

Synergism Effect of Antibacterial Activity of Some Medicinal Plants

Mona Al-Terehi^{1*}, Ali H. Al-Saadi²,HyderTurki Al-musawi³,HaiderK.Zaida²

RussulHikmatBehjet², ZahraaHaleem²

1. University of Kufa -College of Science

2. University of Babylon, College of Science

3.The Green University of Al-Qasim, College of Biotechnology

Mona_diabetis@yahoo.com

Abstract

Synergism between plant extract was carried out in present study using six common plants which extracted by mixture of methanol: water (20:80)%, then it characterized by thin layer chromatography, antimicrobial activity of these extract performed using OD of bacterial growth at 600 nm against *E.coli* , *S.aureus* , *Serratia Spp.* , *Klebsiillapnemoniae*, *Aeromonashydrophillia* isolation from different source in hospital tests were performed as only one extract on every bacterial species then two extract was mixed together for evaluated synergist efficiency on every bacterial species, results show that used one extract only causes decreased in bacterial growth in different level, barley was the more effect on pathogenic bacteria but sage was lower effect, it caused activated bacterial growth. Synergism between plant extract also show different effects level according to mixture forming and types of bacterial species. Present study concluded that mixture of plants used in herbal medicine have disadvantages in addition of its advantage to treatment bacterial infection, in other hand synergism between plants may be more effects.

Keywords: synergism , OD, pathogenic bacteria.

Introduction

Return to the nature is becoming important idea in the last decades, because of increasing side effects of drugs, drug tolerance in patients and new recombinants in genetic materials of bacteria which is responsible of drug resistance. The variation in phytochemicals compounds in different species of plants give us ability to use this materials in different application, like industrial, economic and medical application (Al-Saadi, 2012).

The medical applications of plant are important but it must be under the physicians recommendations for lowering level of its side effects.

Review of literature clarify the plants extracts roles as anti-bacterial agents in different site and pathogenesis. Mahesh and Satish (2008) used methanol leaf extracts of *Acacia nilotica*, *Sidacordifolia*, *Tinosporacordifolia*, *Withaniasomnifer* and *Ziziphmauritiana* showed significant antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Xanthomonasaxonopodispv.malvacearum* by inhibition zone method. Also some Indian traditional plant extracts such as *Ocimumsanctum*, *Ocimumgratissimum*, *Aeglemarmelos*, and *Adhatodavasicaleavess* showed antibacterial activity against human pathogenic Bacteria (Prasannabalajiet al., 2012).

Researches uses different method to extract phytochemicals compounds such as watery (cold and hot), organic solvents, directed and fractionation methods in order to obtain maximum concentrations of compounds.

Plants that used in present study was used in wide range in medical applications, such as anti-bacterial, anti-fungus, antioxidant and anti-mutagenesis. Also studies show that side effects of this plants extracts was low than industrial drugs.

1- Licorice (*glycyrrhizaglabra*)

Licorice (L) was one of the important plants have long history in medical applications, root is important part in plant which is contained flavonoid components (Yamamura et al., 1992). Licorice also contain Phenolic compounds, coumarins, volatile oil, saponine and minerals (AL-Turiahe, 2010).

Fukalet al., (2003) improve that licorice useful in treatment bacterial infections of *Streptococcus* mutants, *Staphylococcus aureus* and *Mycobacterium*. Also it have antibacterial activity against G⁺ and G⁻ bacteria such as *Bacillus subtilis*, MTCC121 , *Salmonella typhi* MTCC733 , *Yersinia enterocolitica* and *Klebsiellapneumoniae* MTCC (Khanuja, et al., 2006) .

2- Barley (*Hordeumvulgare* L.)

Barley (B) is one of the importance food sources in the world , all part of plants fruit, leaves, seeds, grains and whole plants has been used in different medical treatments (Marwat et al., 2012). Barley contain from soluble and insoluble dietary fiber (DF), vitamins E and C , B-complex vitamins, minerals and phenolic compounds, highly viscose of soluble polysaccharide (Mahesh et al., 2010).

Many studies supported using barley in different inflammation such as Intranasal inflammation by use in Iran (Ross, 2005).

3- *ORIGANUM VULGARE*

Origanum vulgare (O) used as antioxidant and anti-mutagenesis activity in many researches also in many studies it used many compounds that extracted by organic solvent such as ethanol, methanol and water from different parts of plants, Ashraf *et al.*, (2011) used equas and chloroform to extract phytochemicals compound from *Origanum vulgare* and used it as antibacterial and antifungal agents .

