Asian Journal of Chemistry; Vol. 24, No. 12 (2012), 5602-5604

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Preparation of Some Oxygenic Compounds and Comparing Their Antibacterial Activity with Pomegranate *Puniea granatum* Peels Extracts†

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AJC-11706

The aim of this study is to prepare and identify a group of oxygenic compounds and compare their antibacterial and antitumor activity with pomegranate peels extracts. Twenty oxygenic compounds were identified as a chalcones. While pomegranate peels extracts divided into cold distilled water extracts, hot distilled water extracts, ethanol extracts and methanol extracts. Statistical analysis used was one way ANOVA. The results indicated that the oxygenic compounds in 10^{-3} M concentration gave antibacterial activity against *E. coli* and *Staphylococcus aureus* ($p < 0.001$). The extracts inhibited the bacteria growth in order (EtOH < MeOH < H_2O) ($p < 0.001$).

Key Words: Preparation, Oxygenic compounds, Antibacterial, Antitumor, Pomegranate extracts.

INTRODUCTION

Pomegranate (*Puniea granatum*) contains many important compounds with medical and biological activity[1]. One of famous and widely used of these compounds is flavonoid compounds, which are responsible in the plant for the dye, especially in the cortex[2]. In addition of using of pomegranate as food, the peel has widelyuse range in the industry such as in tanning and medical treatments *e.g.*, heart disease, which belonging to the activity of Kuandin substance[3]. Pomegranate is used over the centuries in alternative medicine in a wide range of uses, one of these uses as a diuretic[4]. The use of pomegranates cooked seeds with honey in the treatment of mouth sores, stomach, ear ache and sore on the soles of the nose[5], scales are also used after drying in the treatment of stomach ulcers[6]. The therapeutic efficacy of the pomegranate belong to it contain the active compounds in the fruit, seeds and chaff, including semiconductor alkaloids[7]. Which includesa large family of compounds, the most important quinidine, resins the most important cannabis, which is a powerful painkiller[8]. Glucosides including Salesan, is a compound, which is used as analgesic[6]. Volatile oils, which have been used inrecent studies as antifungal and bacteria[9]. Tannins which have medical uses too broad because it includes a large family of compounds and that one of the most important kinds of tanninis most intense flavonoid compounds[10].

EXPERIMENTAL

We used thin layer chromatography silica gel kind with polyamide from Merck and BDH. The papers used in the chromatography paper and filter papers with the numbers[1,3,4] and the type offilter paper (HA) of (0.45 µm) from Whatman.

Culture media: We used MacConkey Agar as a culture media from Mast Co., Blood Agar media and Nutrient Agar media from Oxoide Co. and Mutrin Hinton Agar (MHA) from Maknur Co.

Formic acid, ethyl acetate, ethylmethyl ketone, formaldehyde, petrolum ether, Folin-phenol, methanol and ethanol from BDH Co. Also isobutanol and sulphuric acid from Fluka Co. While hydrochloric acid, acetic acid, benzene from Merck Co. and phosphoric acid from Hopkin & Williams.

Solid: Magnesium turnings, ferricyanide potassium, sodium chloride, glucose and pnitroaniline from Riedal De Haen. Copper-sulphatr penta hydrat from Hopkin and William. Ferric chloride anhydrous, trichloro-acetic acid, pnitroaniline, oxalic acid, potassium carbonate, tannic acid, potassium iodide and sodium tungestatefrom Fluka. Urea, P-toiuenesuiphonic acid, potassium iodate, phenol, sodium hydroxide, vanillin, bismuth subnitrate, sodium nitrite and phosphomolybedic from BDH.

Pomegranate peel used: The work dry pomegranate peels were collected in the same season of this article, where it studied in detail.

†Presented at International Conference on Global Trends in Pure and Applied Chemical Sciences, 3-4 March, 2012; Udaipur, India

INSTRUMENT

Instrument	Company
Tissue Hemogenizertaype Braun Melsungen AG	Potters
Centrifuge type Markiv	Baird & Tatlock
Spectrophotometer type PU 7870Vis/Nir	Philips
Filtration Instrument under critical pressure type AMED	Pfeiffer
FT IR type FT-IR100	Fisher
Sensitive balance AE 160	Mettler
Freeze Drying Machine type BETA	Ehvisa
Fraction Collector type 660	Buchi
Foster for growth of bacteria	Termarks
Autoclave sterilization device type HH-30D	Hirayama
Magnetic Stirrer	IKA-Combimag
pH-Meter type 702005	Kent Eil

Extraction: Solvents distilled water cold and hot, absolute ethanol and methanol used in the extraction processes to extract the various components of pomegranate peel and as follows:

Use of cold distilled water: First extract was obtained by weighing the amount of powdered pomegranate peel 0.5 g was added with 30 mL of distilled water then mixed by tissue mixer for 5 min and then left with constant stirring using a magnetic stirring for 24 h at room temperature, separation of the mixture by using a centrifuge, then filtrate and dried using a drying under pressure at a temperature by freeze drying machine, after that the dry extract was obtained, which saved atfreezing temperature for use later.

