

Phytoconstituents Screened of Grapefruit Peels with Antimicrobial Properties of Naringin and Naringenin Extracted and Isolated from its

Bassam A. Hassan¹, Fadil Mohsen Hamed², Maitham M. Abdulridha³, Farqad M. Baqer⁴

^{1, 2, 4} Lecturer at pPharmaceutical Chemistry Department College of Pharmacy, Thi Qar University, Iraq.

³ Lecturer at Technical Institute of Alshatra, Iraq.

Co-author Email: Bassam_org@yahoo.com : bassam-abd@utq.edu.iq

Abstract

Grapefruit belongs to the Rutaceae family of bioactive citrus fruits. The absence of saponins, coumarins, terpenoids, sterols, proteins and anthraquinones was shown by phytochemical screening of dried grapefruit peels at the beginning of this research. Alkaloids, flavonoids, sugars, tannins, glycosides, and polyphenols are present. Naringenin (flavanone) and Naringin (glycoside flavanone) were subsequently extracted and isolated from dried grapefruit (citrus fruit) peels. Spectra of FT-IR, thin layer chromatography TLC, point of melting and chemical examination testing its calculated. Over time, certain pathogenic bacterial strains isolated from patients such as E. coli, Acinetobacter, Klebsiella, Streptococcus, Staphylococcus, Aeromonas have been studied against the antibacterial activity of Naringenin and Naringin. Naringenin (flavanone) subsequently demonstrated higher biological activity than the drugs Naringin and Cefuroxime.

Key, words: Antimicrobial properties, Naringin, Naringenin grapefruit, and Phytochemical Screening.

1. introduction:

Naringenin, a type of flavonoid, is colorless [1] and flavanone is tasteless [2]. It is identified in a multiplicity of citrus fruits, orange blossom, tomatoes, and vegetable herbs [3] and is the predominant flavanone in grapefruit.

These botanical ingredients have been linked to many biological properties, including anti-inflammatory, antioxidant, antibacterial, antiviral, anti-tumor, anti-lipid, and cardioprotective effect. Most of the knowledge reported was derived in vitro and in vivo studies, from experiments. While there's some psychiatric trials have also been conducted, the primary focus is on the vital activity as well as the cardiac protective function of naringin [4].

In addition, similar biological activity between Naringenin (flavanones) and Naringin (flavanone glycosides) were tested in these experiments. Coli Streptococcus, Acinetobacter Klebsiella, Staphylococcus, Aeromonas, some of which are against the pathogenic bacterial strains that have been detected in patients, and have reported highly significant therapeutic properties of Naringin compared to Naringin and cefuroxime, as tabulated in Table No 4.

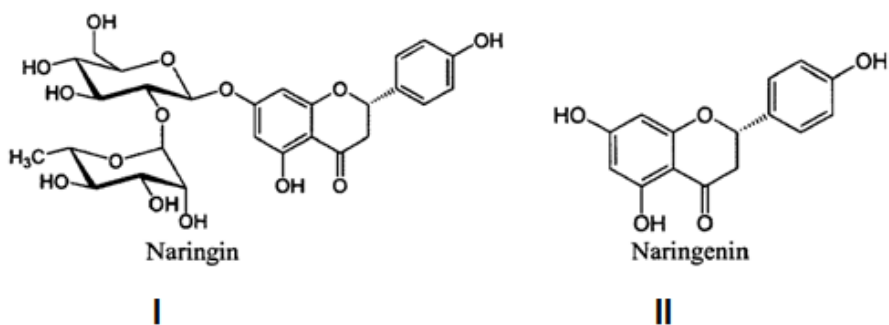


Figure No 1 : (I) structure of Naringin and (II) structure of Chemical Naringenin

