CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW
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1:1 Introduction and Literature review

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants and microbes (Erdogrul, 2002). In the recent years, the use of plants with preventive and therapeutic effects contributes to health care needs (Holetz, et al., 2002). There are three main interesting reasons in treating and healing power of plant extract. First, pharmacological studies have demonstrated that many of plants are known to possess antimicrobial agents; second, people are becoming aware of the side effects associated with the over prescription of traditional antibiotics; third, time to time resistant microorganisms against antibiotics are increasing (Holetz et al., 2002, Melendez and Capriles, 2006, and Naz et al., 2007). Among these plants, pomegranate has an important role in folk medicine. Pomegranate is known as a rich source of pharmacological properties which have been evaluated due to anti parasitic, antibacterial, antifungal, anti proliferative, apoptotic and anti-cancer effects as well as protection against herpes virus, inhibition of LDL oxidation and decrease in atheromatous plaque formation and reduction of systolic blood pressure (Kim et al., 2002, Reddy et al., 2007, and Naz et al., 2007).
Antimicrobial drug resistance in human bacterial pathogens is a continuing worldwide issue and as a consequence, effective treatment and control of such organisms remains an important challenge. Bacterial resistance has appeared for every major class of antibiotic (Lambert, 2005). Since their introduction the emergence of resistance to antibiotics has become increasingly evident, particularly for important pathogens such as *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., *Enterococcus* spp. and *Staphylococcus* spp. (Adesiyun, 2007 and Rodrigo, 2007).

Antimicrobial drug include antibiotics and chemical antimicrobials. Antibiotics these are antimicrobial substances produced by living microorganisms, they include culture extract, filtrates of fungi as penicillin and cephalosporin, and bacteria as *Streptomyces* and *Bacillus species*. Chemical antimicrobials are synthetically produced antimicrobial compounds; include sulphonamides, cotrimoxazole, nalidixic acid and metronidazole.

Antimicrobials act on bacteria at various ways, by inhibiting cell wall formation, damage of cell membrane, inhibit protein production, and inhibit production of nucleic acid. But some antimicrobials act as broad spectrum that means activity against wide range of Gram positive and Gram negative bacteria.

Bacteria become resistant to antimicrobial by genetic mutation, producing enzyme inactivating antimicrobial, change metabolic system, and alert permeability of their cell membrane.
In the recent decades, the interest in evaluating therapeutic effects of plants has increased dramatically (Alanis *et al.*, 2005, and Thuille *et al.*, 2006) as 80% of the world's people rely on complementary and alternative medicine for their health care needs (Magee *et al.*, 2005 and Duraipandian *et al.*, 2006). Phytoplants have been shown to be good alternatives to synthetic chemical antimicrobial agents and antibiotics because of the serious side effects, antimicrobial resistance and the emergence of previously uncommon infections that have been reported to be on the increase due to inappropriate or widespread overuse of antimicrobials (Olila *et al.*, 2002, and Pawar *et al.*, 2010).

Ever since the dawn of civilization man has used plants for his food, shelter, and fodder for his animals. Plants were also identified for use to cure him from innumerable ailments which struck his physical being. They designated these plants as 'medicinal plants'. From ancient literature it is evidence that the various parts of the plants were used in Siddha, Ayurvedha and Unani medicine for the treatment of disease of human beings (Palaniswamy *et al.*, 2008).

Over the last decade research into the antimicrobial properties of traditional plant based medicines has been revisited (Navarro *et al.*, 1996 and Braga *et al.*, 2005). Numerous plants have been screened for antimicrobial properties, for example Holetz and colleagues (Mathabe *et al.*, 2006) tested 13 plants used in Brazilian traditional medicine and they demonstrated activity against bacteria such as *Staphylococcus aureus* and *E. coli*. Meléndez and Capriles., (2006) and Braga *et al.*, (2005) tested 172 plant species used in Puerto Rico and they
demonstrated that 14 of these showed activity against bacteria including *S. aureus* and *E. coli*.

