

The Potential Aqueous Extracts of Medical Plant and Alum against Bacteria, an in Vitro Study

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Abstract--- *Background: The attention for plants as well as to medicinal herbs has increased. It's mainly as key sources for the foundation of medical drugs and a source of effective materials that go into the composition of the drug. Aim: In order to determine the medical significance of (Malvasylvestris, Matricariachmomilla, Salvia officinalis, Quercus infectoria, Curcuma Longa, and Alum)aqueous extract and its anti-microbial activity in contradiction of particular kinds within Gram positive, negative bacteria through incomes degree the distance of the inhibition zone against some types of Gram positive and negative bacteria by means measure the diameter of the inhibition zone (mm) Methods: These bacteria were randomly isolated from clinical samples of people whom Fallen asleep in Al-Hilla teaching hospital, Babylon during a period from October2016 to January 2017. In the present study; the diagnosis of bacterial strains where after its culturing proceeding within appropriate media (morphological feature) and then the diagnosis was performed by using conventional biochemical tests and Epi 20 system at the microbiological laboratory/College of Medicine/Babylon University. These strains were included; E.coli, Enterobacter, Klebsiella, Acinetobacter, proteus, Salmonella, and Pseudomonas. The results of this investigation have exposed that studied extract is effective in discouraging the development of these bacteria outside the body of the organism (Gram positive & Negative bacteria). CONCLUSION: from the above experiment, it was concluded that studied aqueous plant extracts has beneficial effect on tested pathogenic bacteria due to high concentration of energetic biochemical ingredient for antimicrobial action that useful for preventing certain enteric diseases.*

Keywords--- Value.

I. INTRODUCTION

Acquire opposition of bacteria to drugs which are employed as therapeutic agents due to bacterial genetic ability to change (1) made the Finding of new components that are naturally active from plants or plant-based farmed products as a material of concern to many researchers. M. Sylvestris is usually used as vegetable and a medicative plant in Asian nation wherever it's named as Panirak. The plant flowers right-angled measure used as a remedy for cut wound, eczema, dermal infected wounds, bronchitis, organic process issues, and inflammations (2). Relating to the results of wang (2005), anthocyanins from M. sylvestris caused decreases in total sterol and triglycerides of plasma. It's additionally shown that the extracts of some Malva species protected rats from stomachal lesions iatrogenic by fermentation alcohol.

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This anti-ulcerogenic activity is also related to the high mucilage content from the plant species (3) *Matricaria chamomila* (Chamomile) is a herbal medicinal plant of 15 to 50 cm high and from planting stage it flowers, after a few weeks; and used to relieve various body pain, calm headaches and tooth aches, to relieve menstruation pains. It is an anti-inflammatory drug that softens eyelids and eyes (4). *Salvia officinalis* is a returning plant, evergreen, with woody stems, green leaves and violet blue flowers. Shrubberies remain gray-green pigment and in higher superficial layer have crinkles while lower surface stay almost white and considerable shorter soft fluff. Some species of the genus *Salvia* like *salvia officinalis* has a significant therapeutic effect. It was effective in lowering blood sugar, relaxation and so on (5). *Quercus infectoria*, one of the popular therapeutic shrubberies secondhand usually used in a postpartum care and treatment of various ailments. This plant is a small tree or shrub about two meters high and is mainly found in Asia (6) The galls of *Quercus infectoria* have also become pharmacologically recognized to own astringent, antibacterial (7), antifungal (8), larvicidal (9), antidiabetic (10), local anaesthetic (11), antiviral (12), and anti-inflammatory (13) activities.