2. procedures and Material

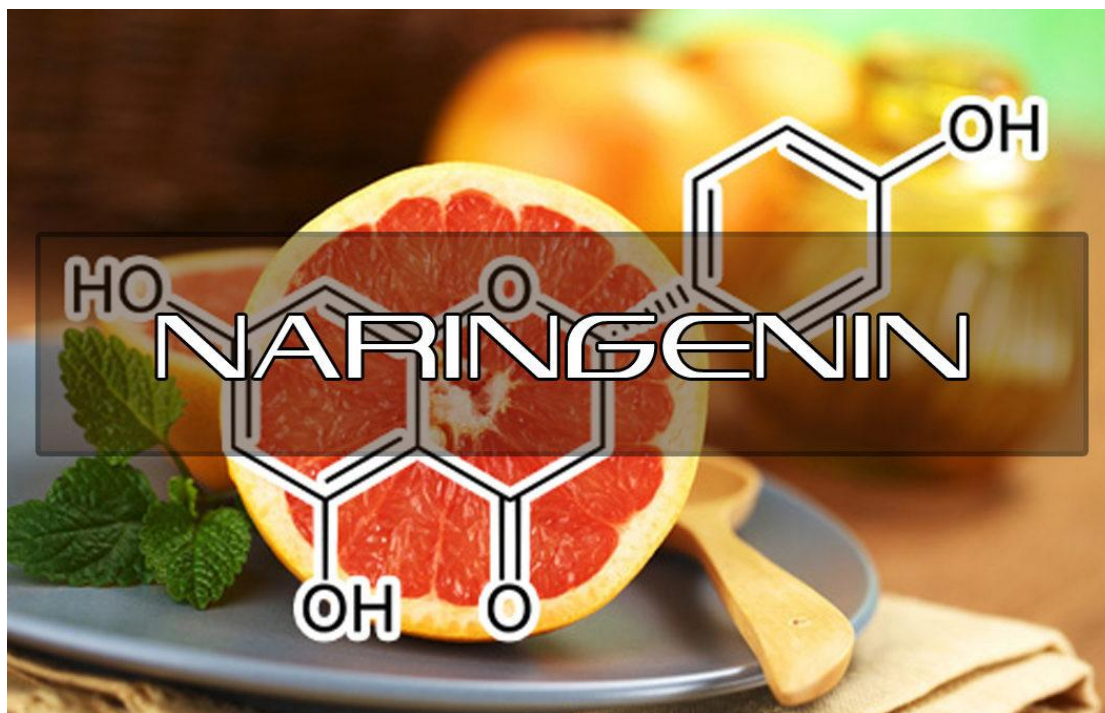
2.1. Equipment:

The grapefruit, from the regional supermarket of Nasiriyaah, was picked. They got removed and then brushed washed with distilled water using a washing machine. Then the grapefruit peel Dried and anchored, it was, properly by using mortar and then to fine pestle by using a seasonal grinder machine. For further details using powder. All the chemicals extracted from the faculty laboratory are collected. The research done at the laboratory of organic chemistry.

2.2. Phytochemical Qualitative Screening

Sr .No	Structure of Molecules	Chemical testing	Testing Result Outcome
1	Carbohydrates	Molish test	+ve
2	Flavonoids	Shinoda test	+ve
3	Alkaloids	Wagner reagent	+ ve
4	Coumarins	diluted of NaOH by Filter paper soaked	Negative (-ve)
5	Glycosides	Fehling's test	+ve
6	Resins	Ethanol 95% +boiling + 4% HCl	+ve
7	Tannins or Poly phenols	10% of lead acetate	+ve
8	Saponin	Shaken of the extraction	-ve
9	Anthraquinons	Borntrager's test	-ve
10	Terpenoids	Salkowski reaction	-ve
11	Sterols	Liebermann burchard	-ve
12	Protein	Ninhydrin test	-ve

Table No 1. screening of phytochemical (where, – absent and + present)



2.3 .1 Naringin flavonoid glycoside extraction :

Extraction by cold method

50 gm of grapefruit peel material 250 ml of petroleum ether, soaked macerated in, two days, test result. Then purified to extract nonpolar materials, such as resin aromatic oil, fatty acid and waxes etc. via a Buuchner tube defatted process. Then it cooled down and dried.

