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Research Article

Assessment of the Antimicrobial activity of Punica granatum Extract against common bacterial Isolates from Dongola hospitals. 2023-2024

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ABSTRACT:

Recently, natural products have been evaluated as sources of antimicrobial agents with efficacies against a variety of microorganisms, Plants have been in use for thousands of years to treat health diseases.

The main aim of this study is that due to increasing concerns about the development of antimicrobial resistance among pathogenic bacteria, so alternative strategies are sought that do not use antibiotics to reduce pathogenic bacteria from foods and patients. Punica granatum has been commonly employed as a crude drug in traditional medicine.

The antibacterial activity of methanolic Extract of Punica granatum Peels, Pith, and Seeds were evaluated using Agar Disc diffusion susceptibility in different concentrations (50 mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml) against twelve multi-drug isolates (S.aureus, E.coli, K.pneumoniae, S.paratyphi A, Shigella spp,, P.vulgaris and P.aeruginosa).

All clinical isolates were observed to be susceptible to all concentrations of peels extract. Pith extract have antibacterial activity against all isolates except S.praratyphi A. Seeds extract show no activity against isolates except P. vulgaris.

Keywords: *Antimicrobial, bacteria, Punica granatum Extract, herbal antimicrobials*

1.INTRODUCTION

The widespread use of commercially available antimicrobials lead to the consequence of emergence of antimicrobial resistant pathogens, with prolonged use it may have negative effect on human, that make clear way for herbal antimicrobials.

Medicinal plants have great possible against infectious agent and can be used for therapeutic purposes to develop an alternative treatment of microbial infection, its beneficial and free of side effect.

1.1 Pomegranate (Punicagranatum)

Pomegranate (Punicagranatum) is one of the oldest edible fruits has been widely used by traditional medicine in different part of the world to treat different types of disease. (Damania *et al.,*2005)

Its cultivation and usages are deeply embedded in human history and its utilization has been found in many ancient cultures as food as well as a medical remedy. (Holland *et al.,*2008)

In the Greek mythology it represents life, regeneration and marriage. In Persian mythology isfandiyar (legendary Persian hero) eats a pomegranate and becomes invincible.

In china it is widely represented in ceramic art symbolizing fertility, abundance, posterity, and a blessed future.

In Christianity it is a symbol of resurrection and eternal life.

In Islam the Quran describes paradise gardens with shade and fruits including the pomegranate (fruit of paradise). (langley *et al.,*2000)

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Pomegranates were found in the tomb of King Tut, evidence that the Egyptians believed the pomegranate would help them into the afterlife. About the same time, the Hebrew slaves in Egypt became acquainted with the pomegranate.

1.1.2 Morphology

Pomegranate tree typically grows 12-16 feet, has many spiny branches, leaves are simple bright green slightly leathery with short petiole, new leaves are colored from reddish to green, they develop opposite to entire margins and have a deciduous habit. The flowers are bisexual, clustered in groups 2-5 at end of new branches, they are big and bright glossy red, bell shaped with petals and sepals. The root system of a pomegranate is very flexible, Two groups of skeletal roots can be identified those growing vertically and those growing horizontally. The major part of the active roots is located within the crown projection.

The ripe Pomegranate fruit can be up to five inches wide with a deep red, leathery skin, is grenade shaped and crowned by the pointed calyx.

The fruit contains many seeds separated by white membranous pericarp, and each is surrounded by small amounts of red juice. It takes 5 to 7 months to be ready as mature fruits. Ripening occurs about six months after flowering in normal pomegranate zones with average varieties, the early varieties taking somewhat less time to ripen. *(*Still *et al.,*2006*)* The best fruits are obtained in those areas where the period of development occurs during the time of high temperatures. The fruit is non-climacteric (will not ripen off the shrub) and should be picked after it has reached maturity. In storage it will dry out a little, causing the sugars to concentrate, which makes it appear sweeter.

1.1.3 Scientific classification

Kingdom *Plantae*
Class *Magnoliophyda*
Order *Myrtales*
Family *Lythraceae*
Genus *punica*
Species *P.granatum*

The order of *Myrtales* most likely originates from *Saxifragales,*the family *of Lythraceae* is likely to be an initial form, which gave the start to the families of *Sonneratiaceae* and *Punicaceae*.

Along with Lecythidaceaeare positioned a bit aside and have gone ahead as compared to *Myrtaceae*which left behind a branch with *Lythraceae*and *Oenotheraceae*(G.M.levin, 1999).

P. granatum did not irradiate a group of closely related taxons and until now remains a practically non-differentiated kind. However, this is also common for many other kinds of similar evolutional fate and systematic isolation.

Rapid evolutional establishment of *Punica* may have also resulted from island and echotopical isolation at relatively high limits of vertical distribution of population.

The majority of existing kinds of pomegranate are the result of artificial selection by people conducted over the course of centuries, and not a particular combination of genes, same as all creations of natural selection.

Pomegranate did not develop genetic alienation as a result of different ways of evolution of cultivated and wild grown plants as was the case with many other cultivated plants.

The first step in creating different sorts of pomegranate certainly was the selection among the populations of wild grown pomegranate, transferring plants into a culture in form of shrubs, shoots, petioles or by planting seeds, which could occur accidentally or on purpose. The method of transferring the best plants from the populations of wild grown pomegranate into a culture has lived up to these days.

1.1.4 Distribution

The pomegranate is native to Central Asia and Persia where its history starts. It was first discovered and exploited as a wild plant; only later people who lived in the hills and valleys of the region learned to domesticate the fruit.

From its centers of origin, the pomegranate spread like a pebble thrown into a still pond the ripples of its culture spread in all directions within sub-tropical and mild temperate zones.

It grows in tropical and sub-tropical area, native to the region from northern India to Iran and Mediterranean. Pomegranate cultivation spread to India from Afghanistan but the earliest. Afghanistan is part of the original native area ofthe pomegranate. The pomegranate spread to Samarkhand and from there to China. Where yellow skinned types were introduced. (G.M.levin, 1999)

The modern natural area of pomegranate distribution almost completely fits within Mediterranean and Iran Turanian floristic regions of the ancient Mediterranean subkingdom, which only partially occupies circumboreal region includes atlantic Europe,

Balkan, Euxinus, and Caucasus provinces and Eastern asiatic region includes SikangYuennan province of Boreal subkingdom of holarctic kingdom. Thus, wildly grown pomegranate is spread from Balkans to North westerns India. Its separate populations can also be found to the west Switzerland, Apennines, Perinea as well as to the east.

In Sudan it grows in marra mountain (western sudan) special gardens supplied with specific plant requirements.

1.1.5 Planting

The plant is mostly drought tolerant, and can be grown in dry areas especially during fruit development stage, prolonged hot and dry climate is mandatory for better growth, it cannot withstand cold weather and easily damaged in humid condition, it prefers sunny and sheltered places, deep alkaline soils are favaurable. Water requirement for the pomegranate is variable depending on when and how much rainfall occurs. On average pomegranates need about 45 inches of water per year.(agriculture.com)

Pomegranates are sun loving plants that react negatively to excessiveshading. They need at least 6 hours of sunlight per day for good fruiting.

Pomegranates need very little fertilizer unless you have a sandy soil. The only element that is commonly needed is nitrogen. The application rate depends on the age of the plant.

Pomegranates are self-fruitful, meaning that you only need one plant to have fruit. But the shrubs will benefit from cross pollination by an increase in production of about 30%. Pollination is usually done by insects as well hummingbirds. Although bees can help pollinate pomegranates, they are not normally the main pollinators. And pomegranates flowers are one of the hummingbird's favorites. There is little evidence of wind dispersal of pollen (Richard Ashton*et al*., 2006).