*Punica granatum*. (*Punicaceae*) referred to in English as pomegranates, belonging to family *punicaeae*, has long been esteemed as food and medicine, and is a diet in convalescence after diarrhea (Nadkarni, 2000). It is used in Siddha, Ayurvedha and Unani. medicine especially for the treatment of gastro-intestinal (GI) diseases. Pomegranate is a fruit of great antiquity and is known to have been cultivated in the Middle East more than 5,000 years ago. The plant is found all over India. Pomegranate has been considered important since prehistoric times as an agency of longevity (Ram, 1998). The fruit is good for dysentery, diarrhoea and gastralgia (Warrier, *et al.*, 2002). Hindoo physicians use the rind of the fruit and flowers, combined with aromatics, such as cloves, cinnamon, coriander, pepper etc as bowel astringent in diarrhea (Blatter, *et al.*, 2001). In addition to its ancient historical uses, pomegranate is used in several systems of medicine for a variety of ailments. In Ayurvedic medicine the pomegranate is considered “a pharmacy unto itself” and is used as an anti parasitic agent, a “blood tonic,” and to heal aphthae, diarrhea, and ulcers (Jurenka, 2008).

The fresh rind of the fruit contains: wax, 0.8; resin, 4.5; mannitol, 1.8; non-crystallized sugars, 2.7; gums, 3.2; inulin, 1.0; mucilage, 0.6; tannin, 10.4; gallic acid, 4.0; and calcium oxalate, 4.0%. Pectin occur to the extent of 2-4 % (Ram, 1998). Pomegranate peel combined with optimum level of aromatic such as cloves is a most useful remedy in chronic dysentery as well as diarrhoea. The
rind is an anti helmintic and an astringent and useful in treating diarrhea, dysentry and gastralgia (Prashanth, et al., 2001). Commonly used as febrifuge and part of the diet in convalescence after diarrhea. Wet and dry fruit is good for heart, stomach and enhances the production of hemoglobin. It is a good diuretic agent and gives strength. Duraipandiyan et al., (2006) reported that dried fruit coat is grounded and mixed with water and taken internally to treat stomachache and diarrhea. Extract of different parts of the fruit exhibited antibacterial activity. Extracts of the whole fruit were highly active against Micrococcus pyogens, S. aureus, E.coli, and Pseudomonas aeruginosa. They were also very effective against intestinal pathogenic bacilli such as Salmonella paradysenteriae III-Z, S. typhi, S. monetevideo, S. scholtmuelleri and Shigella paradysentriae B.H. Alcoholic extracts of the fruit rind and root bark showed activity against Micrococcus pyogens 60% (Ram, 1998). Ingestion of pathogens can cause many different infections. These may be confined to the GIT or initiated in the gut before spreading to other parts of the body. A syndrome characterized by GI symptoms including nausea, vomiting, diarrhoea and abdominal discomfort. Worldwide, diarrhoea diseases are the second leading cause of death; about 25 million enteric infections occur each year. These infections cause significant morbidity and death, particularly in elderly people and children younger than age 5. It has been estimated that 4 to 6 million children die each year from diarrhoea, particularly in developing countries in Asia and Africa. Even in developed countries, significant
morbidity occurs as a result of diarrhoea illness, although acute diarrhoeal syndromes are usually self-limited, some persons with infectious diarrhoea will require diagnostic studies and treatment. The last decade has seen a resurgence of global interest in medicinal plants as therapeutic agents. This traditional treatment approach is of much significance in the world especially in India due to the endemic presence of infective gastrointestinal diseases, which are the major causes of infant and adult mortality. Knowing the activity of *P. granatum* a study has been carried out to know its antibacterial activity, which has been reported in this research.

Pomegranate have been highlighted in many studies as having antimicrobial activity against a range of both Gram positive and negative bacteria (Navarro *et al.*, 2002 and Braga *et al.*, 2005). Silver, (2005) tested a number of extracts of pomegranates against a range of bacteria (*S. aureus*, *E. coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Bacillus subtilis* (*B. subtilis*) and *Salmonella typhi*), and they found activity against all isolates. O'Neill *et al.*, (2003) observed that pomegranate extracts were able to inhibit not only the growth of *S. aureus* but also the production of enterotoxin. The methanolic extract derived from 200 g of dried pomegranate produced bactericidal effects at 1% (v/v) over an extended incubation period (50 hours), demonstrating longevity of action.