Curcuma longa L. which is usually identified as 'turmeric' belong to Zingiberaceae family, is a group of 70 class of rhizomatous thymes. It is a returning basil, refined throughout relatively warm portions of the world. It is recognized universal to its flexible routine in medicine, makeups products, food flavouring as well as textile industries. (14) Turmeric precipitate, extracts, oleoresins are particular of the broadly secondhand profitable of *C. longa* plant. (15)

The oils and the diaryl heptanoid curcumin, which are the major secondary metabolites for *C. longa* have become exposed mainly responsible for the pharmacological actions of turmeric dust, extracts as well as oleoresins. The key actions found to be hepatoprotective, anti-inflammatory, antimicrobial, antiviral, wound healing, anticancer as well as antitumor. Furthermore, it consumes possible healing effects versus neurodegenerative, heart disorder, and autoimmune diseases. (16) The oil of *C. longa* has revealed to possess the anti-inflammatory activity, increase the bile flow and effective against bronchial asthma in a clinical trial (17, 55). Both the curcumin and the oil become revealed to possess wound medicinal possessions and inhibitory actions versus pathogenic fungi both in vitro and in vivo. (18) Alum (aluminum potassium sulfate) the crystallized binary sulfates within formulation $KAl(SO_4)_2 \cdot 12H_2O$ are generally odorless, neutral crystal-like solid which turn white in air. It's mainly used food preservation as well as antiseptic for numerous grounding procedure like preserving and fermentation beside water purification (19). The goal of current investigation was to testing the antimicrobial "in vitro" action of aqueous extracts of all above medical plant against most isolated pathogenic bacteria (*E. coli*, *Enterobacter*, *Klebsiella*, *Acinetobacter*, *proteus*, *Salmonella*, and *Pseudomonas*).

II. MATERIAL & METHOD

Microbial Isolates: eight types of clinical microbial isolates (*E. coli*, *Enterobacter*, *Klebsiella*, *Acinetobacter*, *proteus*, *Salmonella*, and *Pseudomonas*.) were collected from patients who attended to Merjan teaching hospital, Babylon, Iraq.

Plant aqueous extraction

Malvasylvesris leaves were washed several times with distilled water to remove dust particles and then sun dried to remove the residual moisture. Malvasylvestris leaf extract was set by putting 5 g of dried fine cut in 500 ml glass beaker along with 400 ml of sterile distilled water. The combination was then simmered for five minutes till the pigment of aqueous solution altered as of watery to brown-yellow. Then concoction was chilled to apartment temperature followed by filtration with filter paper (Whatman No. 1) before centrifuging at one thousand and two handed rpm for two minutes to eliminate biomaterials. The extract was stored at room temperature in order to be used for further experiments (20) flowers of *Matricariachmomilla* were macerated and centrifuged (3000 rpm/20 min/4 °C), the supernatant was called aqueous extract(21).The plant part of *Salvia officinalis* (leaves) were air dried. The dry powder of plant material was in a mount of (100) g and added to 500 ml of D.W formerly simmered then boiled for one minute to obtain the aqueous extract (22). *Quercusinfectoria* leaves were collected from Babylon province, Iraq 2016. 10 g of sample was cut into small pieces and extracted at room temperature. with onehanded ml of water distilled (twice after 24 h) for water extract.(23)Fresh flower of leaves of, *Curcuma longawere* collected, washed under tap water, then in distilled water, and then kept between folds offilter paper to remove excess of water from exterior superficial layer. Ten gram of original leaves of *Curcuma longa*, was crushed fine, and 100 ml of D.W was add respectively (1:1w/v) then soaked for twenty four hourthen soaked for twenty four hour. Soaked suspension was then filtered through muslin clothes and *Curcuma longa* leaf extract was collected by means of funnel in separate conical flask. Finally filtrate obtained was used against plant pathogens (24). From the local market at Hilla city, Iraq 2016, Alum material was purchased and was identified in the College of Medicine, Department of Biochemistry, University of Babylon. Crystals of alum $KAl(SO_4)_2 \cdot 12H_2O$ dissolved completely in hot (100 ml) distilled water at 92 °C, at pH 3.6(25).