4- *Salvia officinalis* L

Salvia officinalis L (S) or it is known sage, genus from Lamiaceae family, using as flavoring agent in food preparation, and as medicinal plants using in different applications (Stanojevic *et al.*, 2010). Khalil and Li (2011) extract essential oil from *Salvia officinalis* and used it as antimicrobial agent, in the other hand many studies use extract of sage as antibacterial agents against gram+ and gram- bacteria (Behboud *et al.*, 2011). Stefanovic *et al.*, (2012) use synergism between sage and *CICHORIUM INTYBUS* against pathogenic bacteria.

5- *Zingiber officinale*

Commonly known as ginger (G) , rhizome was the important part of plant that use in medical application, studies clarified that extract of ginger consist from phenols, volatile oils (Nanjundiah *et al.*, 2009). Researchers use ginger as anti-inflammation and anti-thrombi (Thomson *et al.*, 2002). Galadima *et al.* (2011) use ginger extract against gastrointestinal track pathogenic bacteria. Kaushik and Goyal (2011) using methanolic and equase extract of ginger against bacterial spp. They found that methanolic extract was more efficient than other extract in disc diffusion methods.

6- Pomegranate

Punicagranatum (P) its known fruit that used in different food applications, the cortex of fruit contain tanins such as punicalin, punicalagin and granatin B also it contain alkaloids like pelletierine, pseudo pelletierine and methyl pelletierine (Al-saadi *et al.*, 2012) literature of review reported the Pomegranate efficiency against pathogenic bacteria *B. coagulans*, *B. cereus*, *B. subtilis* and *S. aureus* (Dahham *et al.* 2010).

The aim of this study is evaluation synergism between plant extract against Pathogenic bacteria *e.coli*, *S. typhi*, *S. aureus*, *Serratia*, *Klebsilla pneumoniae*, *Aeromonas hydrophilla* was isolated from different infection.

Materials and methods

- 1- Plant part that use in present study, licorice deried root, barley seeds, ginger dreid rhizomes, punica cortex, leaf of *ORIGANUM VULGARE* and sage.
- 2- **Plants extract:** The plants extracts of plant was prepared according to the method of Sato *et al.* (1990) with some modification. Specific weight of the plant and it is mixed with the average 1 gm to 3 ml of the solvent solution (20 % methanol: 80 % distilled water), The mixture is uniformed by electric blender for 30 minutes in room temperature. The solution is filtered by using gauze fabric for getting transudate solution. It is deried using incubator at 50C° for 24 hours, The dispers is kept in a dry place until it is used.
- 3- **Plant extract aliquot,** it prepares in 3 concentration (0.1 g/ml) then it sterilized using mellipor 0.2 mm.
- 4- **TLC profile ;** plant extract characterized by TLC, by use solvents (DW: ethylacetate: methanol) (20:60:20 v/v/v) as mobile phase, then bands exam in visible and UV light in 312 wave length to detect retardation factor.
- 5- **Pathogenic bacteria** *E. coli*, *S. aureus*, *Serratia Spp.*, *Klebsilla pneumoniae*, *Aeromonas hydrophilla* was isolated from different source in hospital in suitable media, then antibiotic sensitivity was detected using disc methods to (methicillin ME, amikacine AM, azithromycine AZM, fusidic acid Fa, imipenem IPM, naldixic acid NA, piperacillin PRL, ampicillin/cloxacillin APX, doxycycline DO, nitrofurantion F, oxacillin OX, rifampin RA, clindamycin PA and trimethoprim/sulphamethoxazol SXT).
- 6- **Antibacterial activity of plant extract;** this assay performed using OD of growth culture according to Jabor *et al.* (2013).
- 7- **Synergism of plant extract ;** this assay performed using mixed between two plant extract as, Every mixed in this mixture was experiment against pathogenic bacteria in 5 microgram/ml media for every type of plant extract.

Results

Thin layer chromatography of plants extract show these extracts consist of different compounds as show in figure (1) under visible light and UV light.

The method that used in present study for extract plants is common and used in wide range of researches because methanol and water was high polar solvents may extract polar phytochemicals compounds, also this mixture of

solvent was low toxicity if it used in vivo (Al-turaiheet *al* 2012, AL-saadiet *al* 2013).