Many bacteria have advanced protective mechanisms for the detoxification of heavy metal ions (Strohal *et al.*, 2005). In addition, all strains were sensitive to
AgNO$_3$ over the concentration range studied. Ionic silver salts have been particularly useful as a bactericide at minute concentrations.

The enhancement of the antimicrobial activities exhibited by pomegranate rind extracts by the addition of metal ions has been investigated by (Stewart et al, 1998). Their aim was to develop a rapid screening method for the detection of tuberculosis. They demonstrated that short term exposure to a pomegranate rind extract (PRE)/ferrous salt combination for 3 minutes reduced bacteriophage levels with no effect upon the bacteria. This short duration of exposure, although effective for the bacteriophage assay, was necessary owing to the low stability observed for the PRE/ferrous salt solutions.

*Shigella* species are non motile organism, Gram negative rods or coccobacilli. The main disease that *Shigella* spp caused is bacillary dysentery; shigellosis is caused mainly by *S. dysenteriae* and *S. flexneri*. *Shigella* species are found only in the human intestinal tract, transmitted by the faecal oral route. The highest incidence of shigellosis occurs in areas of poor sanitation and where water supplies are polluted. Lack of personal and domestic hygiene are important in contributing to the spread of infection. Young children usually over 6 months of age are more frequently infected than adult. Antimicrobial activity against *Shigella* include sulphonamides, tetracycline, chloramphenicol, ampicillin and streptomycin. In recent years strains of *shigella* resistant to sulphonylamides and streptomycin have emerged. Major *S. dysenteriae*
epidemics caused by multiple drug resistant strains have been reported from Sri Lanka, Bangladesh, and Central America.

Another Gram negative rod is *Salmonella*, motile organism. Mainly caused enteric fever, food poisoning, bone infections and meningitis. Most *Salmonellae* are found in the intestine of animals, but *S. typhi* and *S. paratyphi* are excreted in the faeces and urine of infected patients and are present in gall bladders of long term carriers. Infection is by ingesting the organism in contaminated food or water, or from contaminated hands. Antimicrobial activity against *Salmonella* includes chloramphenicol, cotrimoxazole, and ampicillin. Chloramphenicol resistant strains have been reported from Malaysia and elsewhere, and in recent years major typhoid epidemics caused by strains showing resistance to several antibiotics have been reported from Mexico and South East Asia.

*Salmonella typhimurium* drug resistant strains have been reported from India and the Philippines, and other serovars showing multiple drug resistance have been reported from several developing countries.

To isolate both organisms from faeces we need selective media, as XLD (xylose lysine deoxycholate) agar, in this medium two organism give pink red colony but *Salmonella* give also black center. DCA (deoxycholate citrate agar) or SSA (*Salmonella_Shigella* agar) both organism give non lactose ferment colony.
1:2 Rationale

The success of antibiotics in so many areas has, ironically, led antibiotics to become an endangered category of drugs. Bacteria have once again demonstrated their enormous genetic flexibility by becoming resistant to one antibiotic after another. At first, bacterial resistance to antibiotics, such as penicillin, did not seem very alarming because new antibiotics were regularly being discovered and introduced into clinical use. In the 1970s, however, a scant two decades after the introduction of the first antibiotics, the number of new antibiotics entering the pipeline from laboratory to clinic began to decrease. Antibiotic discovery and development are expensive, especially considering the speed with which bacterial resistance can arise. And they are becoming more and more difficult to discover and develop. These factors have led pharmaceutical companies to be less and less interested in antibiotic production. One company after another has shut down or cut back on its antibiotic discovery program.

Another reason, most of microbial resistance which is now making it difficult to treat some infectious disease is of genetic origin. It is due to extensive use of antimicrobial drug which have favoured the emergence of resistant strains. The overuse and misuse of antimicrobials have lead to the death of sensitive strains leaving resistant strains to survive, multiply, and infect new hosts.
1:3 Objectives

1:3:1 General objective

To evaluate and detect the activity of Pomegranate rind extract (PRE) as antimicrobial agent against some of GIT Gram negative bacteria.