III. IN VITRO ANTIMICROBIAL ACTIVITY

The screening of antimicrobial activities of each extract on the tested bacteria used in this investigation was determined on Muller Hinton agar media, by the agar diffusion techniques using agar well diffusion method. Loop-full growths from microbial isolates were inoculated into nutrient broth incubated at 37 °C for 18 hours. The microbial suspensions were diluted with normal saline. Adjust the turbidity and compare with standard tube (McFarland number0.5) to yield a uniform suspension containing 1.5×10^8 CFU / ml. Cotton swab was dipped into adjustment suspension and streaked the entire Mueller-Hinton agar (for all tested bacteria) surface of plates and the plates were left for one 5 -15 minutes within room temperature to dry. Wells of 6 mm diameter and 5 mm depth were made on the solid agar using a sterile glass borer. Approximately 20µl of each extract was inoculated onto wells were made in the spread plate culture of each microbial isolates. The plate was then allowed to incubate within (37°C) through overnight. After 12-24 hrs of incubation, each extract was noted for zone of inhibition for all isolates. The diameters of the zone of inhibitions were measured by measuring scale in millimeter (mm) (26, 56, 57, 58).

IV. RESULT AND DISCUSSION

As a general rule, an extract is considered active against both bacteria and fungi, if the zone of inhibition was

greater than 6 mm (27).Antimicrobial activities of aqueous extract of *Malvasylvestris* aqueous extract of *Malvasylvestris* poses antibacterial action versus all tested bacterial isolates with different pattern of inhibition zone(as reviles in figure (1):

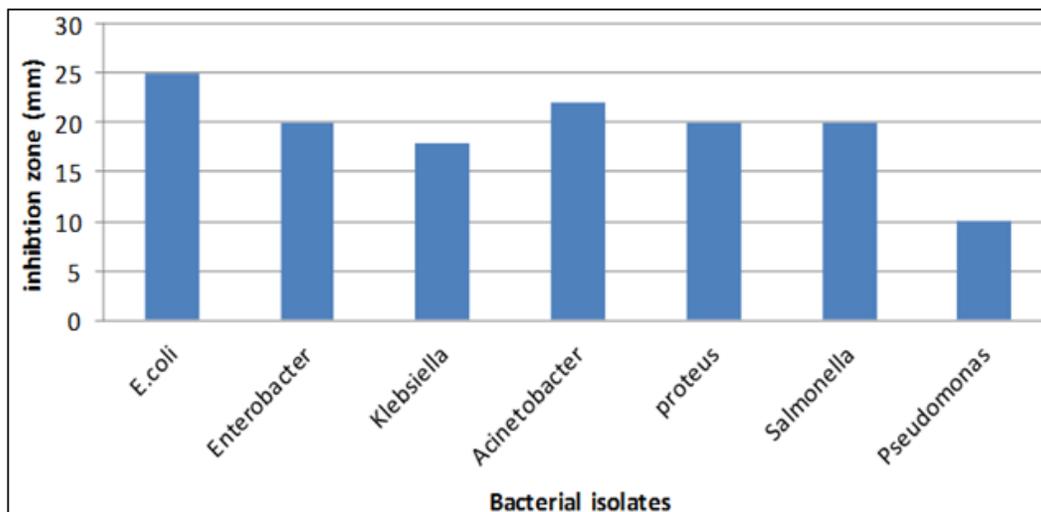


Figure 1: Antibacterial Activity of *Malvasylvestri* against G-ve bacteria

Significant zone of inhibition was revealed against tested G-ve bacteria indicated in this study and the result was accordance with (28), (29) and (30). The energetic elements are originate in the flowers as well as leaves, with rich in mucilage content; which are responsible for treatment due to their expectorant characteristic (31). Antibacterial activity of *Malvasylvestris* was significant also against *Acinetobacter* with (21mm) inhibition zone followed by *Enterobacter*, *proteus*, *Salmonella spp* with (20mm) inhibition zone and (18mm) against *Klebsiella* while lowest antimicrobial activity was against *Pseudomonas* isolate (10mm). The *M. sylvestris L.* has anti-microbial effects, attributable to its flavonoids contents (32) Moreover, the monosaccharide sugar products of the mucilage hydrolysis can be converted to acids (e.g. lactic acid) by the gut bacteria, lowering the gut pH and helping in balancing the intestinal microflora and normalizing the intestinal movement.(33).

