ORIGINAL ARTICLE

Antimicrobial Activity of the Compound 2-Piperidinone, N-[4-Bromo-n-butyl]- Extracted from Pomegranate Peels

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HIGHLIGHTS

- Extraction, isolation, and estimation of PNbb in pomegranate peels.
- Use GC-mas technique to diagnose the PNbb extracted from pomegranate peels.
- The study of the biological efficiency of compound PNbb against three types of clinical pathogenic.
- Demonstrate the biologic activity of the extracted organic compound PNbb by finding the statistical values by finding the statistical values f the extracted organic com

Abstract

Objective: The biological efficiency of one of the 2-piperidinone, N-[4-bromo-n-butyl]- (PNbb) compounds that are isolated from the pomegranate peel extract was studied. **Materials and Methods:** Using organic solvents, seven compounds were isolated from 200 g dried pomegranate peels by Soxhlet extractor. PNbb with the use of solvents such as methanol, chloroform, ethyl acetate, and hexane was applied. The extraction ratio was higher when methanol was used and chloroform exhibits less. All the extracts were analyzed by the gas chromatography–mass spectrometry (GC-MS) to identify and characterize the chemical compounds present in the raw extract in both aquatic and organic layers. Each extracted isolated compound was detected using a number of internationally recognized detection methods. By taking the highest concentration of the PNbb and less concentration of the compound, the biological efficacy of PNbb was investigated against pathogenic microorganisms. The study of activity of inhibition of pathogenic isolates was achieved using the potato culture medium Dextrose Agar. The PNbb has demonstrated excellent bioavailability against clinical pathogenic isolates. **Results:** The GC-Mas detection process showed the presence of seven compounds in the pomegranate peel extract. The same technique demonstrated the possibility of extracted PNbb compound using various solvents, its potential for inhibition evaluation, and the study of its biological effect against clinical pathogenic isolated. **Conclusions:** In the present investigation, seven active compounds have been identified from by GC-MS, one of these compounds as bioactive compound (PNbb) that probably use as against pathogenic microbes. The presence of bioactive compounds in pomegranate peels proved pharmaceutical importance. However, further studies will require to find its bioactivity and toxicity profile.

Key words: Antimicrobial activity, clinical pathogenic isolated, pomegranate peel extract

INTRODUCTION

The 2-piperidinone, N-[4-bromo-n-butyl]- (PNbb), IUPAC Name [1-(4-bromobutyl) piperidin-2-one], is a member of the class of delta-lactams, i.e., 2-piperidinone in which the amide hydrogen is replaced by a 4-bromobutyl group. It is a delta-lactam and an organobromine compound, and due to its selectivity, it is effective against a number of biological activities. PNbb is

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a white powder whose molecular formula has C9H16BrNO, a molecular weight of 234.137 g/mol, it is chemically currently classified as antimicrobial and a pesticide. It can be classified as a biochemist for its high toxicity, as shown in Figure 1.

Electron pairs in the groups Br, N-, O= and bonds of beta-lactam in the PNbb compound are particularly related to bacterial surface molecules of negative charge, which disrupt the bacterial membrane leading to its dissolution and thus cell death. On this basis, the amine groups have attracted high antibacterial efficacy as a new class of antibiotics.

The plant extracts are known to be effective, and the scientific reports indicate the effectiveness of plant extracts as effective antimicrobial agents. This was due to the bad and excessive use of antibiotics. Industrial antibiotics have caused the emergence of chemotherapeutic resistance in some bacteria, especially those of antibiotic resistance when taken for long periods of time.[1-5] These side effects of industrial antibiotics have encouraged many people to use medicinal plant extracts as effective therapeutic agents.[6-8] Due to the pomegranate extract of organic compounds of medical importance, many studies have been conducted to evaluate the total content of the compounds, especially the scales of these fruits. However, modern research is trying to seriously evaluate the organic content of different parts of the fruit at different stages of growth and using more sophisticated techniques such as gas chromatography–mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) technology. Several international and local scientific reports have indicated that the plant contains all parts of it, including polyols, amines, flavonoids, tannins, and organic compounds, which may reach >36 species and have been studied.[9] Most organic extracts are used to control the symptoms associated with gastrointestinal disorders, inhibiting or preventing the activity of different types of bacteria and fungal pathogens and reducing the pathological effects of the digestive system, preventing nausea and reducing gastric acid secretions. The use of the bioactive compound PNbb as an effective bactericidal inhibitor can also be used to treat bladder spasms, shrinkage, peptic ulcer inflammation, and pancreatitis. Getting the compound PNbb as a competitor is resistant to different types of microbes. Several analytical studies have been used to estimate the active compounds in pomegranate fruits and pods; the results were astounding when two types of photovoltaic and fluorescent detectors were used. The efficiency of separation and accuracy increased when the researchers used chromatography in other techniques. The main purpose of this study is to find natural medical alternatives that help to inhibit the growth of bacteria associated with bacterial infection, especially bacteria known to possess defensive means that lead to the failure of therapeutic attempts and reduce the effectiveness of antibiotics noting that there are many factors that stimulate bacterial cells to stimulate the production of colonies where Some bacteria and fungi change the behavior of the genes that regulate this process.[10-13]