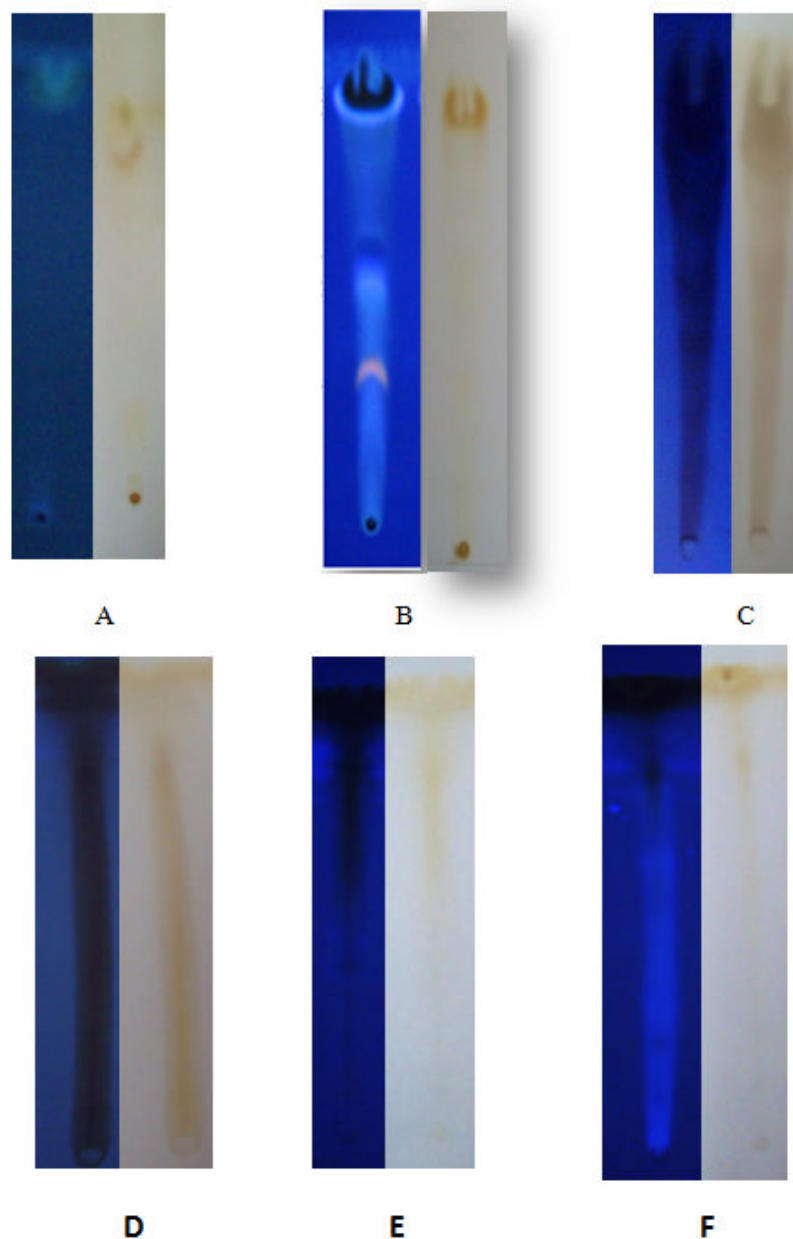


Figure (1) thin layer chromatography of plant extract using as antibacterial activity under visible and UV light **A**, barley ; **B**, Liquors; **C**,*Origanumvulgare* ; **D**, Pomegranate cortex; **E**, Ginger; **F**, sage .

Antibacterial activity

Plant extract used as anti-bacterial activity against five type of patho genic bacteria, include *E.coli*; *S.aureus*; *Klebsillapnemoniae* ; *SerratiaSpp*; *Aeromonashydrophilliawas*, its sensitivity against antibiotics was performed using disc method as show in table (1).

Results show that barley had antibacterial activity against all pathogenic bacteria that used in present study, as show in column 3 in all figures, this may be because it have different compounds in it extract that effect on bacterial growth, studying clarify that barley consist of soluble and in soluble fiber and bioactive constitues such as vitamins and minerals, cellulose beta-glucan, so it a source of tocols compounds (Marwat et al ., 2012).

Barley has historical uses in medical application spatially in inflammation and infection in different country such as renal inflammation, respiratory tract infections and intranasal inflammation. (Ross, 2005; Bussmann et al., 2007). The lower effect from these plant extract was *Salvia officinalis Lit* increased growth of bacteria except *S.aureus* which sensitive to this plant , this may be because these plant have supported compounds for bacterial growth these result in fall in to tow suggestion first , sage can enhancement infection of pathogenic bacteria thus it must be avoided in infection disease, in another hand it need to others studies using different solvent in extract

and nonpathogenic bacteria. Khalil and Li (2011) used Salvia oil as antibacterial against some gram positive and gram negative bacteria, they found The essential oil of *S. officinalis* proved to have antibacterial activity against Gram- positive and negative bacteria. This activity was more obvious against Gram- positive than negative bacteria which deal with present study. This may be due the existence of the outer phospholipid membrane of the gram-negative bacteria.