Use of hot distilled water: The same procedure above but with using a magnetic stirring for a period of 1 h at temperature of 70 ºC.

Use of ethanol and methanol: The weight of 0.5 g of pomegranate peel to 30 mL of ethanol or methanol, mixed by tissue mixer for a period of 5 min and left with constant stirring by magnetic stirring for a period of 24 h, separation of the mixture by centrifuge, keep the filtrate and neglected the sludge, preserved the extracted at freezing temperature until use.

Isolation and identification for types of bacteria: This work done with six negative and positive types of bacteriafor the dye Gram from the laboratories of microbiology and development at the Center for Research Technology House, University of Al-Nahrain and the Ramadi General Hospital and diagnosed using culture media and observation of the form of colonies and coloured dye Gram, then purified on among agricultural different to make sure of its identity.

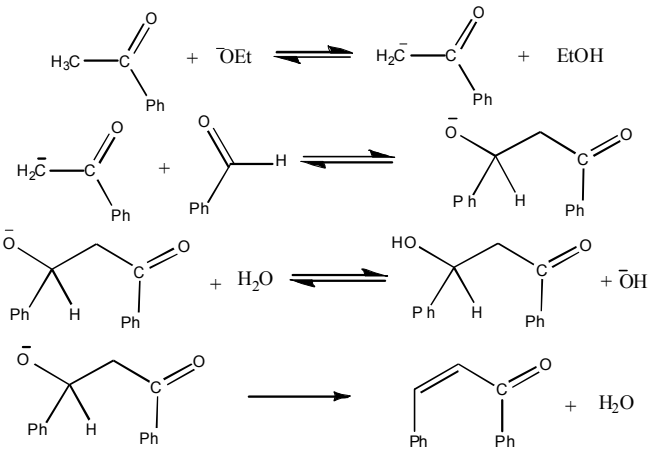
Test the biological activity against bacteria: Method of Power 1966[11] is used in measuring of the biological activity of pomegranate peel extraction and chalcones against the growth of negative and positive dye Gram of pathological bacteria, testing of six types of bacteria, including three types of negative Gram pigment (G-Ve), are: *Vibrio cholerea, Escherichia coli, Serretiamarscesence*. And three types of positive dye Gram (G + Ve) are: *Staphylococcus aureus, Lactobacillus acidophilus, Bacillus cereu*.

Preparation of chalcons[12]: Preparation of alcohol solution of potassium hydroxide (60 %) and cooled to (10 ºC) and gradually added to the mix of aldehyde (0.001 mol) and acetophenone (0.001 mol) in (10 mL) of ethanol. Then left at room temperature for 2 days, then reduce in equivalent amount of water and acidified with cold dilute hydrochloric acid.

We note deposition of the desired chalcons compound then re-crystallized in ethanol and dried to fix physical properties of the resulting compounds.

RESULTS AND DISCUSSION

Infrared spectra: In this research we relied the condensing method of (Claisn-Schmidt) to prepare chalcone that include interaction of equal weights of aceto phenone substituent with benzaldehyde substituents in presence of a base inan alcohol-water solution[12] to give unsaturated ketones and according to mechanical set forth in the **Scheme-I** below which have been diagnosed by FT-IR technique.



Scheme-I: Configuration mechanism of Chalcone by Claisen-Schmidt method

Chalcones prepared compounds have been diagnosed (compounds **1-20**) by infrared spectrum (Table-1), the special absorption package for carbonyl group of chalcones at 1680-1660 cm^{-1}, which is shifted towards low frequency for the known carbonyl groups in organic compounds and is due to the exchange that exists between double bond and aromatic ring. A package appear at the frequency of 1600-1590 cm^{-1}, attributed to the stretching group (C=C) of benzene ring[13]. The hydroxyl groups associated with side rings appear when the frequency absorption package is 3500-3200 cm^{-1}.

UV-visible spectra: prepared compounds (**1-20**) studied by UV-visible technology in (DMSO) and concentration 1×10^{-3} mol and at room temperature this measurements showed the absorption packages back to the first electronic transition (π-π^*) and is located within the range of (240-290), which represent two benzene rings **A** and **B** of prepared compounds. The second package is attributed to the electronic transition (n-π*) and falls within the range of (300-400), which represents a dual movementof electronic for oxygen atom of the carbonyl group of chalcone.