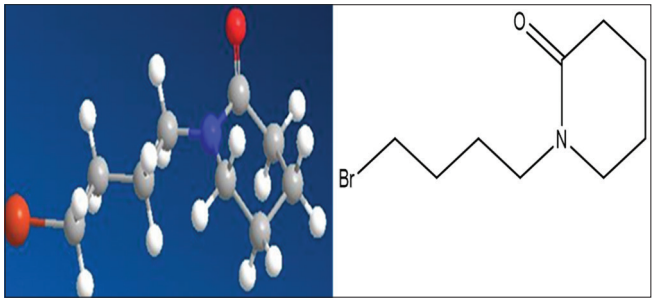

Figure 1: Structure of 2-piperidinone, N-[4-bromo-n-butyl]

MATERIALS AND METHODS

All solvents and reagents were of analytical grade, and all experiments were performed with deionized water (18.2 Ω cm) and the resistivity at 25°C.[14]

Chemicals

- Hexane was obtained from HPLC grade, BDH Chem. Ltd.
- Ethyl acetate, BDH Chem. Ltd.
- Ethanol and methanol, BDH Chem. Ltd.
- Chloroform, HPLC grad. Ltd.
- Potato Dextrose Agar as culture medium, BDH Biochem. Ltd.
- Water was obtained by purification in a deionized water system.

Collection of material

Pomegranate peel material was collected from ripe pomegranate peels and washed to remove the soil with water. The crusts were separated from the rest of the fruit. The crusts were placed on large filtration papers at room temperature. The electric mill is used for drying and grinding of crusts, and dry powder was used for this study.[15,16]

Preparation of pomegranate peel extracts

As per the method described, 200 g of pomegranate peel powder was dissolved in 1 L of water. For increasing the extraction ratio, the mixture was mixed well with an electric mixer and then placed in a shaker incubator at 24°C for 24 h. The filtrate was collected and concentrated in the rotary vacuum evaporator at a temperature of 40°C; the concentrated extract was placed in dishes with a large surface area. The remaining water was dried in an electric oven at a temperature of 40°C until the water completely evaporated and obtained dry powder. The powder is placed in tightly sealed containers and marked with PVC net frozen at 4°C until use.[17,19]

Extraction methods

There are two steps involved in this method using separatory funnel, and in the first step, pomegranate peel powder was mixed with the following solvent ratio of ethanol:chloroform:water (40:40:10). A number of organic compounds were obtained from the extraction process in the organic and aquatic layer; three compounds were obtained in the organic layer (1-docosene, PNbb, and eicosane) and four compounds in aquatic layer (tributyl acetyl citrate [TBAC], O-veratramide, methyl palmitate [MP], butanamide, and N-decyl-N-methyl-).[20,21]

After isolating the two organic and water layers from each other, an isolation was done for the compound PNbb from the organic layer, being the compound to be studied biologically against three types of pathogenic isolates. The specific method for the isolating and purifying the compound PNbb was first accomplished by isolating this compound from the organic layer with different solvents such as methanol, chloroform, ethyl acetate and hexane so that each solvent was used separately from the other.[22,23]

Phytochemical screening of pomegranate peel extracts

The pH value of the pomegranate peel extract was determined by mixing 5 g of dried powder with 25 ml of distilled water, and then, the solution was filtered. The pH was measured by pH meter. After that, some qualitative chemical tests were conducted to determine some of the aggregates and active ingredients.[24] The glycosides have been detected as follows.