Other plant extract have low activity for all species of bacteria such as licorice (lane 2) it cause low effect on bacterial growth. *Hordeum vulgare* more effect on *staph.* And *Aeromonashydrophillia* than others types, *Punicagranutum* effect on *Aeromonashydrophillia* and *staph. aureus*, ginger effect on three species, *Aeromonashydrophillia*, *serratia* and *staph.*, these result deal with many studies in other counties Dahham et al (2010) used different extract of *Punicagranutum* against human pathogenic bacteria they concluded, that results obtained from their study clearly demonstrate broad spectrum antimicrobial activity of pomegranate against seven species of bacteria, More importantly the results indicated that methanolic extracts of pomegranate are more effective against bacteria and fungi than the aqueous extracts so this effect may be because presence of phytochemicals compounds in the extracts including phenols, tannins and flavonoids as major active constituents may be responsible for these activities.

Sebiomoet al (2011) used serial concentrations of different types of ginger extract against *Staphylococcus aureus* and *Streptococcus pyogenes* results show that ethanol extract of both leaf and root can be used alongside conventional antibiotics to fight agents of infections that are so prevalent in the hospitals.

Present study proposed synergism between plant extract against pathogenic bacteria, this suggestion mimic mixtures of herbal medicine have been used in treated disease, and results of present study clarify effect of this synergism against pathogenic bacteria, figure (3) show synergism between plant extract against pathogenic bacteria, in all diagrams in figure (3) lane (1) is positive control, lane (2) is mixture of barley and ginger more effect was against *Aeromonashydrophillia* and *S. aureus*. Lane (3) is barley and Pomegranate the more effect was against *Aeromonashydrophillia* and *Klebsilla pneumoniae* also it has effects on all bacteria it decreased growth, lane (4) is ginger and *Origanum vulgare* more effect of this mixture on *Aeromonashydrophillia* and no effect on *Klebsilla pneumoniae* it activated its growth, so it has low effect against other bacteria, lane (5) is Pomegranate and ginger this mixture causes decreased in all bacterial growth and complete inhibition on *Aeromonashydrophillia*, lane (6) *Origanum vulgare* and barley it causes low effect on all bacterial species, lane (7) sage and barley this mixture also decreased all bacterial growth but more effect was on AREOMOAS, lane (8) is barley and licorice, also *Aeromonashydrophillia* was more affected by this mixture, lane (9) is liquorice and *Origanum vulgare* the result of this mixture was like others in more effect on *Aeromonashydrophillia* and low effect on others, lane (10) is liquorice and Pomegranate causes inhibition in *Aeromonashydrophillia* and decreased in other bacterial species, lane (11) is *Origanum vulgare* and sage also it cause decreased in *Aeromonashydrophillia* and *S. aureus* growth and *E. coli*, lane (12) is liquorice and sage its effect on *E. coli* and *Aeromonashydrophillia* so it effect on others bacteria but in low effect, lane (13) Pomegranate and liquorice it decreased bacterial growth but in low level, lane (14) liquorice and ginger causes complete inhibition on *Aeromonashydrophillia* and most decreased in bacterial growth especially in *E. coli*, lane (15) Pomegranate and sage it caused decreased in all bacterial growth, lane (16) sage and ginger it also decreased in bacterial growth.

These results were resulted from synergism between plants extract mixture which contain phytochemicals compounds may be active if combine with other compounds or these compounds have sequential effect on its target in bacteria, also these compound may be changes microenvironment of bacteria which causes disrupted in nutrient and water transfer, this reasons need to advance studying using electron microscope to understand these effect on bacterial cell wall and in microenvironment.

Some study use synergism between plant extracts and antibiotics, Adwan and Mehanna (2008) use different plant extract with antibacterial had different mechanism such as protein synthesis inhibition: oxytetracycline HCl and gentamicin sulfate; cell wall synthesis inhibition: penicillin G and cephalixin; folic acid synthesis inhibition: Sulfadimethoxine sodium; and nucleic acid synthesis inhibition: enrofloxacin against *Staphylococcus aureus* strains Isolated from Clinical Specimens results of this studying were that synergistic effects between combination of antibiotics and plant extracts with significant reduction in the MICs of the test antibiotics against these strains of *S. aureus*, these results deal with present study results in synergist plant extract with other compounds if it was antibiotics or other extracts.

Present study suggested used synergism between plant extract as antibiotics or used it for make new drugs especially against multi resistant bacteria, which become dispersed in hospital, environment causes different infections and disease.

Synergism between plant extracts in present study also have disadvantage, sometimes it causes activating bacterial growth as show in figure 3 lane (8) against *E. coli* and lane (4) against *Klebsilla pneumoniae* and lane (2) in *serratia* while when it use alone it causes inhibition in bacterial growth, such as barley it cause inhibition in *E. coli*, *Origanum vulgare* when it use alone also it causes activate bacterial growth of *Klebsilla pneumoniae*, so ginger has low effect on this bacteria.

Synergism between plant extract as treatment or protect factors were evaluated in variety studies many of this studies recorded plant extract synergism efficiency in its effected, but these studies on pathogenic bacteria has been little and need to advance techniques in extracted phytochemicals compounds and experience these extract on bacterial genome and plasmid curing.