Extraction by hot method,

The contents were boiling through soxlet for 3 hours with 300 mL in ninety percent alcohol (40-

60°C) after full cold isolation to isolate the polar substances such as glycoside, flavonoids, etc. The filtrate became condensed through the rotary evaporator to a small amount. Acidifying with 20 ml of 6 percent acetic acid (pH 3-4).^[12-13]

2.3.2 Naringenin isolation from Naringin:

Naringin (2 gram) and methyl alcohol (50 milliliter) and 50% hydrochloric acid (2 milliliter) were combined, agitated and boiled for 60 min. The resulting outcome homogeneous mixture was concentrated standardized one to quarter, and frozen and moved with 15 ml of chloroform two times and isolated to the a separator funnel, shake. In order to give the substance Naringenin, the chloroform layer decanted. The following procedure was used to purify naringenin: dissolve with a minimum of acetone, the crude commodity and apply the resulting solution of water (200 milliliter) and ethanoic acid (3 mL) to strongly stirring mix. Precipitated Naringenin was rinsed and chilled in a immersion blender, to get 1.4 gm with water. The physical characteristics shown in table No3 [14].

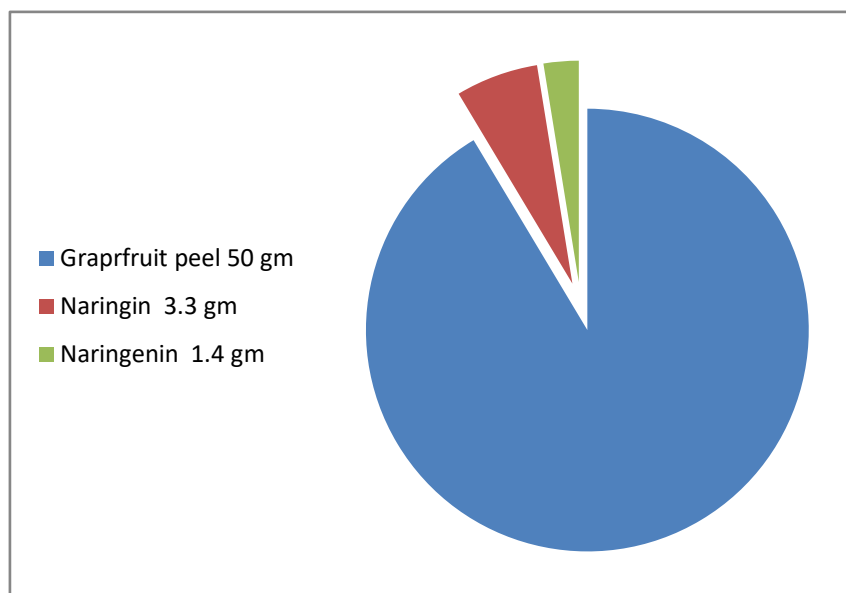


Figure 2 : Graphical representation of Naringenin and Naringin yield .

3. Outcomes and Discussion :

3.1 Naringenin and Naringin Phytochemistry :

Since Naringenin and Naringin extracted by extraction and separation, from grapefruit peel, above that the process was identified chemically verifying the isolated Naringenin and Naringin by breaking down little quantities of a crystals, with one ml of 95 %ethyl alcohol dissolved with in test tube by chemicals such as Molish exam (for generic carbs), Fehling exam for glycosides With the Molish examination and Fehling's glycoside sample, Naringin showed a positive result and also showed a +ve Shinoda exam test and an alkaline flavonone test. Although Naringenin showed -ve result with Molish and Fehling tests, glycoside testing and also +ve Shinoda and alkaline exams were shown. The findings show that naringenin (flavonones) and naringin (glycoside flavonones) were isolated from grapefruit. its.grapefruit peels were isolated_[15] .

Sr. No	Name of the Sample	Molish examination	Fehling's examination	Shinoda examination	Alkaline examination
1	Naringin	Positive ring in Purple	Positive red precipitate	Positive Crimson	Positive Colorless
2	Naringenin	negative	negative	Positive Crimson	Positive Colorless

TableNo2.Naringenin and Naringin phytochemical screening analysis (where,- absent and + present)

Sr. No	Parameter	Observation Naringin	Observation Naringenin
1	Color	Yellowish brown	colorless
2	Odor	The aromatic str	The aromatic str
3	Point of Melting	166 °C	251°C
4	Percent Yield	5.80%	2.40%
5	Rf	0.65	0.78

Table No 3:- Physical Characters of Naringin and Naringenin

3.2 Results of Chromatographic Analysis (TLC): Naringin extracted from grapefruit peel examined with eluted silica gel, butyl alcohol: acetic acid : water (4:1:5) Rf = 0.65. Naringenin, eluted with methyl alcohol chloroform (9.0: 1.0) Rf = 0.78, was chromatographed over Silica gel. Close to the normal commercial Rf value of Naringin (0.57) and Naringenin (0.68) from publication [16,17,18,19].