The plant has anti-toxidants and antibacterial agents (Gil *etal*., 2000). it used as a treatment for parasitic infestations, treat acidosis, dysentery, microbial infections, diarrhea (Ahmad and Beg, 2001), respiratory and oral pathologies. Several studies focused on prevention and treatment of cancer, cardiovascular disease, diabetes (Hertog*etal*., 1997), dental conditions, erectile dysfunction and skin allergy investigations were carried out to determine antioxidant, anti-carcinogenic and anti-inflammatory properties of pomegranate constituents. Pomegranate juice has recently become more popular in Western diet because of the attribution of health benefits. Pomegranate and its derivates such as juice peel and seeds are rich source of several high-value compounds with beneficial physiological activities.

Clinical studies suggest that pomegranate increases the activity of serum high density lipoprotein (HDL) and decreases the low-density lipoprotein (LDL) susceptibility to aggregation and oxidation. Inhibit LDL oxidation in 3 ways (Rosenblat and Aviram2006):

- Pomegranate juice polyphenols inhibit copper ion-induced LDL oxidation, and thus reduces the oxidized LDL content.
- Pomegranate juice polyphenols also increase the activity of serum HDL-associated paraoxonase 1 (PON1).

PON1 can in turn hydrolyse lipid peroxides in oxidized LDL and convert them to a less atherogenic "LDL", leading to a further reduction in oxidized LDL content.

1.1.6 Medicinal uses

Table (1) Chemical composition

Chemical	Concentration
Moisture	78.0%
Calcium	10mg
Protein	1.6%
Phosphorus	70mg
Fat	0.1%
Iron	0.3mg
Minerals	0.7%
Vitamin C	16mg
Carbohydrates	14.5
Fiber	5.1%
Small amount of vitamin B complex	

1.1.7 phytochemicals

Phytochemicals are the secondary metabolites produced by plants as a defense mechanism against environmental threats such as harmful ultraviolet (UV) radiation, pathogens, and herbivorous predators. Plant polyphenols are a major group of phytochemicals and an important class of antioxidants. These compounds are widespread virtually in all plant foods, often at high levels.

Punicagranatum include different types of chemicals gallocatechins, delphinidin, cyanidin, gallic acid and sitosterol (Seeram*etal*., 2006), which had therapeutic properties. Numerous phytochemical constituents have been reported to be present in different parts of the pomegranate plant making it pharmacologically precious.

Different types of phytochemicals have been identified in various parts of the pomegranate tree.

The major class of pomegranate phytochemicals is the polyphenols (phenolic rings bearing multiple hydroxyl groups).

Phenolic compounds are excellent oxygen radical scavengers because the electron reduction potential of the phenolic radical is lower than that of oxygen radicals. Therefore, phenolic compounds can scavenge reactive oxygen intermediates thus preventing further oxidative reactions. (Seeram *et al.,* 2006)

Itplays a major role in fruit color, flavor, texture as well as antioxidant and antibacterial activities. Phenolic compounds can denature enzymes but they can also bind to substrates such as minerals, vitamins and carbohydrates making them unavailable for microorganisms. Phenols can be absorbed to the cell wall, resulting in a disruption of the membrane structure and function.

1.1.8 Pomegranate polyphenols include:

1.1.8.1 Tannins

Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins.

The astringency from the tannins is that which causes the dry and puckery feeling in the mouth following the consumption of pomegranate, red wine, strong tea, or an un-ripened fruit.

The term tannin refers to the use of tannins in tanning animal hides into leather; however, the term is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecules. Tannins have molecular weights ranging from 500 to over 3000.

Tannins are found as shapeless yellowish or light brown masses like powder, flakes or sponge.

Interestingly, tannins are found almost in all plants and in all climates all over the world. The name ‘tannin’ is derived from the French word ‘tanin’ (tanning substance) and is used for a range of natural polyphenols. Lower plants such as algae, fungi and mosses do not contain much tannin. The percentage of tannins present in the plants, however, varies. While they are present in significant proportions in some plants, many others have too little of them.

Tannins are usually found in large quantities in the bark of trees where they act as a barrier for micro-organisms and protect the tree. Apart from tanning, tannins are also used in dyeing, photography, refining beer and wine as well as an astringent in medicines.

Tannins often lower the absorption of some materials into the body, tannins are also often known as anti-nutrients. For example, tannins are found in tea and coffee and consuming too much of these beverages without milk may lead to calcium and iron deficiency through a complex formation with iron when it is in the gastro-intestinal lumen which decreases the bioavailability of iron in the body and often lead to osteoporosis and anemia, adding milk or lemon juice to the tea helps in reducing or neutralizing tannins, consuming food that is rich in vitamin C also helps in neutralizing tannin’s effects on iron absorption.

Humic acids and Fulvic acids these are simplified structures, but they show the complexity of tannins. Gallic Acid, Guaiacyl, Syringyl and Cresylic Moieties are the building blocks of these large organic molecules, structure of the tannin varies, depending on the plant life in a given area.

1.1.8.2 Location of the tannins in various plant tissues:

- Bud tissues - most common in the outer part of the bud, probably as protection against freezing.
- Leaf tissues - most common in the upper epidermis. However, in evergreen plants, tannins are evenly distributed in all leaf tissues. They serve to reduce palatability and, thus, protect against predators.
- Root tissues - most common in the hypodermis (just below the epidermis). They probably act as a chemical barrier to penetration and colonization of roots by plant pathogens.
- Seed tissues - located mainly in a layer between the outer integument and the aleuronic layer. They have been associated with the maintenance of plant dormancy, and have allopathic and bactericidal properties.
- Stem tissues - often found in the active growth areas of the trees, such as the secondary phloem and xylem and the layer between epidermis and cortex.

Tannins are found in leaf tissues, bud tissues, seed tissues, root tissues and stem tissues. An example of the location of the tannins in the stem tissue is that they are often found in the growth areas of trees, such as the secondary phloem and xylem and the layer between the cortex and epidermis.

Tannins may have a role in the growth regulation of these tissues. They are also found in the heartwood of conifers and may be contribute to the natural durability of wood by inhibiting microbial activity.
Usually divided into two major groups: (Hassanpour et al, 2011)

- Condensed tannins (flavonoids).
- Hydrolysable tannins (non-flavonoids).

1.1.8.3 Medicinal Uses of tannins:

Tannins may be employed medicinally in anti-diarrheal, haemostatic, and Anti-hemorrhoidal compounds. The anti-inflammatory effects of tannins help control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders. Diarrhea is also treated with an effective astringent medicine that does not stop the flow of the disturbing substance in the stomach; rather, it controls the irritation in the small intestine.
Tannins not only heal burns and stop bleeding, but they also stop infection while they continue to heal the wound internally. The ability of tannins to form a protective layer over the exposed tissue keeps the wound from being infected even more.
Tannins are also beneficial when applied to the mucosal lining of the mouth.
Tannins can also be effective in protecting the kidneys.
Tannins have been used for immediate relief of sore throats, diarrhea, dysentery, hemorrhaging, fatigue, skin ulcers.
Tannins can cause regression of tumors that are already present in tissue, but if used excessively over time, they can cause tumors in healthy tissue. They have been also reported to have anti-viral, anti-bacterial and anti-parasitic effects.
When incubated with red grape juice and red wines with a high content of condensed tannins, the poliovirus, herpes simplex virus, and various enteric viruses are inactivated.