1:3:2 Specific objectives

1. The aim of this study to detect the activity of the PRE as antimicrobial agent against Salmonella and Shigella bacteria, that showed resistance for some antibiotics.

2. To find out the phytochemical components of Punica granatum through the use of device GCMS.
CHAPTER TWO

MATERIALS AND METHODS
CHAPTER TWO

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Materials and Methods

2:1 Study location

Laboratory of Sudan University of Science and Technology.

2:2 Type of the study

Descriptive cross sectional study.

2:3 Study duration

From April to October in 2014.

2:4 Microorganisms

Clinical isolates of *Salmonella paratyphi B* and *Shigella spp.* were obtained from the Microbiology Laboratory of Ribat National University. Each of the bacterial specimens was inoculated into sterile normal saline and incubated at room temperature for 5 minutes, then measured to a 0.5 Mc Farland dilution (standard concentration). Then the agar plates with methanolic extract of *P.granatum* rind was incubated overnight at 37°C.

2:5 Identification of microorganisms

Including stain reaction, organism morphology, growth condition, colonial appearance on different media, and biochemical characteristics.
2:5:1 Gram staining technique

After smear preparation, the fixed smear was covered by crystal violet for one minute. The smear washed with tap water, and covered with Gram iodine solution for one minute. After that smear was washed with Acetone alcohol for few seconds, then washed with tap water. Finally smear covered with sufranine solution for two minutes, washed with tap water and allowed to dry then examined with oil emersion lens.

2:5:2 Biochemical tests

The differentiation of suspect organisms colonies using motility indole urea (MIU) medium and kligler iron agar (KIA) and other biochemical tests. Results are showed in table 2:1.

Table 2:1 Biochemical results for *Salmonella* and *Shigella* bacteria

<table>
<thead>
<tr>
<th>Species</th>
<th>Lact</th>
<th>Glu</th>
<th>Ox</th>
<th>Cit</th>
<th>Mot</th>
<th>Ind</th>
<th>Urea</th>
<th>Slope</th>
<th>Butt</th>
<th>$H_2S$</th>
<th>GAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shigella species</td>
<td>_</td>
<td>+</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>D</td>
<td>_</td>
<td>R</td>
<td>Y</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Salmonella paratyphi B</td>
<td>_</td>
<td>+</td>
<td>_</td>
<td>+</td>
<td>+</td>
<td>_</td>
<td>_</td>
<td>R</td>
<td>Y</td>
<td>+</td>
<td>D</td>
</tr>
</tbody>
</table>

2:6 Preparation of Muller_Hinton agar

It was prepared by dissolving 38g Muller Hinton powder in one liter of distilled water, then sterilized by autoclaving at 121C for 15 minutes, then cooled to
about 50°C and poured into sterile petri dishes in 15ml amount, the poured media were left to solidify at room temperature (Cheeshrough, 1991).

2:7 Plant Materials

The fruit of *P. granatum* was selected for this study based on their traditional practices by Indians. Fresh fruits were collected from the local market.

2:8 Preparation of methanol extract

Extraction was carried out according to method described by Sukhdev et al. (2008).

Hundred of *P. granatum* rind was grinded using mortar and pestle and extracted with 70% methanol using soxhelt extractor apparatus. Extraction was carried out for about eight hours till the solvent returned colorless. Solvent was evaporated under reduced pressure using rotary evaporator apparatus. Finally extract was allowed to dry in air in petri dishes till complete dryness and the yield percentage was calculated as followed:

Weight of extract obtained/ weight of plant sample 100

**Table 2:1 Yield percent of extract**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight of sample</th>
<th>Weight of extract</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>100g</td>
<td>40.8 g</td>
<td>40.8%</td>
<td></td>
</tr>
</tbody>
</table>
2:9 Antibacterial Tests

Methanolic Pomegranate rind extract (MPRE) was tested at different concentrations using a standard diffusion technique: 100%, 50% and 25%. The tested organism was inoculated on Muller-Hinton agar using sterile swab. Then three wells were made using cork porer device with equal space between each other and the diameter of plate was 9cm. The three wells were labeled by the following number: 1, 2 and 3. Well (1) filled with MPRE100%, well (2) filled with MPRE50% and well (3) filled with MPRE25%. Finally, the diameter of inhibition zones were measured in millimeter after 24 hours incubation at 37ºC.