Alkene (1-decosene) was detected using a nitrous detector, and a similar amide (PNbb) was measured at 260 nm with an ultraviolet (UV) spectrometer against the blank. The Eicosane compound was measured using a spectral UV spectrophotometer at fixed wavelength 375 nm. The absorption of the polyhydric compound (TBAC) was measured at a wavelength 410 nm. The Hydroxyl-carbamide (O-vertamide) was measured at 230 nm. The compound (MP) was estimated at the UV maximum wavelength that determined experimentally at 310 nm. The aliphatic amide (Butan amide) and (N-decyl-N-methyl-) were estimated at the maximum wavelength 210 nm.[25]

A similar identification of amide PNbb

Using a standard solution of 2-bisperididone, N-[4-bromo-n-butyl], the plant extract was directly measured in volume 1.0 mL and different concentrations (2.0, 4.0, 6.0, 8.0, and 10.0 µg/ml). The 2-piperidinone compound, N-[4-bromo-n-butyl]-, was estimated at a peak absorption of 260 nm with UV spectrometer against the deionized water as a blank. The amount of PNbb was calculated from the linear regression equation obtained from quercetin calibrations. Total polyphenol contents were determined using the linear regression equation obtained from the standard plot of PNbb. This compound was calculated as mean standard deviation (SD) ($n = 3$) and was expressed in mg/g from PNbb equivalent of the dry extract.

GC-mass analysis

In general, studies indicate that all extracting compounds can be analyzed more precisely by applying GC-MS (MS, MSDCHEM \ 1 \METHODS\ MUAFAQ.M) to determine negative M/Z. Fragments allow easy identification of composite parts by mass spectrum. To shorten the total time for GC analysis of these compounds, the cavity column should be long and narrow as 10 m × 0.250 im, i.d, 0.25 µm.

Percentage obtained from extracts

Approximately 20 g (5%) dry mass of all extract components was obtained from extracting 400 g of pomegranate peels after 16 h of continuous hot extraction in Soxhlet extract using ethanol as solvent. The Copshan division method was used for raw alcohol extracts. Table 1 shows the different extraction ratios when using different solvents such as hexane, chloroform, ethyl acetate, and methanol.

PNbb crude

The weight ratio of PNbb is calculated using the following linear regression equation obtained from the standard PNbb.

Determination of antimicrobial activity

The susceptibility of the compound (PNbb) was tested on three types of common bacteria and yeast associated with *Pseudomonas aeruginosa, Proteus mirabilis*, and *Candida albicans,* and the ATCC number of species is as follows: *P. aeruginosa* (ATCC 27853), *P. mirabilis* (ATCC 2792), and *C. albicans* (ATCC10231). The activated bacteria were suspended according to the method described by Lahlali R *et al*. and Kamou NN *et al.*[26,27] (106 cells/ml) and used the method of dissemination (250, 500, and 1000 µg/disk); the incubation of the dishes was carried out for 24, 48, and 72 h at a temperature 37°C, after incubation, the diameter of

Table 1: Amount and percentage yield of organic and aquatic extracts from pomegranate peels

Extract	Amount (g)	Yield (w/w) %
Methanol	9.5	47.5
Chloroform	0.5	2.5
Ethyl acetate	2	10
Hexane	8	40

Table 2: Miscellaneous extraction compounds in organic and aquatic layers

Phase	1-Docosene	2-Piperidinone, N-[4-bromo-n-butyl]	Eicosane	TBAC	O-Vertamide	MP	Butanamide, N-decyl-N-methyl-
Organic layer	+	+	+	–	–	–	–
Aquatic layer	–	–	–	+	+	+	+

TBAC: Tributyl acetyl citrate, MP: Methyl palmitate

the inhibition zone was measured in millimeters of bacterial strains, and the test was performed on three replicates.[28-33]

Data analysis

Data analysis is expressed as mean ± SD. The (t) test was done of ANOVA statistical values and was used to analyze the level of statistical significance between groups. $P \leq 0.001$ was considered to be statistically significant. The weight ratio of PNbb is calculated using the following linear regression equation obtained from the standard PNbb.

$$Y = 0.0011x+0.030, R^2=0.9178$$

Where y is absorbance and x is the amount of PNbb that calculated by the microgram unit. The crude extract of PNbb in methanol, chloroform, ethyl acetate, and hexane was 819 ± 0.335, 80.33 ± 0.322, 79.14 ± 0.311, and 78.15 ± 0.301, respectively, and *P* was considered ($P \leq 0.001$) (follow the statistical values in the tables of the manuscript index).