1.1.8.4 Flavonoids

Are polyphenolic molecules containing 15 carbon atoms and are soluble in water. They consist of two benzene rings connected by short three carbon chain. One of the carbons of this chain is connected to a carbon in one of benzene rings, either through an oxygen bridge or directly, which gives a third middle ring. (De Groot*et al,1998)*
Flavonoids can divide into six major subtypes, which include:

- Flavones.
- Isoflavonoids.
- Flavanones.
- Chalcones.
- Anthoxanthins.
- Anthocyanins.

Many of these molecules, particularly the anthoxanthis give rise to the yellow color of some petals, while anthocyanins responsible for the red color of buds and the purple red color of autumn leaves.
Falvonoids are abundant in plants, in which they perform several functions.They are essential pigments for producing the color needed to attract pollinating insects. In higher order plants, flavonoids are required for UV filtration, nitrogen fixation, cell cycle inhibition, and as chemical messengers, can display vitamin p activity.Some flavonoids inhibit certain spores to protect against certain plant disease.
Flavonoids are ubiquitous in plants and are the most common type of polyphenolic compound found in the human diet. The abundance of flavonoids coupled with low toxicity relative to other plant compounds means they can be ingested in large quantities by animals, including humans.

1.1.8.5 Health benefits to humans

Flavonoids are important anti-oxidants and promote several health effects, anti-viral, anti-cancer, anti-inflammatory, anti-allergic. One flavonoid called quercetin can help to alleviate eczema, sinusitis, asthma, and hay fever.
Some studies have shown that flavonoids intake is inversely related to heart disease, with this molecule inhibiting the oxidation of low-density lipoproteins and therefore reducing the risk of atherosclerosis developing. Pomegranate is rich in flavonoids and their consumption is thought to lower levels of triglycerides and cholesterol in the blood.
Isoflavones also lower cholesterol as well as protecting against osteoporosis and alleviating the symptoms of menopause.
Flavonoids found almost in all fruits, vegetables and herbs. They also found in other food sources including dry beans, grains and green and black teas.
Oranges are an exception to the rule because the flavonoids contained in this fruit are mainly found in the white and pulp interior of the skin.
Certain drugs do interact with flavonoids. Studies have shown that the enzyme cytochrome p450, which is involved in the metabolism of drugs, is inhibited by flavonoids.
Flavonoids have also been shown to interact with certain nutrients. They can bind to non-heme iron, thereby decreasing its absorption in the intestine.

1.1.8.6 non-flavonoids

The non-flavonoids are all based on a single C6 benzene ring compared to flavonoids which have two C6 rings as mentioned above. The non-flavonoids are based upon either hydroxyl benzoic

acid (C6-C1 backbone), or from hydroxyl cinnamic acid (C6-C3 backbone). molecules with a polyol (D-glucose) as a central core. The hydroxyl groups of these carbohydrates are partially or totally esterified with phenolic groups like gallic acid (gallotannin) or ellagic acid (ellagitannin). Hydrolysable tannins are usually present in low amounts in plants. HT are easily hydrolysed by mild acids and bases to yield carbohydrate and phenolic acids.
With the addition of extra functional groups on the basic structure, C6-C1 compounds include gallic, vanillic, salicylic acids. The better known C6-C3 compounds include coumaric and caffeic acids. These non-flavonoid acids are not usually present unattached, they more often form esters with sugars or alcohols to give rise to a wide range of phenolic compounds.

1.1.9 Common isolates

Escherichia coli

It is form part of normal flora of intestinal tract of human and animal, also it can be found in water, soil and vegetation.
It is causes urinary tract, infection, wound infection, appendicitis, peritonitis, bacteremia, meningitis especially of new born, diarrheal disease.
E.coli is gram negative motile lactose fermenters. It is grown aerobically and facultative anaerobe in 35-37 °C.
On kligler iron agar medium produce yellow butt yellow slant with gas production but no hydrogen sulphiteproduction.It is indole test positive urease test negative and citrate utilization test negative. (Monica Cheesbrough 2000, 2006)

Klebsiellapneumoniae

It is found in intestinal tract of human and animal, also it can be found in water and soil. Also, it is found as a commensal in the mouth and upper respiratory tract.
It causes chest infection (severe pneumonia), urinary infection septicaemia and meningitis also wound infection and peritonitis.
It is gram negative, non-motile lactose fermenter capsulated produce mucoid colonies on blood agar and MaCconkey agar medium.
On kligler iron agar medium produce yellow butt yellow slant with gas production and no hydrogen sulphite production. It is urease test positive citrate utilization test positive and indole test negative. (Subhash, 2012)

Proteus vulgaris

proteus species are found in intestinal tract of human and animal, also it can be found in water, soil, sewage. They are frequent contaminants of culture. It is cause urinary tract infection, abdominal and wound infection .It is gram negative, motile, non-lactose fermenter non-capsulated .
When culture aerobically produce characteristic swarming growth over the surface of blood agar and several other culture.
On kligler iron agar medium produce yellow butt red slant with hydrogen sulphite production. It is indole test positive and andfastly urease production (Monica Cheesbrough 2000, 2006).

Salmonella paratyphi A

It is found in intestinal of animal especially pigs, cows, goats' sheep, rodent hens duck and other poultry.
It causes paratyphoid fever is generally milder than typhoid caused by *S.typhi.*
It is gram negative rods, actively motile, non-lactose fermenter it can grow in DCA, XLD, SS media produce non-lactose fermenting colonies.
On kligler iron agar medium produce yellow butt, red slant with gas production. It is indoe test negative, urease test negative citrate utilization test negative. (Subhash, 2012)

Shigelladysenteriae

It is found only in human intestinal tract and causes bacillary dysenteriae (shigellosis).
It is gram negative non-motile rods, non-lactose fermenter, it grows in selective media such as DCA, XLD, SS.
On kligler iron agar media produce yellow butt red slant with hydrogen sulphite production no gas production citrate indole urease tests are negative. (Monica Cheesbrough 2000, 2006)

Pseudomonas aeruginosa

It is found in water, soil, sewage and vegetation also can be found in the intestinal tract.
It causes urinary tract infection, skin infection, respiratory infection, external ear infection, eye infection and septiceamia.
It is gram negative rods, non-lactose fermenter aerobically and usually recognized by yellow to green pyocyanin pigment.
On kligler iron agar medium produce red butt red slant no gas no hydrogen sulphite production citrate utilization test positive indole and urease tests negative (Monica Cheesbrough 2000, 2006).

1.1.10 Rationale

Antimicrobial susceptibility testing is crucial for the guidance of clinical management.Isolates from many part of the world are now multidrug-resistant (MDR). For this reason we evaluated the antibacterial activity of natural substance, different part of *punicagranatum*extract against enterobactericae (*E. coli, K. pneumoniae,*

SallmonellaParatyphiA,Shigellaspp,Proteusvulgaris).And other bacteria *S.aureus*and *P.aeruginosa*.

1.1.11 Objectives

1.1.11.1 General objective

To study the efficiency of antimicrobial activity of plant material punicagranatum on common bacterial isolates from different hospitals in Khartoum states.

1.1.11.2 Specific objective

To investigate the potential effects of antibacterial activities of different part of *punicagranatum*extract against enterobactericae(*E.coli, K.pneumoniae, SallmonellaParatyphiA, Shigellaspp, Proteus vulgaris*)and other like *S.aureus* and *P.aeruginosa*.

