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Some plants extracts Synergism effects in Pathogenic bacteria

Mona Al-Terehi^{1*}, Ali H. Al-Saadi², HaiderK.Zaidan²,
Russul Hikmat Behjet², Zahraa Haleem²

¹University of Kufa -College of Science, ²University of Babylon, College of Science, Iraq.

Abstract: Synergism between plants extracts were 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.*, *Klebsilla pneumoniae*, *Aeromonas hydrophila* isolation from different source in hospital tests were performed as only one extract on every bacterial species then tow 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.

Key words: 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 patinas and new recombinants in genetic materials of bacteria which is responsible of drugs 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 application².

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 Satish¹⁴ used methanol leaf extracts of *Acacia nilotica*, *Sidacordi folia*, *Tinospora cordifolia*, *Withania somnifera* and *Ziziphus mauritiana* showed significant antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Xanthomonas axonopodis pv .malvacearum* by inhibition zone method. Also some Indian traditional plant extracts such as *Ocimum sanctum*, *Ocimum gratissimum*, *Aeglemarmelos*, and *Adhatoda vasica* leaves showed antibacterial activity against human pathogenic Bacteria¹⁷.

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 rang 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.

Licorice (*glycyrrhiza glabra*) (L) was one of the important plants have long history in medical applications, root is important part in plant which is contained flavonoid components²⁴. Licorice also contain Phenolic compounds, coumarins, volatile oil, saponine and minerals³.

Fukalet *al.*¹⁷ improve that licorice useful in treatment bacterial infections of Streptococcus mutants, Staphylococcus auras and Mycobacterium. Also it have an antibacterial activity against G⁺ and G⁻ bacteria such as Bacillus subtilus, MTCC121, Salmonella typhi MTCC733, Yersinia enterocolitica and *Klebsiella pneumoniae* MTCC¹².

Barley (*Hordeum vulgare* L.) (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 polysaccharide¹³.

Many studies supported using barley in different inflammation such as Intranasal inflammation by use in Iran¹⁸.

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.*⁴ used equous and chloroform to extract phytochemicals compound from *Origanum vulgare* and used it as antibacterial and antifungal agents .

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^{22,11} 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⁻ bacteria⁵. Stefanovi *et al.*²¹ use synergism between sage and *Cichorium intybus* against pathogenic bacteria.

Zingiber officinale is 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 oils¹⁶. Researchers use ginger as anti-inflammation and anti-thrombi (Thomson et al, 2002). Galadima *et al*⁸ use ginger extract against gastrointestinal track pathogenic bacteria. Kaushik and Goyal¹⁰ 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.

Pomegranate, *Punica granatum* (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 pelletirine and methyl pelletirine² literature of review reported the Pomegranate efficiency against pathogenic bacteria *B. coagulans*, *B. cereus* *B. subtilis* and *S. aureus*⁶.

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

Materials and methods

1. Plant part that use in present study, licorice deride root, barley seeds, ginger dried 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 dried using incubator at 50C° for 24 hours, The dispert 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 pnemoniae*, *Aeromonas hydrophillia* 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, ampicilline/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*⁹.
7. Synergism of plant extract ; this assay performed using mixed between tow 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^{3,2}.