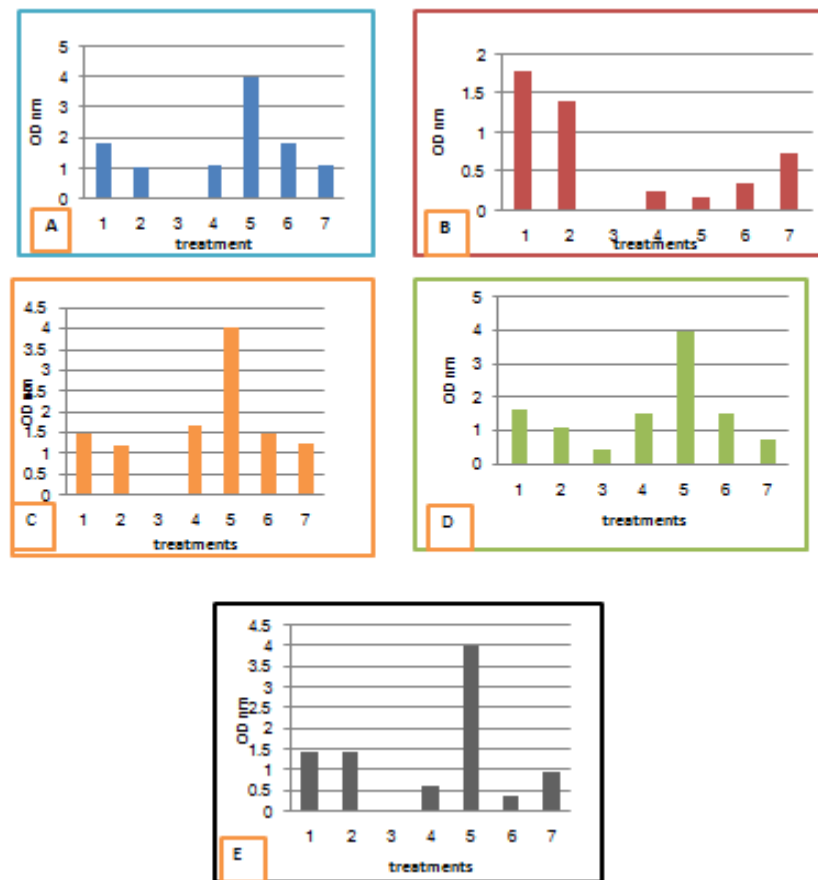
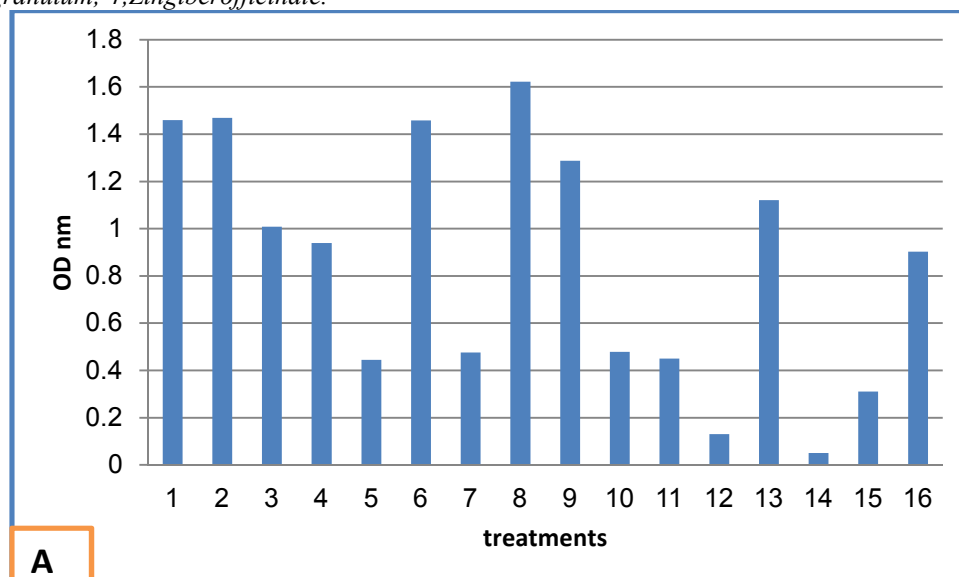
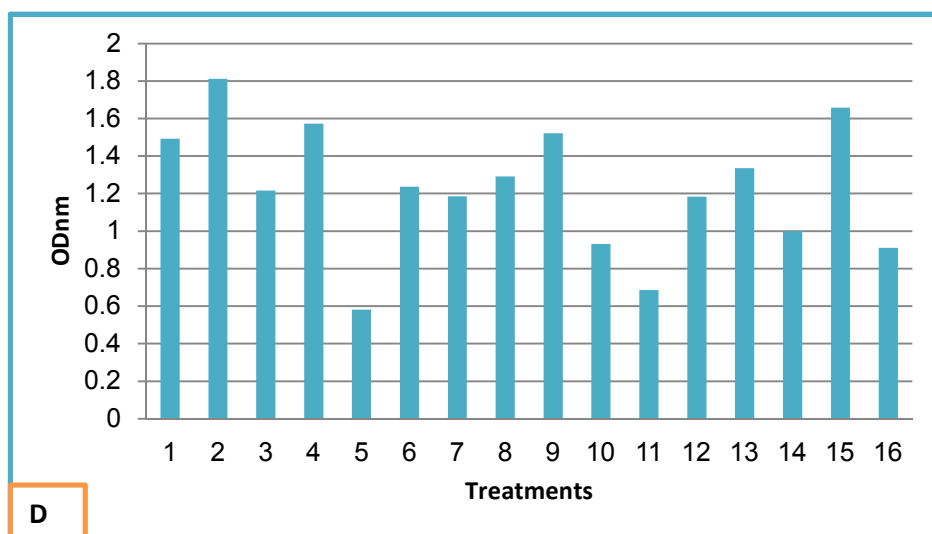