The antimicrobial actions of *Matricariachmomilla* was significant versus all tested bacterial isolates except *Klebsiella* isolate as reveals in figure 2:

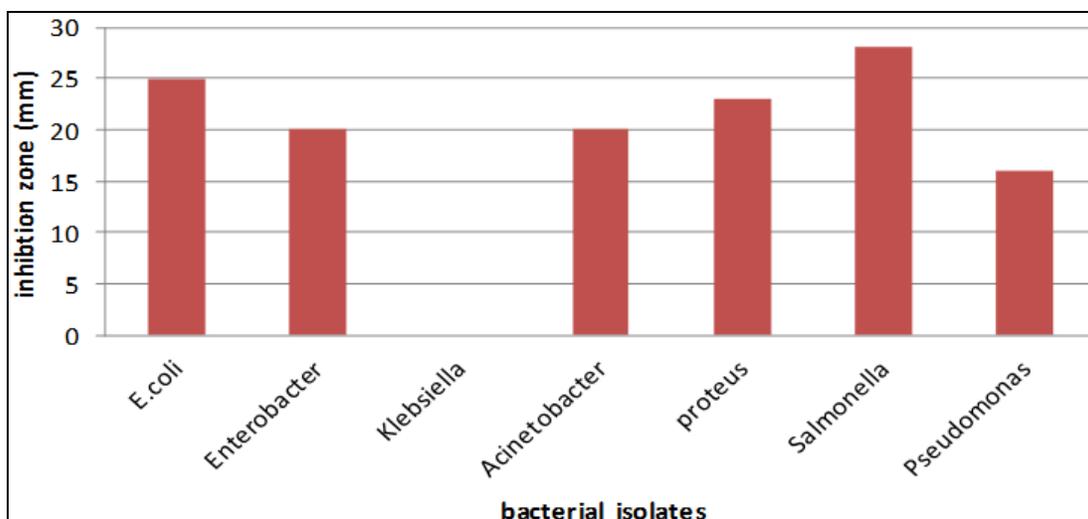


Figure 2: Antibacterial Activity of *Matricaria chamomilla* against G-ve bacteria

The highest activity was against *Salmonella* with (28mm) followed by (25mm, 23mm, 20mm, 16mm) inhibition zone against (*E.coli*, *proteus*, *Acinetobacter* and *Enterobacter*, *Pseudomonas*) respectively. The obtained results of *Matricaria chamomilla* extract have pointed that this extract have different compounds such as triterpens, triterpenoids, tannins glycosides and phenol, while these compound were having antimicrobial actions of several bacterial growth specially on *Pseudomonas aeruginosa* (34). Some reports confirmed that phenolic complexes (tannins & flavonoids) possess great antimicrobial action due to their content of hydroxyl group (-OH) in their chemical structure which are able to binding to hydrogen of the proteins which leads to disruption of bonds of sulphuric, hydrogen abundant within tertiary structure proteins that existing in bacterial cell (35).