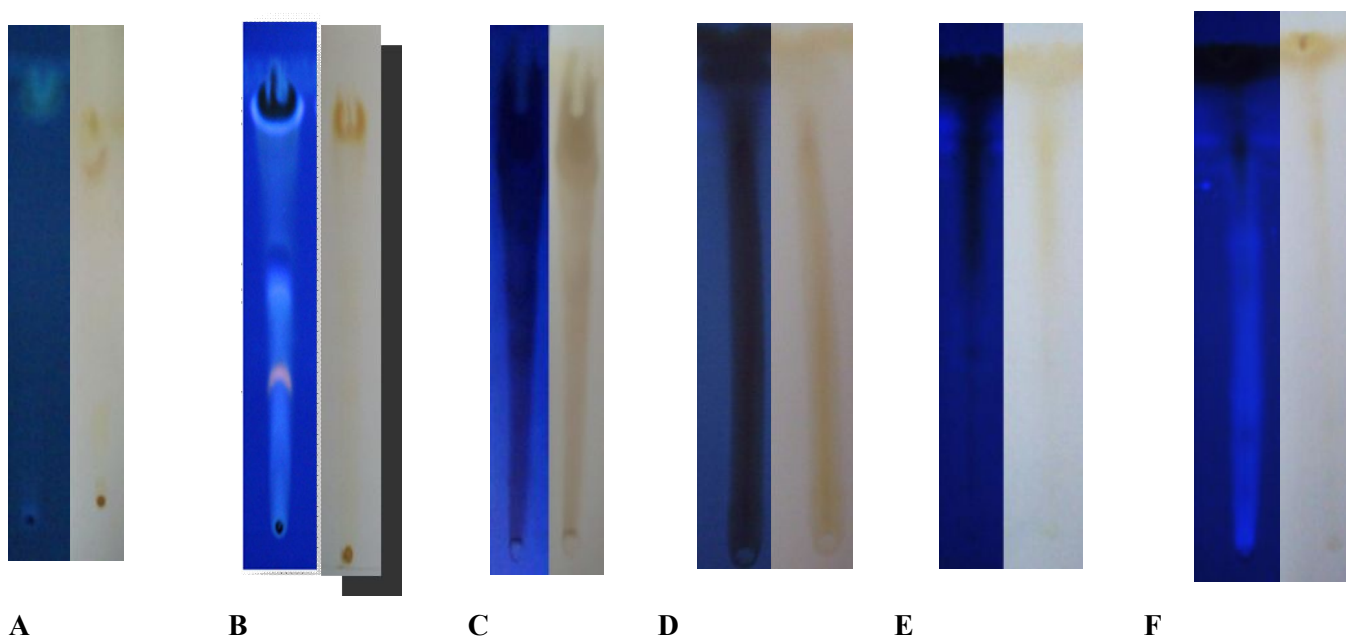


Figure (1) thin layer chromatography profile of plant extract using as antibacterial activity under visible and UV light (312 nm) A, barley ; B, Liquors; C, *Origanum vulgare* ; D, Pomegranate cortex; E, Ginger; F, sage .

Antibacterial activity

Plant extract used as anti-bacterial activity against five type of patho genic bacteria, include *E.coli*; *S.aureus*; *Klebsilla pnemoniae* ; *Serratia Spp*; *Aeromonas hydrophillia* was, 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 constitutes such as vitamins and minerals, cellulose beta-glucan, so it a source of tocols compounds¹⁵.

Barley has historical uses in medical application spatially in inflammation and infection in different country such as renal inflammation, respiratory tract infections and intranasal inflammation¹⁸. (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. ¹¹Khalil and Li 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 *Aeromonas hydrophillia* than others types, *Punica granatum* effect on *Aeromonas hydrophillia* and *staph* aurous , ginger effect on three specious , *Aeromonas hydrophillia*, *serratia* and *staph*, these result deal with many studies in other counties Dahham *et al*⁶ used different extract of *Punica granatum* 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*²⁰ 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 *Aeromonas hydrophillia* and *S* aurous. Lane (3) is barley and Pomegranate the more effect was against *Aeromonas hydrophillia* and *kliepsella* also it has effects on all bacteria it decreased growth, lane (4) is ginger and *Origanum vulgare* more effect of this mixture on *Aeromonas hydrophillia* and no effect on *Klebsilla pnemoniae* 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 *Aeromonas hydrophillia*, 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 *Aeromonas*, lane (8) is barley and liquorice, also *Aeromonas hydrophillia* was more affected by this mixture, lane(9) is liquors and *Origanum vulgare* the result of this mixture was like others in more effect on *Aeromonas hydrophillia* and low effect on others, lane (10) is liquorice and Pomegranate causes inhibition in *Aeromonas hydrophillia* and decreased in other bacterial species , lane (11) is *Origanum vulgare* and sage also it cause decreased in *Aeromonas hydrophillia* and *S aureos* growth and *E coli*, lane (12) is liquorice and sage its effect on *E.coli* and *Aeromona shydrophillia* so it effect on others bacteria but in low effect, lane (13) Pomegranate and liquorice it decreased bacterial growth but inn low level , lane (14) liquorice and ginger causes complete inhibition on *Aeromonas hydrophillia* and most decreased in bacterial growth especially in *E.coli* , lane (15)Pomegranate and sage it cause 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 microenviromental 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 Mehanna¹ use different plant extract with antibacterial had different mechanism such as protein synthesis inhibition: oxytetracycline HCl and gentamicin sulfate; cellwall synthesis inhibition: penicillin G and cephalexin; folic acid synthesis inhibition: Sulfadimethoxine as 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 pnemoniae* 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 pnemoniae*, 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.