2:10 Gas Chromatography of Methanolic Pomegranate Rind Extract

In gas chromatography, the mobile phase (or moving phase) is a carrier gas, usually any phase inert gas such as helium or an un reactive gas such as nitrogen. The stationary phase is microscopic layer of lipid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column (a homage to the fractionating column used in distillation). The instrument used to perform gas chromatography is called a gas chromatograph (or acrograph, gas separator).

The gaseous compounds being analysed interact with the walls of the column, which is coated with a stationary phase. This causes each compound to elute at a different time, known as the retention time of the compound. The comparison of retention times is what gives GC its analytical usefulness.
2:11 Methylation steps

Two grams from the sample was taken and transferred in a test tube, then 7ml from alcoholic sodium hydroxide was prepared by (2g (NaOH) to 100ml (methanol)), and 7ml of alcoholic sulphuric acid was added which was prepared by (1ml ($H_2SO_4$) TO 100ml (methanol)). After that sample was put for overnight in the room temperature.

Then a suitable volume from normal hexen (n-Hexen) was added, also a suitable of super saturated sodium chloride was added. Then it was shaken gently, after that two layers were appeared, the upper one is organic layer and the lower is aqueous layer. Finally a volume of the organic was taken and diluted by diethylene and injected in Gas chromatography Mass spectorometer (GC-MS) by using 1micrometer syringe.
CHAPTER THREE

RESULTS
CHAPTER THREE

RESULTS

3-1 Results

After 24 h, zones of inhibition were observed against *Salmonella* and *Shigella*.

The results on antimicrobial screening of the extracts of the *P.granatum* are shown in tables 3:1, 3:2, 3:3 and 3:4.

**Table 3:1 Zone inhibition diameter of Methanolic Pomegranate Rind Extract (MPRE) against *Salmonella* .**

<table>
<thead>
<tr>
<th>Concentration of MPRE</th>
<th>100%</th>
<th>50%</th>
<th>25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>16mm</td>
<td>16mm</td>
<td>9mm</td>
<td>4mm</td>
</tr>
<tr>
<td>10mm</td>
<td>10mm</td>
<td>6mm</td>
<td>5mm</td>
</tr>
<tr>
<td>10mm</td>
<td>10mm</td>
<td>8mm</td>
<td>6mm</td>
</tr>
<tr>
<td>14mm</td>
<td>14mm</td>
<td>10mm</td>
<td>8mm</td>
</tr>
<tr>
<td>16mm</td>
<td>16mm</td>
<td>11mm</td>
<td>5mm</td>
</tr>
<tr>
<td>15mm</td>
<td>15mm</td>
<td>10mm</td>
<td>4mm</td>
</tr>
<tr>
<td>19mm</td>
<td>19mm</td>
<td>11mm</td>
<td>5mm</td>
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<tr>
<td>17mm</td>
<td>17mm</td>
<td>8mm</td>
<td>5mm</td>
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<tr>
<td>15mm</td>
<td>15mm</td>
<td>10mm</td>
<td>6mm</td>
</tr>
<tr>
<td>15mm</td>
<td>15mm</td>
<td>11mm</td>
<td>6mm</td>
</tr>
</tbody>
</table>
The antibacterial activity of the extract resulted in clear inhibitory zones of at least 10mm for *Salmonella spp.* in concentration 100%. It was found that all concentrations of MPRE (100%, 50% and 25%) inhibited *Salmonella*. In addition, the concentration of 100% was the most effective extract against *Salmonella* compared with the others.

MPRE 50% resulted in clear inhibition zone at minimum 6mm, and maximum 11mm. This concentration made inhibition zone less than MPRE 100%.