RESULTS AND DISCUSSION

The present study was conducted with an aim to find out antimicrobial properties of pomegranate peel extract. Amount of the identified compounds that was extracted from pomegranate peels of the organic or aquatic layer show in [Table 2]. The chemical detection results showed the presence of seven active compounds in the organic layer and water layer which were extracted from pomegranate peels. Four hydrolysis compounds are TBAC, O-veratramide, MP, and butanamide, N-decyl-N-methyl- and three organic extracts are 1-docosene, PNbb, and ecosane. The results of the analyses were agreed with the GC-MS spectra and the diagnostic findings of the UV spectrum. The optimal conditions for separation and diagnosis of all extraction compounds are listed in Table 3. The compounds were studied through GC-mass [Table 3 and Figures 2-4] to create a molecular ion for each extract. It has been found that its molecular ion equals the weight of the formula of the minus one or more. The graphs confirm the weight of all compound molecules that give a good indication of the isolation and identification of all chemical compounds extracted separately. A number of biologically active compounds were obtained from the organic and aquatic layers in the pomegranate peels extract as shown in Table 1 and Figure 2. The selection of different solvent is an important step for obtaining extracts with acceptable yields and strong antioxidant activity. The

Table 3: Data analysis parameters for separation and specific determination of pomegranate peel extract in GC-MS

Column	HP-5MS, 5% Phenyl methyl Silox (1629.5), 30 m×0.250 µm I.D.×0.25 µm, SS., Inlet He
EMV mode	Gain factor (1.00)
Resulting EM voltage	1306
Low mass	28.0
High mass	441
Threshold	150
Minimum quality for all compounds	90–97%
Flow rate	1 ml/min
Run time	24 min
Hold up time	1.5388 min
Solvent delay	3.00 min
Average velocity	36.796 cm/s
Temperature	Initial 70°C to Maximum 375°C
Pressure	8.81 Psi

GC-MS: Gas chromatography mass spectrometry

yields of extract from different solvents were obtained in the order methanol, chloroform, ethyl acetate, and hexane. The process was performed using different solvents. Active extract PNbb was obtained with high bioefficacy against three types of clinical pathogenic isolated. The methanol extract was found to contain the highest amount of PNbb compound followed by hexane, ethyl acetate, and chloroform. However, little quantity PNbb compounds were detected in chloroform extracts, as shown in Table 1. Pomegranate, a member of the family Punicaceae, is a delicious fruit, also high in nutrition, and rich in antioxidants and phytochemicals, and pomegranate is one of the most powerful, nutrient-dense foods for overall good health. Pomegranate peel has been used for its much health-promoting qualities and also having antimicrobial activity, anticancer property, antiatherosclerotic property, and antioxidative property.[34,35] Table 4 and Figure 5 show the results of the antibiotic test (*P. aeruginosa*, *P. mirabilis,* and *C. albicans*) using concentrations of PNbb compound (250, 500, and 1000 ppm) referred to 1, 2, and 3, respectively, and it is noted that the highest inhibition of the compound was on the *P. mirabilis* bacteria to form a large area of the dish followed by *P. aeruginosa*, Where the diameter of the inhibition of the sample (concentration 1) by 27 mm, while the diameter of the sample inhibition (concentration 2) Of the same bacteria is 34 mm,