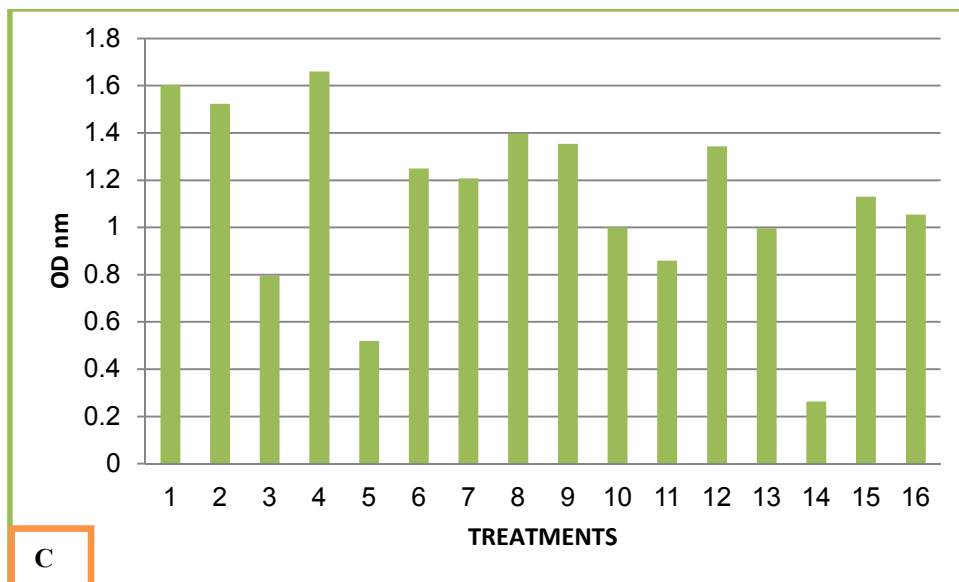
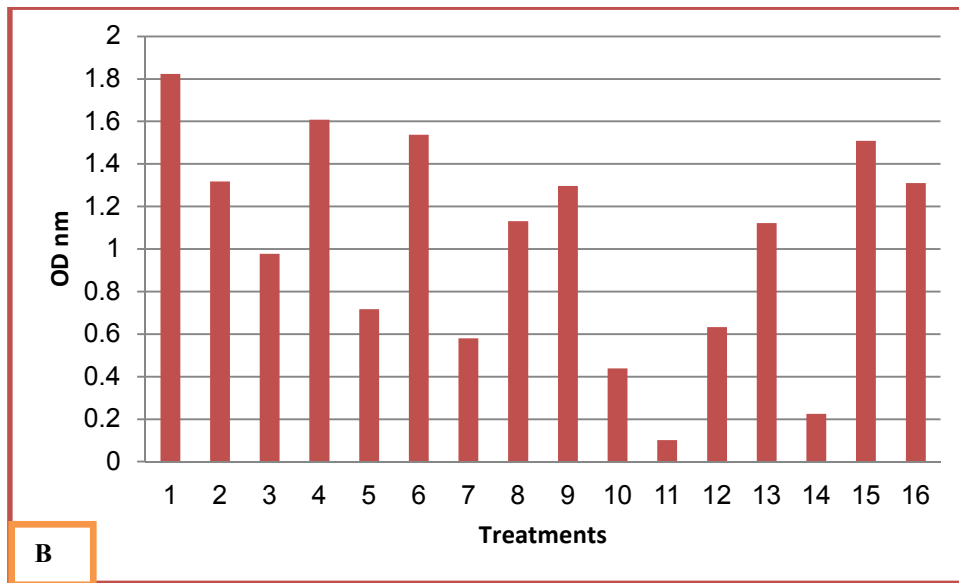