Biological activity of the prepared compounds (1-20): Many of chemical compounds contain set of qualities that make them useful in important therapeutic areas in the killing of microorganisms and some types of microorganisms like sickness bacteria. In the current study we tested biological activity of prepared compounds on six types of bacteria, three types of negative dye Cram (G-Ve) are: *Vibrio cholerea, Escherichia coli, Serretiamarscesence* and three types of positive dye Gram (G + Ve) are: *Staphylococcus aureus*,

TABLE-1
UV-VIS AND FT-IR SPECTRUM ABSORPTION
OF PREPARED COMPOUNDS

No	UV/VIS (nm)	ν(C-O)	ν(C-H)	ν(C=C)	ν(C=O)	ν(O-H)
1	295, 354	1236	843	1597	1655	3412
2	295, 325, 383, 400	1244	850	1546	1630	3412
3	290, 320, 437	1225	831	1513	1633	3363
4	290, 311	1223	865	1508	1630	3411
5	270, 290	1233	865	1542	1605	3400
6	275, 355	1235	865	1452	1659	3409
7	270, 360	1236	832	1545	1653	3426
8	250, 284	1236	832	1545	1654	3425
9	280, 335, 385	1212	819	1551	1635	3264
10	280, 361, 393	1240	820	1563	1654	3431
11	280, 339, 460	1215	817	1525	1628	3427
12	290, 285, 371	1214	819	1525	1625	3421
13	275, 300, 355	1227	840	1568	1652	3366
14	270, 350	1217	832	1559	1650	3223
15	280, 330, 419	1213	816	1562	1678	–
16	275, 340	1223	837	1541	1689	3381
17	270, 341, 360	1210	842	1595	1688	3357
18	275, 340, 430	1221	823	1596	1689	3402
19	255, 277	1228	805	1438	1686	–
20	255, 280, 336	1235	856	1522	1689	–

Lactobacillus acidophilus and *Bacillus cereus*. Found through testing that there is ability to discourage the growth of bacterial colonies and all the extracts and prepared compounds (Chalcones) The results were as follows:

In the low concentrations (10^{-5}) mol all types of negative dye gram bacteria gave resistant against compounds that have been tested with bacterial activity. The positive dye gram bacteria were also resisted with the exception of *Staphylococcus aureus* was sensitive to some of compounds.But when concentration increase to (10^{-3}) all prepared compounds gave effective against bacterial, when working on two types of bacteria negative for the first character valued *Escherichia coli* and the second cationic dye *Staphylococcus aureus*.

Biological activity of extracts from pomegranate peel: We extract pomegranate peel by using solvents as hot and cold distilled water, ethanol and methanol, then measure the biological activity of four pomegranate peel extracts on two types of pathogenic bacteria. It was found that the all extracts have opposite effect of two types of negative dye gram bacteria representing in *Escherichia coli* and the cationic dye gram bacteria representing in *Staphylococcus aureus* bacteria, this is agree with the previous study of the researchers[14]. To measure the power of biological activity we measure the diameter of inhibition (an area where there is no growth of bacterial) of all extracts where we found that the diameter of inhibition of the cold water extract for pomegranate peel is bigger than it is to the rest of the extracts of other and this indicates a clear indication that the aqueous extract is more effective extract of the other.

As for alcohol extract (ethanol, methanol), the cause of poor efficiency these extracts compared with water cold extract of pomegranate peel may be due to biodegradable tannins where it disintegrate when exposed to alcoholic solvent, so it's best to exclude alcohol in the extraction of these tannins[15]. From the results it can be concluded that the higher polarity of the used solvent to extract that will increase biological activity as the inhibition diameter will increases with polarity of the solvent as follows: EtOH < MeOH < H_2O.

The obtained results have been significantly analysis statistically (one way ANOVA) it show the following results: Fig. 1 the effect of groups of compounds and extracts under study on percentage of inhibition of bacteria (*Staphylococcus*), as seen from the figure that the extracts, especially water has had a significant effect ($P < 0.001$). This is followed by the percentage of inhibition of the moral effects of the compounds under study and extracts on bacteria (*E. coli*) and shown in Fig. 1 that extracts specially water extract had a significant effect ($P < 0.001$).

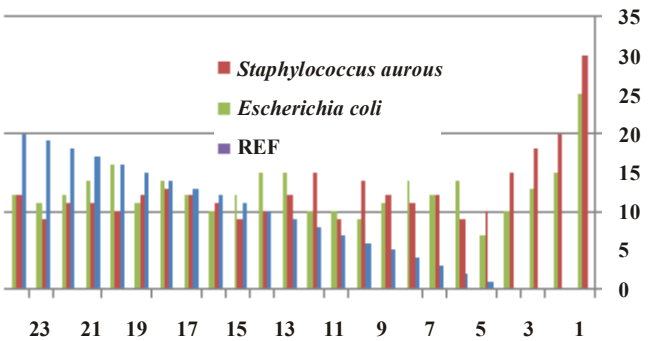


Fig. 1. Effect of extracts and prepared compounds on the pathogenic bacteria

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