Also the phenols are able to destruction of cell wall then increase of its permeability for these compounds leading to denaturation of cell proteins (36) as well as to inhibition of its biochemical activity due to their ability to bind with cell enzymes. The consequences of in vitro testing of antimicrobial action of *S. officinalis* aqueous extracts shown in figure (3):

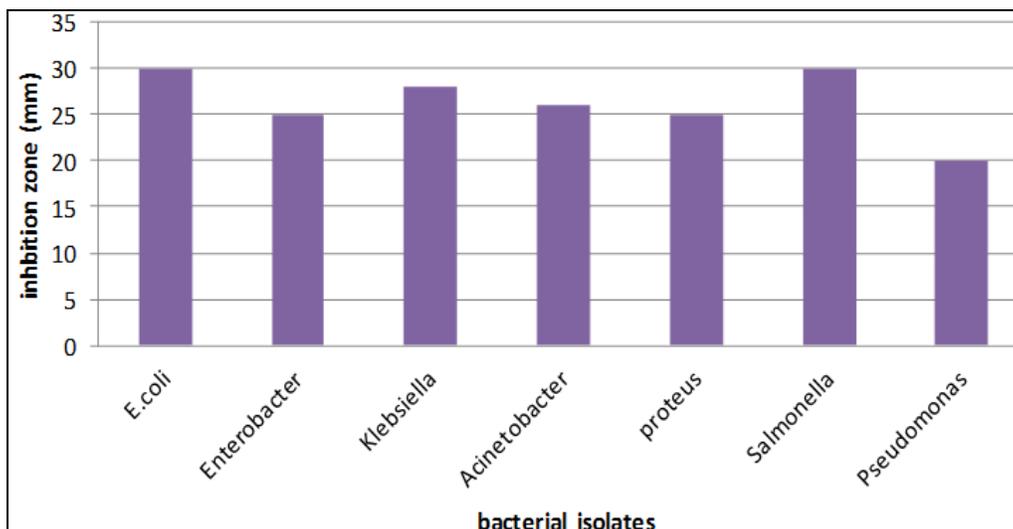


Figure 3: Antibacterial Activity of Salvia Officinalis against G-ve bacteria

Salvia Officinalis extract proved to be the most effective studied extracts. It inhibited all of bacteria; most susceptible were containing sulfur compounds can be effective against development of Salmonella and useful in food products to prevent its pathogenicity (38). Plants are basis of different chemical compounds, which make them of a medicinal importance. Two types (inert and active constituents) compounds are find depending on their activity.

The inert ingredients are distinct compounds that possess no therapeutic or physical effects like subrine, lignin and cellulose while active constituents have these effects and divided into other kind (proteins, glycosides, saponins, tannins, flavonoids, vitamine C, as well as many phenolic compounds, alkaloids and steroids) according to their physical and chemical properties as well as chemical analysis of sage (*S. officinalis*) extract aqueous exposed certain of these ingredients (tanins, steroids, glycosides, terpens, flavonoids and saponines). Aqueous-soluble polysaccharides complex from *S. officinalis* composed of galactose, glucose, mannose, xylose, and fructose have shown an immunomodulatory activity in the comitogenicthymocyte test, which is inferred as an in vitro associate of adjuvant action as well as to their mitogenic activity (39). The results of in vitro testing of antimicrobial actions of aqueous extracts shown in fig (4):