1.2 Literature review

K. Kanoun, B. Abbouni, S. Gabbés, 4S. Dellani and N. Zizi, investigate the antibacterial activity of aqueous decocted and methanolic pomegranate peels extract against two pathogenic multidrug resistant Gram-negative and positive bacteria (*Salmonella typhi, Enterobacter cloacae Staphylococcus aureus ATCC 43300, Bacillussubtilis)* by disc diffusion method; the aqueous decocted extract showed strong antibacterial activity against *Staphylococcus aureus ATCC 43300* and *Bacillus subtilis*compared to methanolic extract, otherwise the inhibition zones produced at 50 mg/ml were respectively 28±0 and 24±0 mm for aqueous decocted extract and 26.76±1.36, 22±0mm for methanolic extract ; for Gram-negative bacteria,it has been observed a decrease in the activity of the same extracts compared to Gram positive bacteria, the diameter of inhibition zone at 50 mg/ml of *Enterobacter cloacae* and *Salmonella typhi*tested with methanolic extract was respectively 14±0 and 9.76±1.36 mm, while the inhibition zone produced tested with aqueous decocted extract were respectively 20.5±0.5 and 10.5±0.5mm. Laboratory of Molecular Microbiology Health and Proteomics, Department of Biology,Algeria 2014.

J. janani and D. Esthelydia study activities of punicagranatum against oral microorganism and their result show pomegranate whole fruit has bioactivity against oral pathogens 2013 PG food chemistry and food processing Department of chemistry, India.

TianchaiNuamsetti study the antibacterial activity of different extract of pomegrnate fruit peels and arils(with seeds) were investigated by agar well diffusion and broth dilution against food related bacteria .The solvents use as extractants and the total phenolic content were also evaluated and there result show that all pomegranate extracts contained high levels of phenolic and exhibited all antibacterial activity against all bacteria tested Department of microbiology Faculty of science, king Mongkuts university 2012.

Tianchainuamsetti, petladadechayuenyong, sukontantipaibulvunt, in vitro antibacterial activities of different extracts of pomegranate fruit peels and arils (with seeds) were investigated by agar-well diffusion and broth dilution methods against four food-related bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonellatyphimurium*). The solvents used asextractants in this study were hot water, 95% ethanol, and acetone. Their total phenolic contents were also evaluated. All pomegranate extracts contained high levels of phenolics and exhibited antibacterial activity against all bacteria tested. The hot water extract of the peels was the most potent with the minimal inhibitory concentration of 207 mg/ml against E. coli and less than 103.6 mg/ml against the other bacteria. Gram-positive bacteria were generally more sensitive to the extracts than Gram-negative ones-faculty of king mongkut, Bangkok, 2012.

Vasudhapai, thangjamrubeechanu, and mamathainvestigate*the antibacterial activity of*Pomegranate rind extracts (alcoholic and aqueous) against various enteric pathogens. BothStandard strains and clinical isolates of Vibrio cholerae, Entero toxigenic E. coli, Enteropathogenic E. coli, Entero aggregative E.coli, *Salmonella* and *Shigella* species along with fewstrains of Candida were used in the study. Antimicrobial susceptibility testing was performed following standard procedure (Kirby-Bauer's diffusion method) by the punch well technique.The results obtained were encouraging as the ethanolic extract showed greater zones of inhibition against the various enteric pathogens tested in comparison with the aqueous extract.Most significant inhibitory effect was seen against Shigellaflexneri and *Aeromonashydrophila*manipal university, india, 2011.

H. Kadi, A. Moussaoui,H. Benmehdi, A. Benayahia, N. Nahalbouderba.Investigate the antibacterial activity of aqueous and ethanolic extracts of *Punicagranatum* obtained by decoction and maceration. The different extracts of *Punicagranatum* have been tested for antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus stearothermophilus*) and Gram-negative bacteria (*Escherichiacoli*, *Pseudomonas aeruginosa*) by disc diffusion method. The ethanolic macerate extract showed the strong invitroantibacterial activity against *Pseudomonas aeruginosa*with zone inhibition of 24.4 mm. However, the results tests by disc diffusion method revealed the effectiveness of ethanolicdecoctate against Gram-positivebacteria (*Staphylococcus*

*aureus*and*Bacillusstearothermophilus*) with diameter zone of inhibition varying with 21.1mm and 23.75 mm respectively 2011.
SaadSabbarDahham, Mir Naiman Ali, Hajera,Tabassum and Mazharuddin Khan study the antibacterial and antifungal activities ofpomegranate peel extract (rind), seed extract, juice and whole fruit on the selected bacteria and fungi. The peelextract has shown highest antimicrobial activity compared to other extracts. Among the selected bacterial andfungal cultures, the highest antibacterial activity was recorded against *Staphylococcus aureus*and among fungihigh activity against *Aspergillusniger*was recorded, Department of Microbiology, Osmania University, Hyderabad, India 2010.
Dr. Raga Eltyeb Osman, prof. Elamin Ibrahim Elnima, prof. mohammedElfatih A omer study the antibacterial activity of promegranate extracts against diabetic wound bacteria. The result indicted that punicagranatum have high activity in wound healing- Khartoum university, Sudan 2010.
AhmetD.Duman, Mustafa Ozgen, KenanS.Dayisoyly, Nurcan Erbil and CoskunDurgac, They use arial of six pomegranate varieties grown in the Mediterranean region of turkey were tested for their antimicrobial properities by the agar diffusion and minimum inhibitory concentration method against seven bacteria (*Bacillus megaterium*, *Corynebactreiumxerosis* UC9165, Escherichia coli DM, *Enterococcusfaecalis*. it has been observed that the extracts had antimicrobial effect giving inhibition zones ranging in size from 13 to 26 mm, university of kahramanmarassutcu imam, turkey 2009.

3.1 METHODOLOGY

3.1.1 Study design
This is a prospective experimental study.
3.1.2 Study subjects
Clinical isolated from human where directorate to the hospital include:

- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Escherichiae coli*
- *Klebsiella pneumoniae*
- *Proteus vulgaris*
- *Salmonella paratyphi A*
- *Shigella spp*

3.1.3 Sample size
The study include twelve isolated bacteria which is clinical isolate Three types of punica granatum extract (peels , pith, seeds) from super market in central market in Khartoum state .
3.1.4 Data collection
Thirteen species of clinical isolate were identified based on :

- Kligler iron agar test
- ureas test
- Citrate utlization test
- oxidase test
- Motility test
- Indole test
- voges-proskauer test
- Methyle red test

3.2 plant material
3.2.1 The origin source of *Punica granatum*
Imported from Egypt-Aseoat
3.2.2 Preparation of plant material
Punica granatum fruit was open from the crown (upper site), The fruit was separated to peels, seeds and pith.
Peels and pith were cut to small pieces by hand then each of them placed in a plastic tray and allowed to dry in shade for seven days.
3.2.3 Extraction method
The method named as marcertion (which is cold method). Extraction was carried out according to method described by Sukhdev*et.* (2008). Specific weight of each sample, 100g of peels, 100g of seeds, and 80g of pith powder was extracted by soaking in 1000 ml 80 % methanol for seven days with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus and the extract of each part combined together.
3.3 Antimicrobial susceptibility test:
3.3.1 Disc diffusion method:
This method depends on diffusion of an antibacterial from the disc though solidified agar layer in petri dish.
The growth of test organisms is inhibited in a circular area around the disc.
3.3.2 Material used for susceptibility test
1-Three extract of punicagranatum (peels, pith, seeds)
2-Muller –Hinton agar medium
3-Normal saline
4-Commercial control antibacterial
5-Dimethyle sulfoxide (DMSO)
6-Whatman 1 filter paper
7- Muller-Hinton broth
8 polycarbonate syringe filter

3.3.3 Preparation of plan disc for susceptibility test
1-First cut the whatman1filterpaper by paper borer to give diameter of six mm
2-we put them in sterile in screw capped biujo bottles.
3-we sterilized them in hot air oven at 170°c for 60 min.

