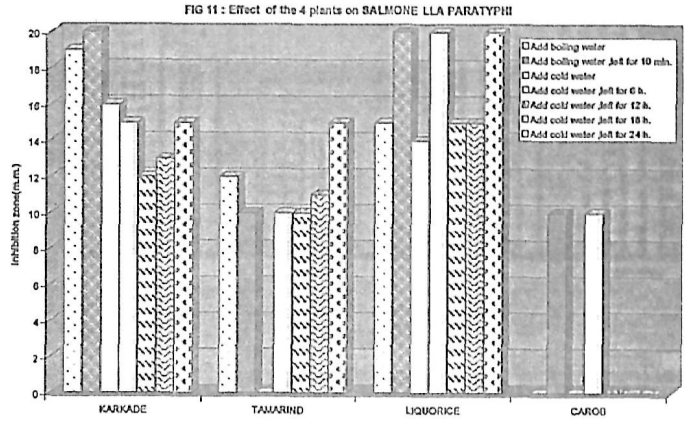

Figure 11

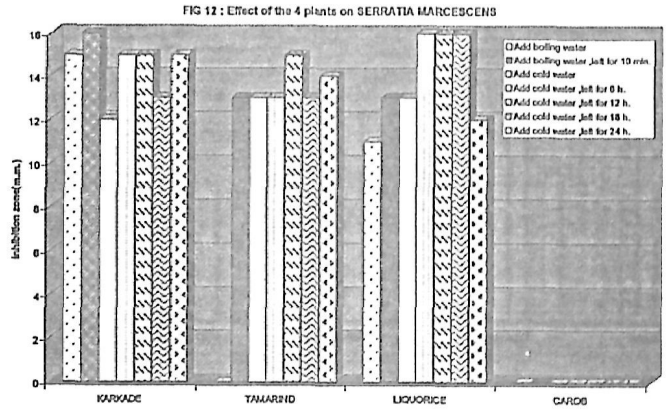

Figure 12

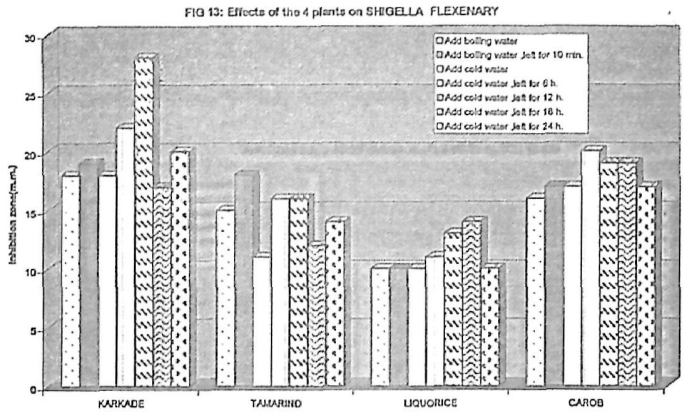

Figure 13

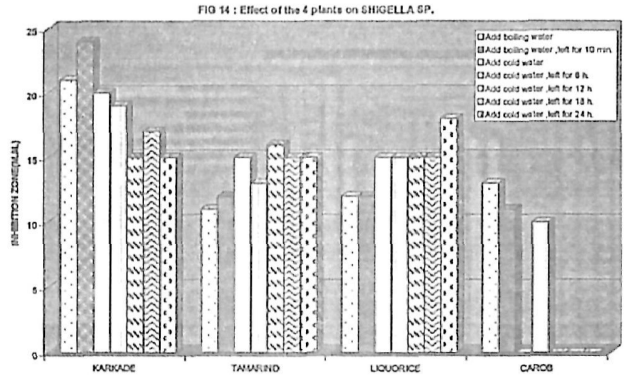

Figure 14

Acknowledgement

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التاثير المضاد للميكروبات فى بعض النباتات المصريه

عفاف على امين ، عاطف حسين السيد ، مرفت احمد فؤاد ، اسامه عبد العاطى رشوان

الملخص العربى

فى هذه الدراسه تم فحص تاثير ٢٨ مستخلص خام لاربع نباتات مصريه وهى الكركديه ، التمر هندى ، العرقسوس و الخروب ضد ١٠ سلالات بكتيريه ممرضه .

وجد ان بكتيريا باسيلس سيريس ، شيجيلا فلكسنارى وشيريشيا كولاى حساسه لكل هذه المستخلصات الثمانى والعشرين وتراوح قطر التثبيط لكل منها : من ١٣ – ٤٢ مم ، من ١٠ – ٢٨ مم و من ١٠ – ٢١ مم على التوالى . بينما كانت سلالة الشيجيلا المختبره حساسه لاربع وعشرين مستخلص فقط بقطر تثبيط يتراوح بين ١٠ – ٢٤ مم .

وظهر ان ٢٣ مستخلص منع نمو كل من سلالة السودوموناس و السالمونيلا تيفيموريوم بقطر تثبيط بين ١١ الى ٢١ مم و من ١٠ الى ٢٣ مم على التوالى .

كما ان السالمونيلا باراتيفى و ايروباكتر ايروجينيس وجد انهما حساستان لاثنين وعشرين مستخلص (قطرمنطقة التثبيط من ١٠ و من ١١ الى ٢٠ مم على التوالى) . و ميكروب السيراتيا مارسيسنس وايروموناس هيدروفيلا منع نموهما بواسطة ٢٠ مستخلص وتراوح قطر التثبيط من ١١ ومن ١٠ الى ١٦ مم بالتوالى .

اظهر الكركديه نشاط مضاد واضح ضد جميع السلالات البكتيريه المختبره . وكان المستخلص بعد ٢٤ ساعه من النقع على البارد ثم الترشيح افضل طريقه لاستعمال النبات حيث منع نمو ٩ من الميكروبات المختبره العشره .

اظهرت الدراسه ان بكتيريا الشيريشيا كولاى والشيجيلا فلكسنارى مقاومه لمستخلصات العرقسوس . و ان مستخلص العرقسوس بعد ١٢ ساعه من النقع على البارد ثم ترشيحه احسن الطرق المستخدمه فى الدراسه للنبات فهو يمنع نمو ٨ ميكروبات من العشرة المختبرين .

ثم تؤثر مستخلصات التمر هندى على بكتيريا ايروباكتر ايروجينيس . وظهر ان مستخلص التمر هندى بعد ١٢ ساعه من النقع على البارد ثم الترشيح احسن الطرق بالنسبه لهذا النبات حيث اظهر نشاطا ضد ٧ ميكروبات من العشره .

كان لنشاط مستخلصات نبات الخروب محدود ، فقد اثر كل من هذه المستخلصات فقط على نمو الباسيلس سيريس والشيجيلا فليكسنارى (ميكروبين فقط من العشرة المختبرين) .

تشجع النتائج الاستخدام التجارى لمستخلصات هذه النباتات فى مقاومة العدوى البكتيريه وفى حفظ الاغذيه من التلوث البكتيرى .