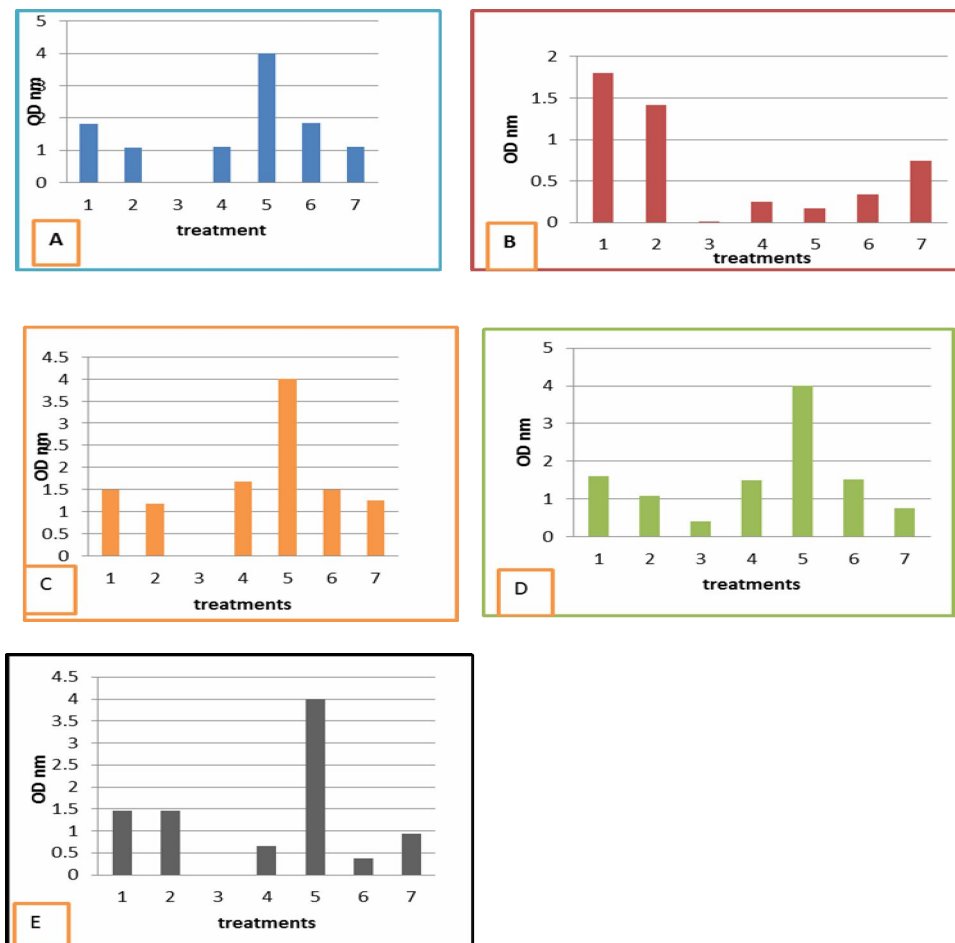


Figure (2) antibacterial activity of plants extract against different pathogenic bacteria
 A, *E.coli*; B, *S. aureus*; C, *Klebsilla pnemoniae* ; D,, *Serratia Spp*; E; *Aeromonas hydrophillia was*.
 1,positive control; 2, glycyrrihza glabra;3, *Hordeum vulgare*;4, *Origanum vulgare*; 5,*Salvia officinalis*;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