3.3.4 Method of extract dilution

Three type of *punciagranatum* (peels,pith,seeds) were used.

Serial dilution of different extract types was prepared.

-in sterile small screw capped bottle 1g of extract is added to 200ul of dimethyle sulfoxide then addd 1800ul from deionized distilled water.

- put it in hot air oven at 30°c for 15min to complete dissolve and to make the suspension homogenous.

- Filter the suspension using polycarbonate syring filter

-Serial dilution is made from each type of extract by the following: -

-in sterile four screws capped bottles add 2ml deionized distilled water.

-Transfer 1ml from starting tube (1000mg/ml) to tube No (1) to made double dilution (500mg/ml).

-From tube No (1) to No (2) concentration become (250mg/ml) also transfer 1ml, then from tube No (2) to tube No (3) concentration become (125 mg /ml), then from No (3) to No (4) concentration become (62.5mg/ml).

- Lastly discharge 1ml from tube No (4).

3.3.5 Impregnation of plan discs

Impregnate the plan disc in each concentration and incubate it at room temperature for 30min.

- put each concentration in separate sterile petridish and labeled each of them with it is concentration.
- put it in hot air oven at 30°c for 24 hr.

3.3.6 Preparation of Inoculums

3.3.7 Preparation of colony suspension

All isolates under test were sub-cultured into non inhibitory nutrient agar plates at 37°c for 24hr.

From this culture, weprepared a suspension in 0.9%NaCl saline solution, the bacterial suspension was well homogenized and its opacity should be approximately equivalent to

McFarland stander (10^{7}_ 10^{8} CFU).

3.3.8 Susceptibility Testing (Agar Diffusion Method: Disc method)

The tested bacteria were inoculated in muller-hinton agar.

Discs saturated with different concentration of each extract were placed into the inoculated media and disc saturated with DMSO as a negative control.

The inoculated plates were left for 30min at room temperature for diffusion of extract on agar surface then incubated at 37°c for 24hrs.

Using the same method, the different strains of bacteria were tested for antibiotic susceptibility by 16 types of commercial discs.

Table (2) various antibiotics used in this study

Abbreviation	Antibiotic name	Concentration	Gram type focused
Ak-30	Amikacin	30ug	Gram negative & Gram positive
AMC-30	Amoxicillin	20/10ug	Gram negative & Gram positive
CTC-30	Cefotaxime	30ug	Gram negative & Gram positive
CTC-40	Cefotaxime/clavulanic acid	30/20ug	Gram positive & Gram negative
CRO- 30	Ceftriaxone	30ug	Gram negative & Gram positive
CX- 5	Cloxacillin	5ug	Gram positive
CIP- 5	Ciprofloxacin	5ug	Gram negative & Gram positive
FA -10	Fusidic Acid	10ug	gram positive & gram negative
IPM - 10	Imipenem	10ug	Gram negative & Gram positive
k -30	kanamycin	30ug	Gram positive & gram negative
MEM- 10	Meropenem	10ug	Gram negative & gram positive
ME-5	Methicillin	5ug	Gram positive
NA- 30	Naldixic Acid	30ug	Gram negative
F -300	Nitrofurantoin	300ug	Gram positive & Gram negative
TE- 30	Tetracycline	30ug	Gram negative & Gram positive
SXT- 25	Trimethoprim/sulfa-methoxazole	1.25/23.75ug	Gram negative & Gram positive

3.4 Determination of Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC):

The evaluation of the MIC is to determine the lowest concentration of an antimicrobial agent required to inhibit completely the bacterial growth, it was found by the use of a dilution range of the culture's series of added agent, its determination was performed from the measurement of the turbidity induced by studied microorganisms' growth, the inhibition is due to a lack of visible culture growth.

3.4.1 Material used for determination of minimum inhibitory concentration

- two extracts of *punica granatum* (peels- pith)
- Normal saline

- Muller Hinton agar
- Muller Hinton broth

3.4.2 Preparation of inoculum

Organisms under test were sub cultured on Muller Hinton agar and incubated aerobically for 24hr at 37°C.

From this culture, we prepared a suspension in 0.9% NaCl saline solution, the bacterial suspension was well homogenized and its opacity should be approximately equivalent to McFarland stander (10^7_ 10^8 CFU).

3.4.3 Method of determination MIC

On five sterile test tube 1ml of Muller Hinton broth was added ,serial dilution was prepared by transferred 100uL of extract suspension from starting tube with concentration 500uL/ml to tube No (1)the concentration become 250uL/ml and from No(1) to No(2) the concentration become 125 uL/ ml and from No(2)to No(3) the concentration 62.5 and No(3)to No(4) the concentration become 31.2uL/ml and from No(4) to No(5) the concentration become 15.6 uL/,ml 1 ml of each bacterial suspensions was added to each concentration ,all tubes was incubated aerobically at 37°c for 24 hr.

MBC was determined by sub-culture the tubes show no turbidty.

3.1.5 Data analysis:

Data were analyzed by APSS (23).

3.1.6 Ethical consideration:

Every hospital was been informed before collection of the isolates and we were obtained the agreement in a consent form.

4. 1. RESULTS

The antibacterial effectiveness of three pomegranate extracts (peels, pith and seeds) against the twelve isolates was evaluated via determination of the surrounding inhibition zones, using different concentration of extracts.

Inhibition zones using different extract concentration (50mg/ml, 25mg/ml, 12.5 mg/ml, 6.25mg/ml) are shown on tables (3, 4, 5).

All isolates show no clear zone with the negative control (DMSO).

Peels extract

All isolate show susceptibility against different concentration of peels extract used, with maximum inhibitionzone against the two strains of *P.vulgaris* inhibition zone (23mm and 20 mm) and *S.dysentery* (20mm).

The tested isolates of *S.aureus, E.coli, K.pneumoniae,* and *P.aeruoginosa (2)* show inhibition zones between (18 ±10 mm). *S.paratyphyi A* and *P.aeruoginosa (1)* show inhibition zones between (15 ± 10).

Table No (3), figure (1).

Pith extract

All isolates show susceptibility to different pith extract concentration except *S.praratyphi A* with no inhibition zone to all concentrations of extract.

Most isolates show inhibition zones varying from (17±10), maximum inhibition zone was shown by *S.aureus (2)* and *P.vulgaris* (19 mm).

Table No (4) figure (2).

Seeds extract

Seeds extract have no susceptibility against all tested isolates except *P.vulgaris (1)* which show inhibition zone from (9±5).

Table (5) figure (3).

-The susceptibility of all isolate to the different extract concentration were represent as susceptible or resistance to presence of inhibition zone or not?? In a percentage table (6).

-Minimum inhibition concentration and Minimum bactirocidal concentration were estimated using Macrotitre method for the two extracts (peels extract, pith extract). table (5).

Susceptibility of the isolates to antibiotics:

-The Susceptibility of all isolates were evaluated against multiple antibiotics agents, show table (7). The result was considered sensitive, intermediate and resistant according to the Manufaur (Bio analysis), show table (8).