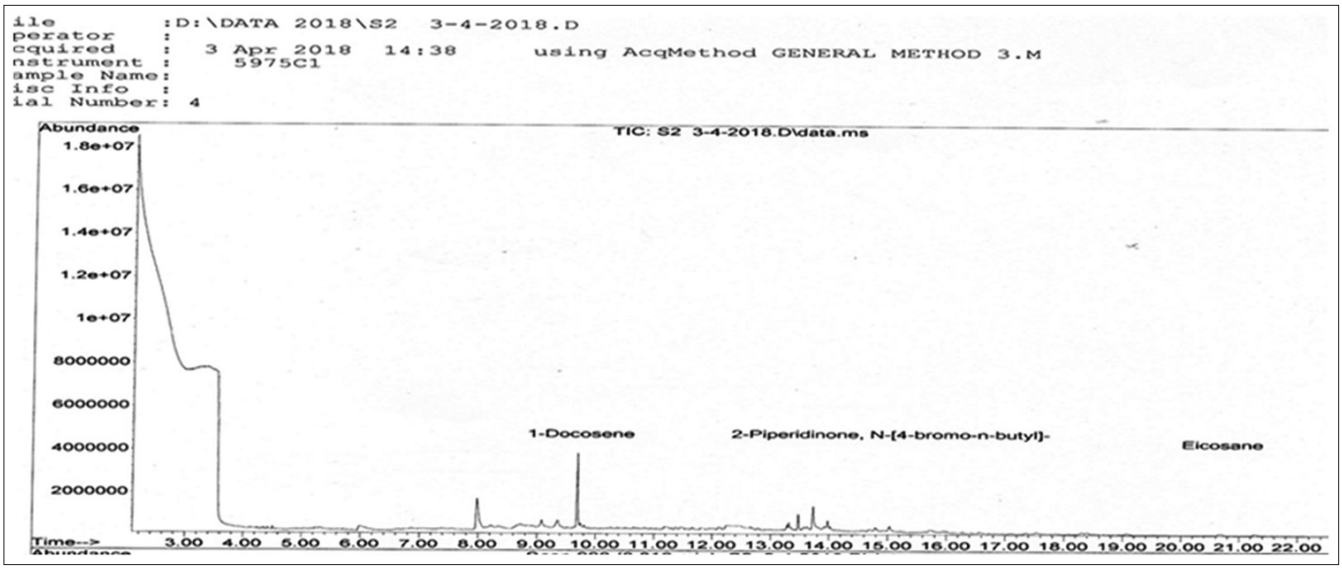

Figure 2: Gas chromatography–mass spectrometry for three compounds in organic layer