Figure (2) antibacterial activity of plants extract against different pathogenic bacteria
A, *E. coli*; **B**, *S. aureus*; **C**, *Klebsilla pneumoniae*; **D**, *Serratia Spp*; **E**, *Aeromonashydrophillias*.
 1, positive control; 2, *glycyrrhiza glabra*; 3, *Hordeum vulgare*; 4, *Origanum vulgare*; 5, *Salvia officinalis*;
 6, *Punicagranatum*; 7, *Zingiber officinale*.





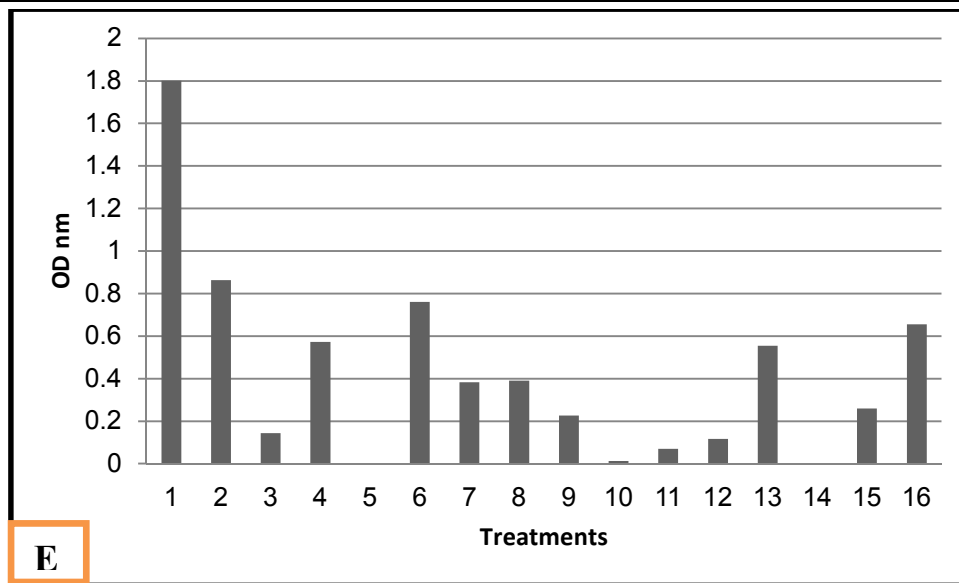


Figure (2) antibacterial activity of plants extract against different pathogenic bacteria
A, *E.coli*; B, *S.aureus*; C, *Klebsillapnemoniae* ; D, *SerratiaSpp*; E, *Aeromonas hydrophilliawas*.

1-Positive control , 2- B+G, 3- B+P, 4- O+G, 5- P+G,6- P+O, 7- B+S, 8- B+L, 9- L+O, 10-L+P, 11- O+S, 12- L+S, 13- O+P, 14- L+G, 15- S+P, 16- S+G.