Table (3) Inhibition zones (mm) with various concentrations of peels extract (mg⁄ml)

	Concentration /inhibition zone			
Species	**50 mg⁄ml**	**25mg⁄ml**	**12.5mg⁄ml**	**6.25mg⁄ml**
ZOI	**mm**	**mm**	**mm**	**mm**
S.aureus 1	18 mm	17 mm	15 mm	14 mm
S.aureus 2	18 mm	17 mm	14mm	12 mm
E.coli 1	18 mm	17 mm	16 mm	10 mm
E.coli 2	15 mm	15 mm	14 mm	11 mm
K.pneumoniae 1	18 mm	17 mm	12 mm	11 mm

K.pneumoniae 2	17 mm	14 mm	13 mm	11 mm
P.vulgaris 1	23 mm	20 mm	18 mm	15 mm
P.vulgaris 2	20 mm	19 mm	18 mm	16 mm
S.paratyphyi A	13 mm	12 mm	11 mm	10 mm
S.dysentery	20 mm	16 mm	15 mm	12 mm
P.aeruoginosa 1	15 mm	14 mm	13 mm	10 mm
P.aeruoginosa 2	18 mm	16 mm	14 mm	11 mm

Table (4) Inhibition zones (mm) with various concentrations of pith extract (mg /ml)

Species	**concentration/Inhibition zone(mm)**			
	50mg/ml	**25mg/ml**	**12.5mg/ml**	**6.25mg/ml**
S.aureus 1	16 mm	15 mm	12 mm	10 mm
S.aureus 2	19 mm	18 mm	12 mm	10 mm
E.coli 1	17 mm	16 mm	14 mm	12 mm
E.coli 2	16 mm	15 mm	14 mm	12 mm
K.pneumoniae 1	16 mm	15 mm	14 mm	12 mm
K.pneumoniae 2	17 mm	15 mm	12 mm	11 mm
P.vulgaris 1	16 mm	13 mm	12 mm	11 mm
P.vulgaris 2	19 mm	17 mm	14 mm	12 mm
S.paratyphyi A	0	0	0	0
S.dysentery	14 mm	12 mm	10 mm	5 mm
P.aeruoginosa 1	15 mm	10 mm	10 mm	9 mm
P.aeruoginosa 2	15 mm	13 mm	11 mm	10 mm

Table (5): Inhibition zones (mm) with various concentrations of seeds extract (mg/ml)

species	**Concentration/Inhibition zone(mm)**			
	50mg/ml	**25mg/ml**	**12.5mg/ml**	**6.25mg/ml**
S.aureus 1	No	No	No	No
S.aureus 2	No	No	No	No
E.coli 1	No	No	No	No
E.coli 2	No	No	No	No
K.pneumoniae 1	No	No	No	No
K.pneumoniae 2	No	No	No	No
P.vulgaris 1	9 mm	7 mm	5 mm	No
P.vulgaris 2	No	No	No	No
S.paratyphyi A	No	No	No	No
S.dysentery	No	No	No	No
P.aeruoginosa 1	No	No	No	No
P.aeruoginosa 2	No	No	No	No

Table (6): Percentage of sensitive and resistant isolates to different types of extract

Extract	**No. of sensitive**	**No. of resistant**	**Percentage of sensitive**
peels	12	0	100 %
pith	11	1	91.6 %
seeds	1	11	8.3 %

Table (7): MIC and MBC of *punica granatum* for peels & pith extract

Species	**Peels extract**		**Pith extract**	
	MIC mg⁄ml	**MBC mg⁄ml**	**MIC mg⁄ml**	**MBC mg⁄ml**
S.aureus 1	3.12	*6.25*	*3.12*	*6.25*
S.aureus 2	*3.12*	*6.25*	*3.12*	*6.25*
E.coli 1	*3.12*	*6.25*	*3.12*	*6.25*

E.coli 2	*3.12*	*6.25*	*3.12*	*6.25*
K.pneumoniae 1	*3.12*	*6.25*	*6.25*	*12.5*
K.pneumoniae2	*6.25*	*12.5*	*6.25*	*12.5*
P.vulgaris 1	*1.56*	*3.12*	*3.12*	*6.25*
P.vulgaris 2	*1.56*	*3.12*	*1.56*	*3.12*
S.paratyphi A	*12.5*	*25.0*	-	-
S.dysentery	*1.56*	*3.12*	*3.12*	*6.25*
P.aeruginosa 1	*3.12*	*6.25*	*3.12*	*6.25*
P.aeruginosa 2	*3.12*	*6.25*	*3.12*	*6.25*

Table (7) A & B: Inhibition zones (mm) of commercial antibiotic discs

A

Antibiotic	**AK- 30**	**AMC-30**	**CTX-30**	**CTC-40**	**CRO-30**	**CX-5**	**FA-10**	**IPM-10**
ZOI	**mm**	**mm**	**mm**	**mm**	**mm**	**mm**	**mm**	**mm**
S.aureus 1	15 mm	7 mm	0	0	0	0	10 mm	9 mm
S.aureus 2	14 mm	9 mm	0	0	0	0	0	0
E.coli 1	26 mm	12 mm	12 mm	22 mm	21 mm	-	-	25 mm
E.coli 2	18 mm	0	0	23 mm	14 mm	-	-	22 mm
K.pneumoniae 1	22 mm	24 mm	20 mm	34 mm	26 mm	-	-	29 mm
K.pneumoniae 2	25 mm	12 mm	26 mm	30 mm	29 mm	-	-	24 mm
P.vulgaris 1	15 mm	17 mm	0	19 mm	14 mm	-	-	21 mm
P.vulgaris 2	18 mm	19 mm	0	21 mm	15 mm	-	-	24 mm
S.paratyphi A	20 mm	15 mm	30 mm	32 mm	32 mm	-	-	30 mm
S.dysentery	23 mm	17 mm	29 mm	30 mm	34 mm	-	-	31 mm
P.aeruginosa 1	21 mm	-	0	0	0	-	-	27 mm
P.aeruginosa 2	20 mm	-	0	0	0	-	-	31 mm

B

Antibiotic	**CIP-5**	**K-30**	**MEM-10**	**NA-30**	**F-300**	**TE-30**	**SXT-25**	**ME-5**
ZOI	**mm**	**mm**	**mm**	**mm**	**mm**	**mm**	**mm**	**mm**
S.aureus 1	0	0	11 mm	-	25 mm	0	17 mm	7 mm
S.aureus 2	0	0	9 mm	-	26 mm	0	18 mm	16 mm
E.coli 1	0	10 mm	33 mm	0	15 mm	0	18 mm	-
E.coli 2	15 mm	18 mm	30 mm	0	16 mm	25 mm	0	-
K.pneumoniae 1	29 mm	17 mm	38 mm	20 mm	13 mm	21 mm	18 mm	-
K.pneumoniae 2	26 mm	16 mm	32 mm	21 mm	14 mm	23 mm	24 mm	-
P.vulgaris 1	0	0	29 mm	0	12 mm	0	0	-
P.vulgaris 2	0	0	34 mm	no	8 mm	0	0	-
S.paratyphi A	30 mm	20 mm	32 mm	25 mm	25 mm	0	0	-
S.dysentery	27 mm	12 mm	30 mm	9 mm	24 mm	0	0	-
P.aeruginosa 1	39 mm	-	37 mm	-	-	10 mm	0	-
P.aeruginosa 2	40 mm	-	35 mm	-	-	10 mm	0	-