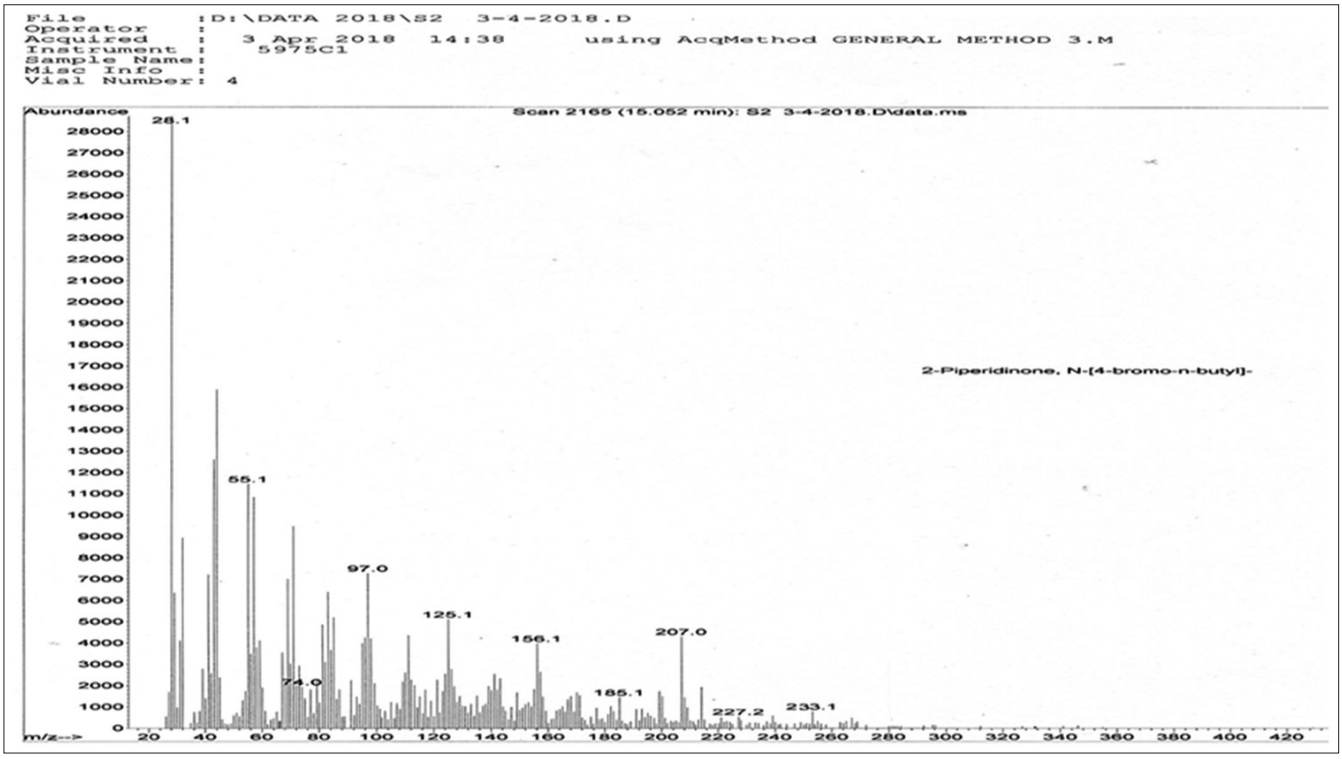

Figure 3: Mass spectrum for 2-piperidinone, N-[4-bromo-n-butyl]

Table 4: Inhibition zone diameter of tested PNbb extracts on *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Candida albicans*

Tested pathogens	PNbb extracts		
	C1	C2	C2
Pseudomonas aeruginosa	27 mm	34 mm	37 mm
Proteus mirabilis	28 mm	30 mm	32 mm
Candida albicans	12 mm	17 mm	22 mm

PNbb: 2-Piperidinone, N-[4-bromo-n-butyl]-

while the diameter of the sample inhibition (concentration 3) of the same bacteria is 37mm using the same amount of inhibitor. For *P. mirabilis*, it was observed that they were close to the concentration (1, 2, and 3) by 28, 30, and 32 mm, respectively, while the effect of inhibition of the compound for three concentrations on *C. albicans* with concentration (1, 2, and 3) was 12, 17, and 22 mm, respectively, compared with other types of bacteria under study. Many medicinal plant extracts have been known to possess antimicrobial effects.[36,37] Pomegranate peels possess novel biologically active compounds. The