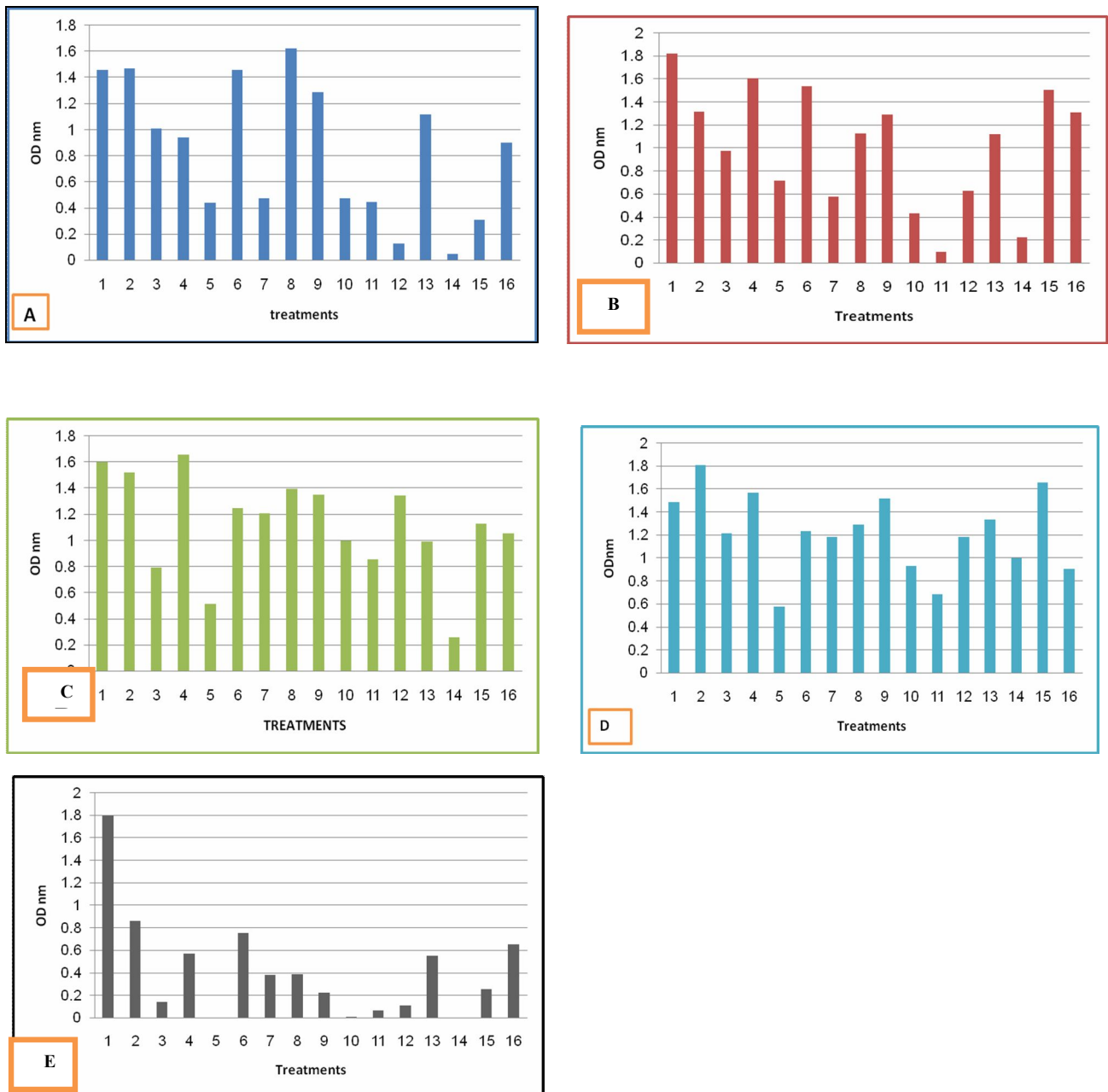


Figure (3) antibacterial activity of plants extract against different pathogenic bacteria A, E.coli; B ,S.aureus; C, Klebsilla pnemoniae ; D, , Serratia Spp; E; Aeromonas

Hydrophillia was.

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.

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