The minimum inhibitory zone created by the 25% conc, of MPRE was 4mm and maximum are 8mm.

**Table 3.2 Zone inhibition diameter of Methanolic Pomegranate Rind Extract (MPRE) against *Shigella* spp.**

<table>
<thead>
<tr>
<th>Concentration of MPRE</th>
<th>100%</th>
<th>50%</th>
<th>25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>6mm</td>
<td>6mm</td>
<td>4mm</td>
<td>-</td>
</tr>
<tr>
<td>6mm</td>
<td>6mm</td>
<td>4mm</td>
<td>-</td>
</tr>
<tr>
<td>4mm</td>
<td>4mm</td>
<td>2mm</td>
<td>-</td>
</tr>
<tr>
<td>6mm</td>
<td>6mm</td>
<td>3mm</td>
<td>-</td>
</tr>
<tr>
<td>7mm</td>
<td>7mm</td>
<td>4mm</td>
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<tr>
<td>5mm</td>
<td>5mm</td>
<td>4mm</td>
<td>-</td>
</tr>
<tr>
<td>3mm</td>
<td>3mm</td>
<td>1mm</td>
<td>-</td>
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<td>3mm</td>
<td>3mm</td>
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<tr>
<td>2mm</td>
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</tr>
<tr>
<td>2mm</td>
<td>2mm</td>
<td>_</td>
<td>-</td>
</tr>
</tbody>
</table>

20
After evaluating the antibacterial effects of three different concentrations of MPRE, it was found that all concentrations of MEPGP not effective, some showed no markable inhibitory effect. Only two MPRE concentrations of 100% and 50% is effective against Shigella bacteria, but there was no significant difference between these concentrations. In addition, the concentration of 100% MPRE was the most effective extract against Shigella compared with the others, that made at more clear zone inhibition with diameter 7mm.

In other hand, concentration of MPRE 50% show activity against Shigella and made zone inhibition with diameter at more 4mm, and at least some considered as resistant. The concentration MPRE25% showed no activity against Shigella, that means zone of inhibition was not made. So, MPRE25% was in effective against Shigella.

**Table 3:3 The Mean of the zone inhibition of MPRE of Salmonella and Shigella bacteria**

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Salmonella</th>
<th>Shigella</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>14.70</td>
<td>4.40</td>
</tr>
<tr>
<td>50%</td>
<td>9.40</td>
<td>2.30</td>
</tr>
<tr>
<td>25%</td>
<td>5.40</td>
<td>.00</td>
</tr>
</tbody>
</table>
According to Interpretative Chart for Antibiotic, specifically regard to cotrimoxazole and chloramphenicol, the *Salmonella* bacteria was sensitive at concentration 100% but other concentration and *Shigella* bacteria showed resistant.

Figure 1: Mean of *Salmonella* and *Shigella* with different conc.
3:2 Gas chromatography result

Gas chromatography results (table 3:3) (figure 3) revealed that *Punica granatum* contain 20 phytochemical components. The active ingredient was not evident in this study.