Table (8): Standard zones for determination of antibiotic activity

Abbreviation	Antibiotic name	Bacteria	Interpretive Standards (mm)		
			Resistant	Intermediate	Susceptible
Ak-30	Amikacin	Enterobacteriaceae P.aeruginosa staphylococcus	≤14	15-16	≥17
AMC-30	Amoxicillin	Enterobacteriaceae P.aeruginosa	≤14	_	≥21
CTC-30	Cefotaxime	Enterobacteriaceae P.aeruginosa	≤22 ≤14	23-25 15-19	≥26 ≥23
CTC-40	Cefotaxime/clavulanic acid	Enterobacteriaceae staphylococcus	≤12	13-15	≥18
CRO- 30	Ceftriaxone	Enterobacteriaceae staphylococcus	≤19 ≤13	20-22 14-20	≥23 ≥21
CX- 5	Cloxacillin	staphylococcus	≤10	11-12	≥13
CIP- 5	Ciprofloxacin	Enterobacteriaceae P.aeruginosa staphylococcus	≤15	16-20	≥21
FA -10	Fusidic Acid	staphylococcus	≤14	15-22	≥23
IPM - 10	Imipenem	Enterobacteriaceae P.aeruginosa staphylococcus	≤19 ≤13	20-22 14-15	≥23 ≥16
k -30	kanamycin	Enterobacteriaceae staphylococcus	≤13	14-17	≥18
MEM- 10	Meropenem	Enterobacteriaceae P.aeruginosa staphylococcus	≤19 ≤13	20-22 14-15	≥23 ≥16
ME-5	Methicillin	Staphylococcus	≤9	10-13	≥14
NA- 30	Naldixic Acid	Enterobacteriaceae	≤13	14-18	≥19
F -300	Nitrofurantoin	Enterobacteriaceae staphylococcus	≤14	15-16	≥17
TE- 30	Tetracycline	Enterobacteriaceae P.aeruginosa staphylococcus	≤11 ≤14	12-14 15-18	≥15 ≥19
SXT- 25	Trimethoprim/sulfa-methoxazole	Enterobacteriaceae P.aeruginosa staphylococcus	≤≥10	11-15	≥16

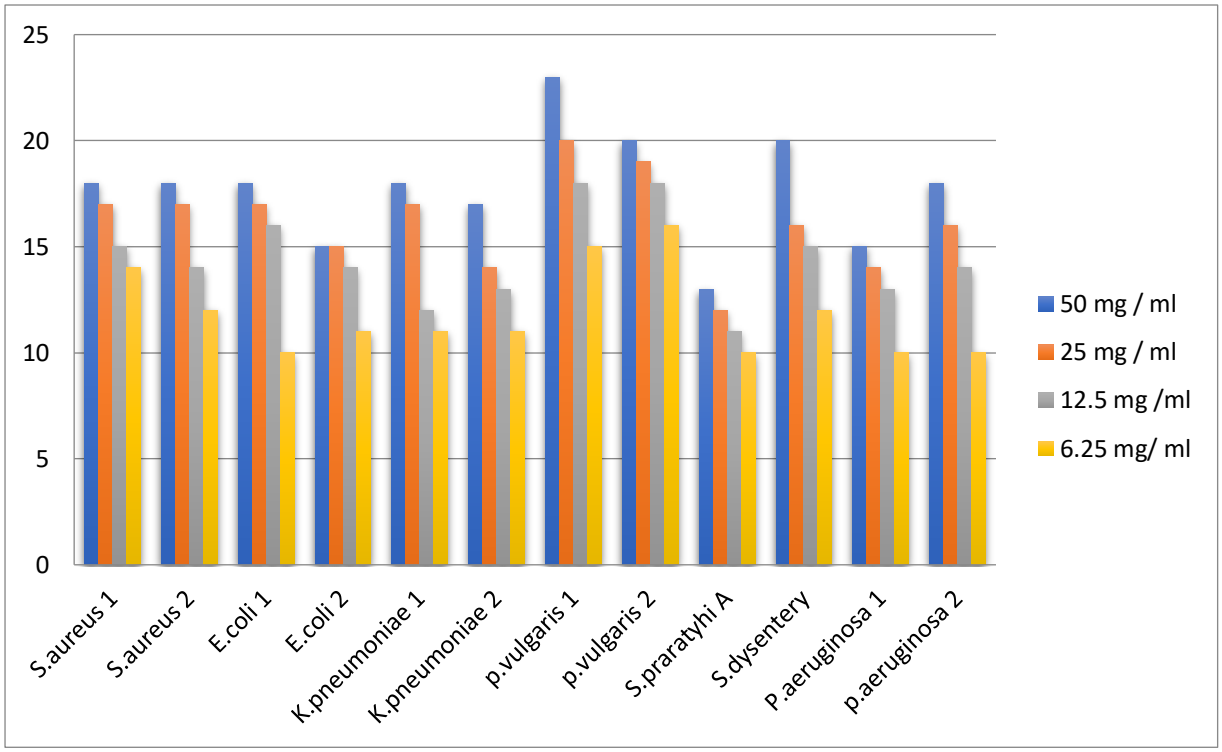

Figure (1): Antibacterial activity of peels extract in different concentrations.

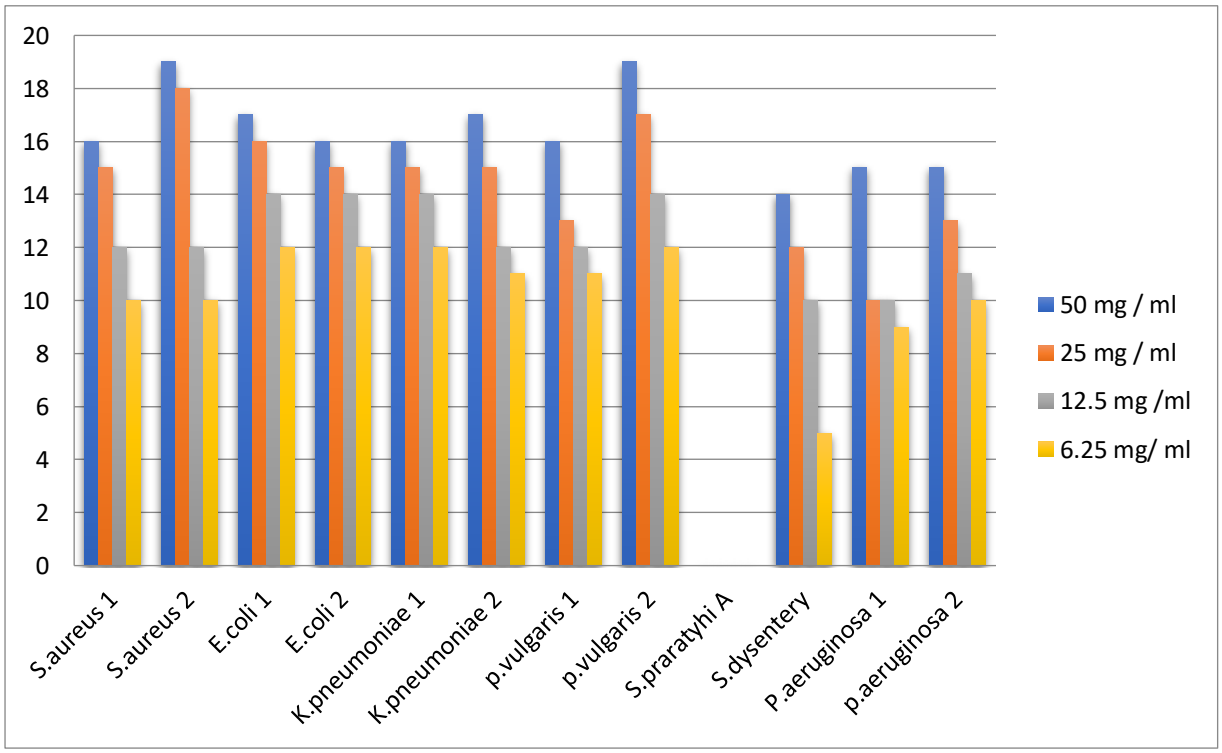

Figure (2): Antibacterial activity of pith extract in different concentrations.