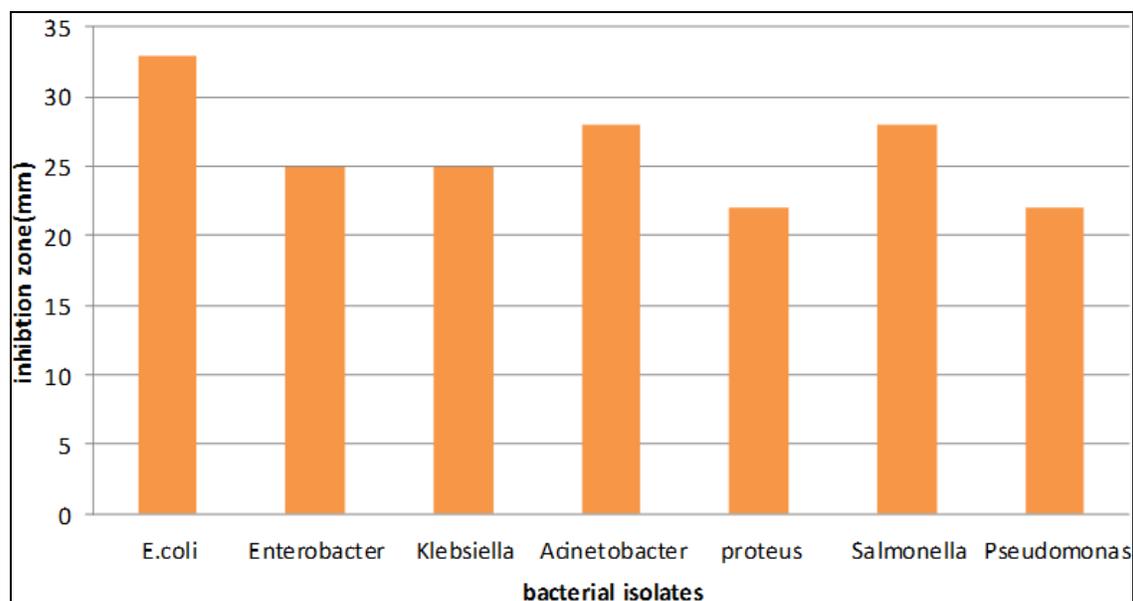


Figure 4: Antibacterial Activity of Quercus infectoria against G-ve bacteria

Inhibition was detected versus all bacterial isolates by aqueous extract of *Quercus infectoria*. It was (33mm, 25mm, 25mm, 28mm, 22mm, 28mm, 22mm) zone of inhibition versus bacterial isolates (*E.coli*, *Enterobacter*, *Klebsiella*, *Acinetobacter*, *proteus*, *Salmonella*, and *Pseudomonas*) respectively .the attendance of gallic acid, tannic acid, ellagic acid, syringic acid, sitosterol, amento flavonehexamethyl ether, essential oils, isocryptomerin, starch, anthocyanins, methyl-betulate, methyl- oleanate, hexagalloyl-glucose and polygalloyl-glucose (40) (41) in extract of *Q. infectoria* explains antimicrobial actions.

Tannin is widely known as one of compound that belong to phenolic groups that is easily dissolved in water gives precipitate with protein in a membrane of the cell (42). Concerning antibacterial activity against tested

bacterial isolates by Curcuma Longa ; Inhibition was detected versus all experienced bacterial strains. The highest inhibition zone was against Salmonella, isolates (30mm) followed by E.coli (28mm).the result was accordance with (43),(44).

Turmeric is well recognized as native herbal medicine that having many biological activities (45). Various health beneficial effects of turmeric are because it contains amount of minor metabolites such as: monoterpenoids, sesquiterpenoids and curcuminoids which include: curcumin (also called diferuloylmethane), demethoxycurcumin, bisdemethoxycurcumin and tet

These are yellowish pigments that have anti – inflammatory effects (46). The turmeric antibacterial property also credited to the occurrence of an alkaloid and veleric acid, a consequence of curcumin production (47). Curcumin is consider as maximum significant portion that accountable for a extensive range of organic activities of turmeric (48) such as antifungal, antiinflammatory and antibacterial activities (49).

Curcumin is considered as molecular constituent powerhouse, containing 20 different antibiotic molecules, due to variety of potentially therapeutic properties, such as wound healing (50). Turmeric rhizomes contain two modules of minor metabolites, curcuminoids and essential oils (51). C. longa composition, structure and functional groups was studies and documented that possess an important role in determining the antibacterial activity, usually composites along with phenolic collections are most effective (52).