**Table 3.4 Gas chromatography analysis of *Punica granatum* rind extracts**

<table>
<thead>
<tr>
<th>Peak</th>
<th>Retention time</th>
<th>Area</th>
<th>Area%</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.362</td>
<td>893665</td>
<td>1.59</td>
<td>1,2,3-propanetriol (CAS) Glycerol</td>
</tr>
<tr>
<td>2</td>
<td>4.877</td>
<td>808183</td>
<td>1.44</td>
<td>1-penten-3-one (CAS) Ethyl vinyl ketone</td>
</tr>
<tr>
<td>3</td>
<td>6.074</td>
<td>393396</td>
<td>0.70</td>
<td>Silanol, trimethyl-</td>
</tr>
<tr>
<td>4</td>
<td>7.172</td>
<td>5703057</td>
<td>10.17</td>
<td>2-Furancarboxaldehyde (CAS) Furfural</td>
</tr>
<tr>
<td>5</td>
<td>7.968</td>
<td>851603</td>
<td>1.52</td>
<td>2-Furanmethanol (CAS) Furfuryl alcohol</td>
</tr>
<tr>
<td>6</td>
<td>8.766</td>
<td>1084381</td>
<td>1.93</td>
<td>Butanoic acid, 2-ethyl-, methyl ester</td>
</tr>
<tr>
<td>7</td>
<td>10.174</td>
<td>302130</td>
<td>0.54</td>
<td>1,2-Cyclopentanedione</td>
</tr>
<tr>
<td>8</td>
<td>10.836</td>
<td>381988</td>
<td>0.68</td>
<td>2,5,Furandione, 3-methyl-</td>
</tr>
<tr>
<td>9</td>
<td>11.255</td>
<td>353999</td>
<td>0.63</td>
<td>4-oxo-5-methoxy-2-penten-5-olide</td>
</tr>
<tr>
<td>10</td>
<td>11.464</td>
<td>513066</td>
<td>0.91</td>
<td>2-Furancarboxaldehyde, 5-methyl-</td>
</tr>
<tr>
<td>11</td>
<td>11.941</td>
<td>686870</td>
<td>1.22</td>
<td>2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one</td>
</tr>
<tr>
<td>12</td>
<td>14.240</td>
<td>439291</td>
<td>0.78</td>
<td>Pentanoic acid, 4-oxo-</td>
</tr>
<tr>
<td>13</td>
<td>15.324</td>
<td>577328</td>
<td>1.03</td>
<td>Methyl 2-furoate</td>
</tr>
<tr>
<td>14</td>
<td>17.040</td>
<td>411139</td>
<td>0.73</td>
<td>Pentanoic acid, 4-oxo(CAS) levulinic acid</td>
</tr>
<tr>
<td>15</td>
<td>17.267</td>
<td>5030694</td>
<td>8.97</td>
<td>4H-Pyran-4-4one,2,3-dihydro-3,5-dihydroxy-6-methyl-</td>
</tr>
<tr>
<td></td>
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<td>-----------------------------------------------------------------</td>
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<tr>
<td>16</td>
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<td>Salicylic Acid</td>
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<td>5.59</td>
<td>1,2,3-Benzene triol</td>
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</table>
CHAPTER FOUR

DISCUSSION
CHAPTER FOUR

DISCUSSION

4:1 DISCUSSION

Results of the present study showed that MPRE was effective against *Salmonella*, but not very effective against *Shigella*. Our result are similar to that (Pradeep *et al*., 2008) who found that *Punica granatum* was effective against *Salmonella*, but our result are different in case of *Shigella*, because in their study they found that *Shigella* bacteria are sensitive, while in our study *Shigella* was resistant. Pradeep *et al*., (2008) in their study showed antibacterial activity of the crude extract of both ripened and unripened pericarp using two solvent (methanol and acetone) extracts, but our study was just active at ripened pericarp extracts using only methanolic extracts. Frdoos *et al*., (2014) in her study showed that ethanolic extracts possessed strong antibacterial activity directed against Gram negative *Escherichia coli type (1)*, and this was important for the first time. This study showed that *Punica granatum* water extract wasn’t equally active as organic extracts. Both aqueous and ether petroleum extracts from different parts of the plant studied (pericarp, leaves, flowers, seeds) did not have antibacterial effect, while ethanol extracts produced disparate zones of inhibition against *Escherichia coli type (1)*. Of the parts studied, the most active extracts were those obtained from pericarp as seeds of *Punica granatum*. The results indicated the presence of zone of inhibition of 24 mm diameter of ethanol extract of pericarp as seeds, and 15
mm of ethanol extract of flowers as leaves. The activity of ethanol extracts from *Punica granatum* was similar to Amikacin.

**CONCLUSION**

Many herbs have preventive or therapeutic potentials. Therefore, further studies are required to find these effects in order to replace synthetic medications with natural remedies. According to the results of this study, the extract of *Punica granatum* might be used in the control of common pathogens. On the other hand, further photochemical studies are required to determine the type of compounds responsible for the antibacterial effect of pomegranate.
REFERENCES


(Punica granatum) for human breast cancer. Breast Cancer Res Treat Feb; 71 3:203-17


APPENDIX