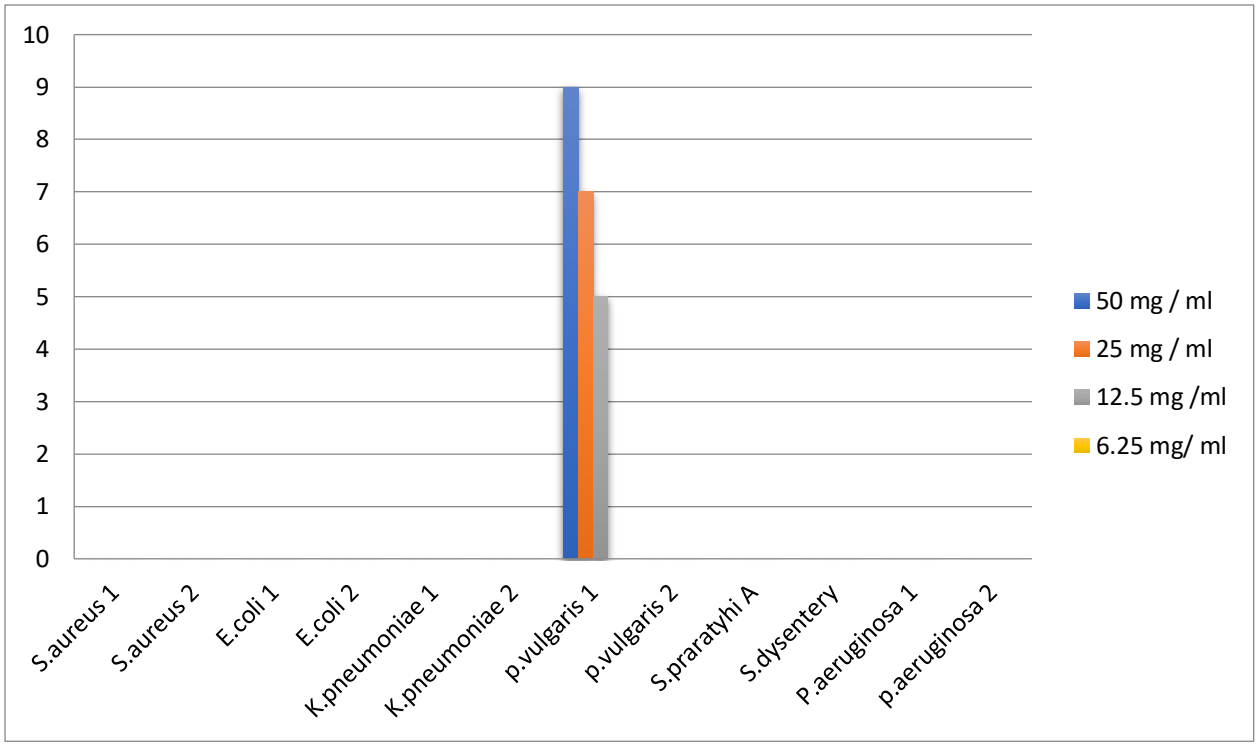

Figure (3): Antibacterial activity of seeds extract in different concentrations

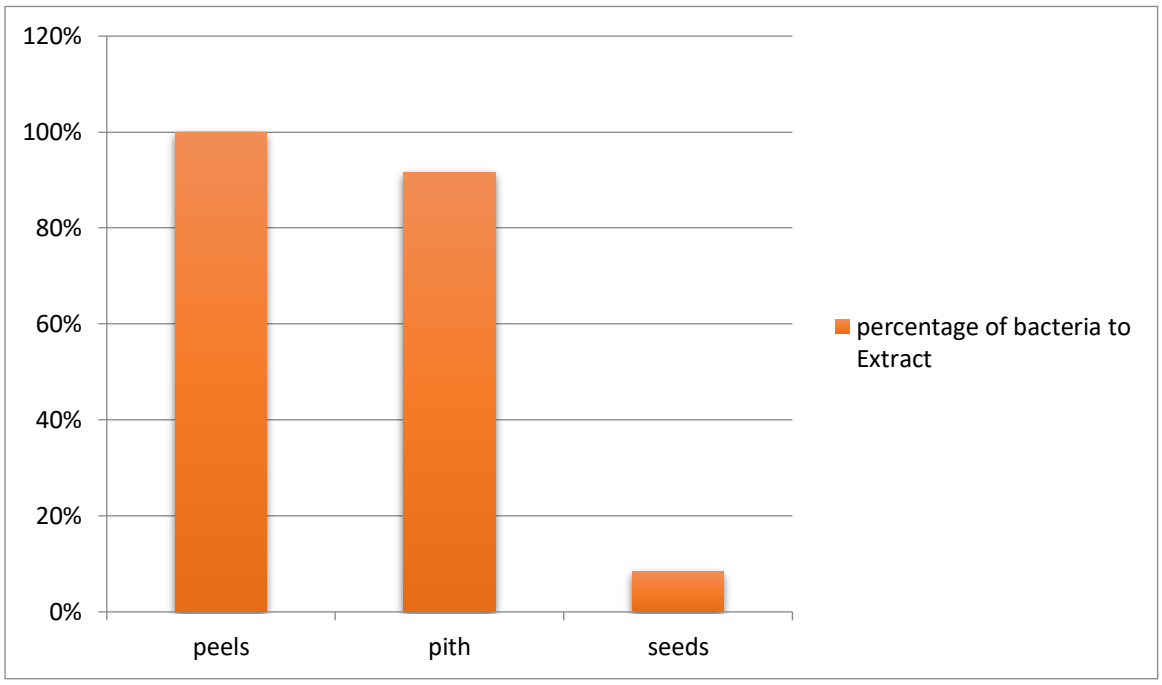

Figure (4): percentage of bacteria to different types of Extract

CONCLUSION:

Extract of punica granatum have antibacterial activity against member of Enterobacteriacae and *pseudomonas aeroginosa* and *staphylococcus aureus* with different diameter zone of inhibition. The antibacterial activities of plant extract possibly due to the secondary metabolites such as tannians, phenolic compounds or saponins that were abundant in the peels because it activated by sunlight and it may be used as medicine for humans. This reduces

the antibiotics missuses, multidrug resistance cost and antibiotic consumption. Furthermore, it could provide health benefits and may be employed in pharmaceutical purposes.

Recommendations:

1. For similar research we recommend to assist the antibacterial activity of punica granatum against *Helicobacter pylori, Bacillus cereus, clostridium* perfringens.
2. Further attention and research to identify the active compounds responsible for the plant biological activity.
3. We recommend to use punica granatum in food preservations.
4. Further studies should be undertaken to elucidate the exact mechanism of action by which extracts exert their antibacterial effect.
5. We recommend using punica granatum in treatment of bacterial infections.
6. We recommend filtrating any plant extract using polycarbonate membranes filter that because turbidity causing by extract is confused with turbidity causing by bacteria especially in determination of MIC.
7. We recommend using of magnifying lens to see the inhibition of bacterial growth when testing antibacterial activity of plant extract against pigment producing organism (like pseudomonas aeuroginosa) because combination of organism pigment with color of plant extract because that will confuse reading the zone of inhibition.

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