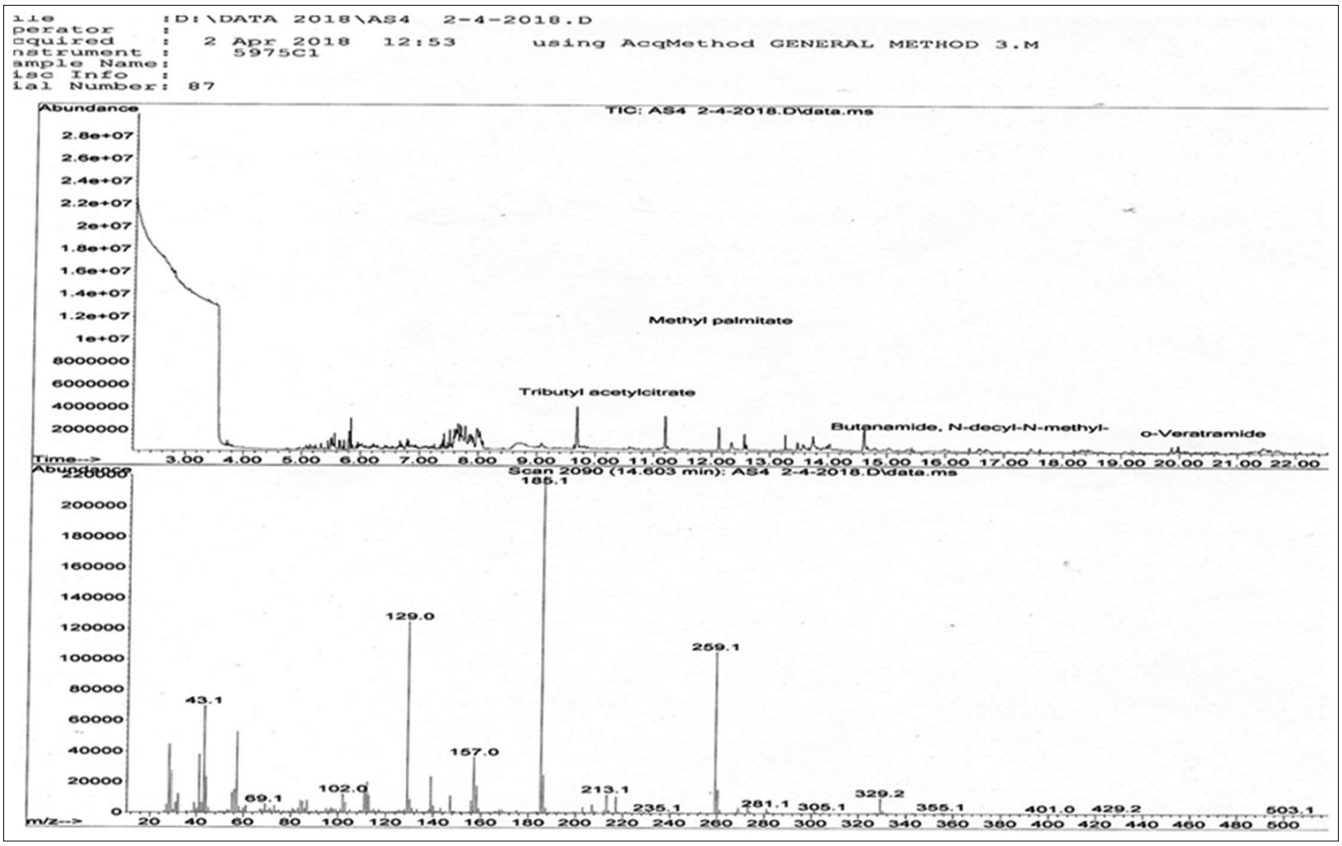

Figure 4: Gas chromatography-mass spectrum for four compound in aquatic layer

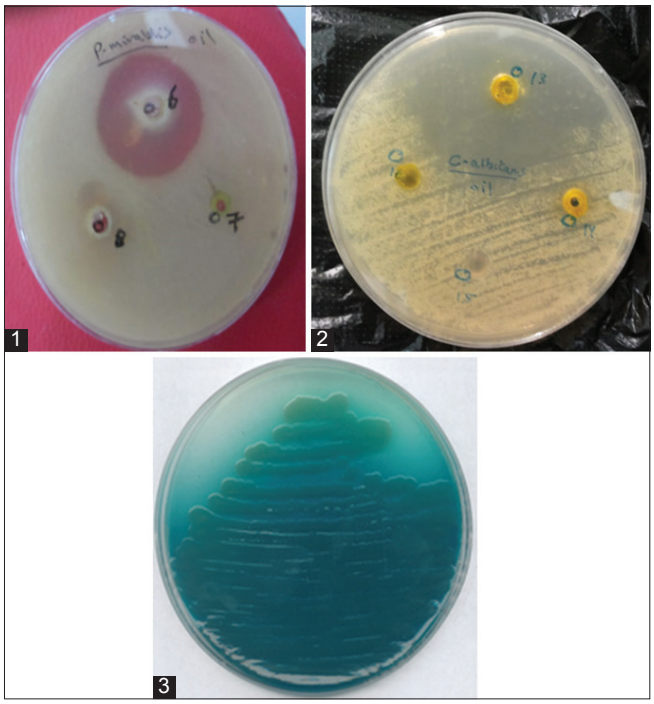

Figure 5: The results of the antibacterial examination (1 - *Pseudomonas aeruginosa*, 2 - *Proteus mirabilis*, and 3 - *Candida albicans*)

extracts from pomegranate peels have been reported to possess inhibition action against human.[38,39]

CONCLUSIONS

This study indicates that the effectiveness of compound PNbb, one of the seven compounds extracted from pomegranate peels. The compound PNbb has a strong effect on the pathogenic microorganisms, especially type *P. aeruginosa* bacteria, followed by type *P. mirabilis* and then Candida type *C. albicans*, indicating that this compound can have multiple drug uses as an alternative to antibiotics and sterilizers. Methanol extraction was better than chloroform extraction.

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AUTHOR'S CONTRIBUTIONS

This research was done individually in the laboratories of the College of Pharmacy, University of Basrah. This research

was completed over a period of 3 months with serious and continuous work, and therefore, excellent results were obtained in finding an easy and sensitive way to estimate the PNbb, compound in the extract from pomegranate peels, and study its biological effect.

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