References

- Al-saadi, A.; Breesam, B. and al-turaihe, M. (2012) medical plants, AL radwan publisher.
- Mahesh, B. and Satish, S. (2008). Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogens. World Journal of Agricultural Sciences 4 (S): 839-843.
- Prasannabalaji, N.; Muralitharan, G.; Sivanandan, R.N.; Kumaran, S. and Pugazhvendan, S.R. (2012). Antibacterial activities of some Indian traditional plant extracts. Asian Pacific Journal of Tropical Disease, S291-S295.
- Yamamura, Y.; Kawakami, J. and Sanata, T. (1992) Pharmacokinetic profile of glycyrrhizin in healthy volunteers by a new high-performance liquid chromatographic method. J. of Pharm. Sci. 81, 1042-1046.
- Al-Terehi, M.; Al Saadi, A. H. Al Ameri, Q. (2012). *In vivo* study of antimutagenic and antioxidant activity of *Glycyrrhizaglabra* root extract. research in pharmacy, 2, 27-34.
- Fukai, T.; Satoh, K.; Nomura, T. and Sakagami, H. (2003). Preliminary evaluation of antinephritis and radical scavenger activities of glabridin from *Glycyrrhizaglabra*. Fitoterapia 74, 624-629.
- Khanuja, K. (2006). Antimicrobial potential of *Glycyrrhizaglabra* Root. CIMAP Com. J. 39.
- Marwat, S.K., M. Hashimi, K.U. Khan, M.A. Khan, M.M. Shoaib and Rehman, F.U. 2012. Barley (*Hordeumvulgare* L.) A Prophetic Food Mentioned in Ahadith and its Ethnobotanical Importance. American-Eurasian J. Agric. Environ. Sci. 12: 835-841.
- Mahesh G.; Abu-Ghannam, N.; and Gallagher, E. (2010). Barley for Brewing: Characteristic Changes during Malting, Brewing and Applications of its By-Products. Comprehensive Reviews in Food Science and Food Safety.
- Ross, I.A., 2005. Medicinal Plants of the World: Chemical Constituents, Traditional, 3: 236-37.
- Ashraf, Z.; Muhammad, A.; Imran, A.; Tareq, A. H. (2011). In Vitro Antibacterial and Antifungal Activity of Methanol, Chloroform and Aqueous Extracts of *Origanumvulgare* and Their Comparative Analysis. International Journal of Organic Chemistry, 1, 257-261.
- Syanojevic, D.; Comic, L.; Stefanovici, O. and Sukdolak, S. S. (2010) IN VITRO SYNERGISTIC ANTIBACTERIAL ACTIVITY OF *SALVIA OFFICINALIS* L. AND SOME PRESERVATIVES. Arch. Biol. Sci., Belgrade, 62 (1), 175-183.
- Khalil, R. and Li, Z.G. (2011) Antimicrobial activity of essential oil of *Salvia officinalis* L. collected in Syria. African Journal of Biotechnology, 10, 8397-8402.
- Behboud, J.; Amirreza, E.; Mohammad, M. (2011). Antibacterial effect of *Salvia officinalis* Lam extract. Annals of Biological Research, 2011, 2 (6): 532-535.
- OLGICA D. STEFANOVIĆ*, DRAGANA D. STANOJEVIĆ and LJILJANA R. »OMIĆ
- Stefanovic, O.; Stefanovic, D.D.; Omia, L.R. (2012). SYNERGISTIC ANTIBACTERIAL ACTIVITY

OF SALVIA OFFICINALIS AND CICHORIUM INTYBUS EXTRACTS AND ANTIBIOTICS. *Acta Poloniae Pharmaceutica ñ Drug Research*, 69, 457- 463.

- Nanjundaiah SM, Annaiah HNM, Dharmesh SM (2009). Gastroprotective effects of Ginger rhizome (Zingiberofficinale) Extract : Role of Gallic acid and Cinnamic acid in H-ATPase/H. Pylori inhibition and antioxidative mechanism. *eCAM.*: 1-13. <http://ecam.oxfordjournals.org/cgi/reprint/nep060v1.p>.
- Thomson, Z.; Al-Qattan, K.K.; Shalaby, R.B. and Ali, M. (2006). Anti-diabetic and hypolipidaemic properties of ginger (Zingiberofficinale) in streptozotocin-induced diabetic rats. *British Journal of Nutrition*, 96, 660–666.
- Kaushik P, Goyal P (2011). Evaluation of various crude extracts of Zingiberofficinale rhizome for potential antibacterial activity: A study in vitro. *Adv. Microbiol.* 1,7-12
- Galadima, A.; Bassey, J.U.; Olowoniyi O.D.; Moses, O. and Yako A. B. (2011). Antimicrobial properties of the ethanolic extracts of Zingiberofficinale (Ginger) on *Escherichia coli* and *Pseudomonas areuginosa*, *Annals of Biological Research*, 2 , 307-311.
- Dahham, S.S; Ali, M.N. ; Tabassum; H. and Khan, M. (2010) Studies on Antibacterial and Antifungal Activity of Pomegranate (*Punicagranatum L.*). *American-Eurasian J. Agric. & Environ. Sci.*, 9 ,273-281.
- Sato, T. ; Onse, Y. ; Nagase, H. and Kito, H. (1990). Mechanism of antimutagenicity of aquatic plant extracts against (benzo (a)pyrene) in the *Salmonella* assay. *J. Mut. Res.* 241,283-290 .
- Jebor, M.; AL-saadi, A.; Hikmat, R.; AL-terehi; Zaidan, H.K. and AL-Saadi. M. (2013). Characterization and antimicrobial activity of barley grain (*Hordeum vulgare*) extract. *Int. J. Curr. Microbiol. App. Sci.* ,2(8): 41-48.
- Sebiomo, A.; Awofodu, A. D .; Awosanya, A. O. ; Awotona, F. E .and Ajayi, A. J. (2010). Comparative studies of antibacterial effect of some antibiotics and ginger (Zingiberofficinale) on two pathogenic bacteria, *Journal of Microbiology and Antimicrobials*, 3, 18-22.
- Adwan, G. and Mhanna, M. (2008) Synergistic Effects of Plant Extracts and Antibiotics on *Staphylococcus aureus* Strains Isolated from Clinical Specimens. *Middle-East Journal of Scientific Research*, 3 , 134-139.