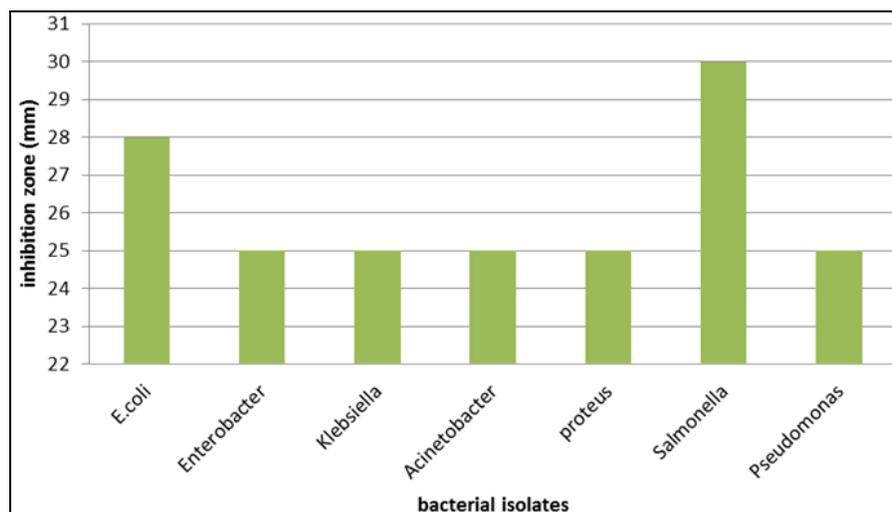


Figure 5: Antibacterial Activity of Curcum against G-ve bacteria

In the current study investigation antimicrobial effects of Alum aqueous extract which are valuable materials source of medicinally useful compounds that had traditionally usage for several applications. Aqueous extracts were being good sources for the bioactive compounds that exhibited good antimicrobial properties (53) .against tested bacterial isolates, the antimicrobiall activity was (35mm) inhibition zone against E.coli, Klebsiella, and Salmonella while 33 mm against Acinetobacter,30mm against proteus,28mm against Enterobacter, and 25 mm against Pseudomonas isolates.

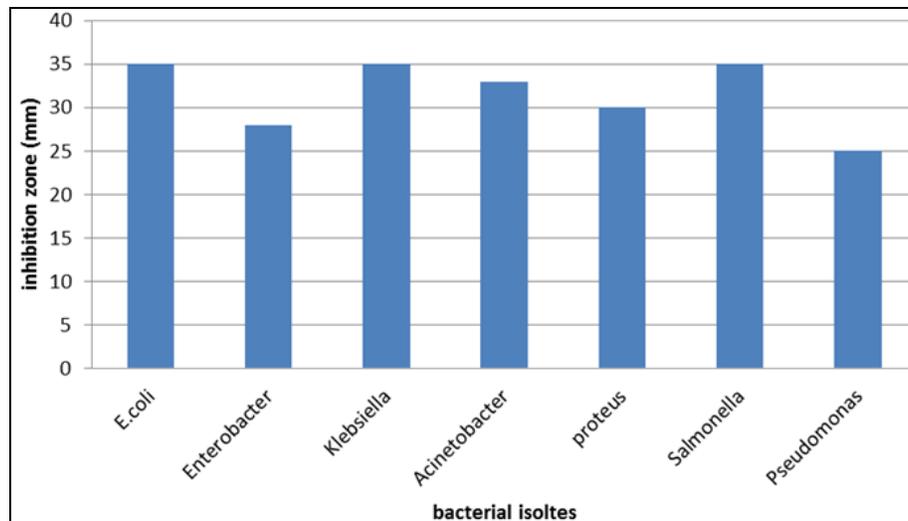


Figure 6: Antibacterial Activity of Alum against G-ve bacteria

Alum is an effective solution for elimination of the smear layer when used as a final rinse. No significantly change in the construction of dentinal tubules in Alum aqueous extract (54). High level of alum solution could cause distraction of gum tissue, Kidney damage and high mortality rate due to intestinal bleeding. Finding in the current study is promising and warrants further laboratory experiments on dissimilar kinds of bacteria, including strict anaerobic and species has been significantly found to persist after treatment procedures. However more studies about alum and its physical and antioxidant properties if there; and toxicity are necessary.

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