3.3 Point of melting:

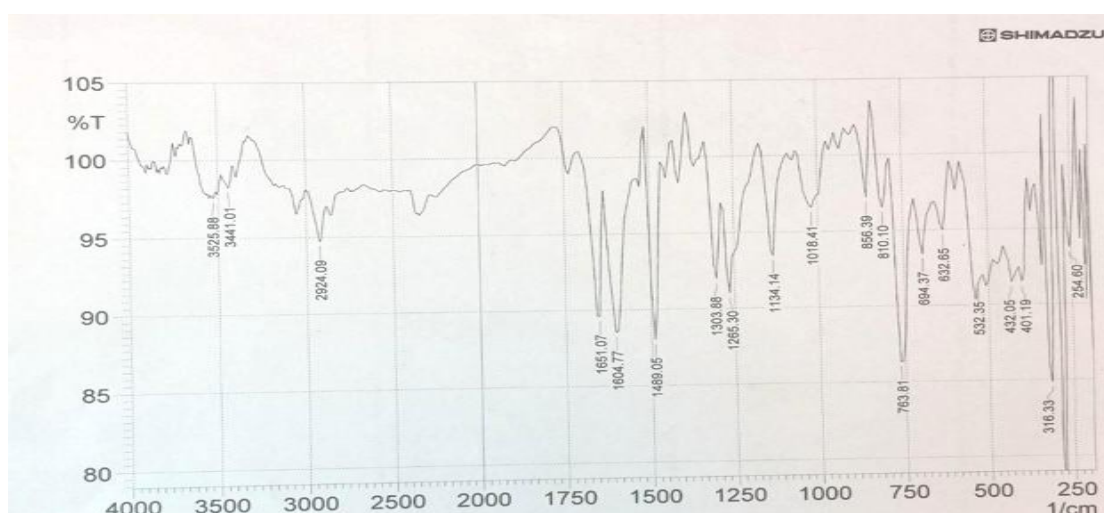
SMP3I apparatus melting point. A melting Naringin 2540C point, Whereas,, Naringenin 2260C melting point, which appeared near the literature's melting of Naringin and Naringenin 2570C, 2210C.

3.4 Spectral analysis by FTIR:

Ft-IR spectra were registered as CsI disks that used a Shimadzu

Ft-IR spectrophotometer, in the range (4000-200) cm⁻¹.

The Ft ir naringin spectrum displays a characteristic band of absorption stretching. 35025cm⁻¹(O-H str) ,3065cm⁻¹(C-H str).(aromatic arene , 2924cm⁻¹(CH str (aliphatic carbon)), 1651 (C = O str), 1604(C=C str. aromatic) [19,20,21,22] as shown in figure No3.



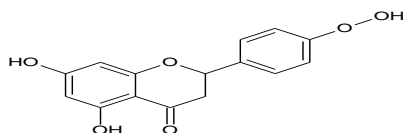


Figure No 3 Spectra of .FT-IR of Naringenin.

Past data of phytoconstituents, TLC and melting point and FTIR spectra have shown impressive results to suggest the extraction and isolation of naringenin (flavonones) and naringin (flavonones glycoside) from grapefruit peels.

4. Minimum Preparing of solution for inhibit concentration:

It was achieved by dissolving 0.4 gm in 95 % ethyl alcohol to get a 40 mg / ml MIC combination of Naringenin and Naringin, which was the minimal inhibitor, the amount checked as seen in the table (No3).
Castration was performed by 0.45 mm and 0.22 mm filtration goods via a Millipore.

5. Biological Action :

In Naringenin and its Naringin's vitro antibacterial activity towards certain pathogens bacterial isolates from patients. are using agar cups process. The outcomes are summarized in table No3
(*Staphylococcus*, *Aeromonas*, *Streptococcus*, *E.coli* and *klebsiella*, *Acinetobacter*)^[23].

Table No 3: Effects of the Naringin and Naringenin effects

Microbial Types	Diameter inhibition region (mm)		
	Antimicrobial Agent		
	Naringenin	Naringin	CEFUROXIME
<i>Streptococcus</i>	30 mm	12 mm	9 mm
<i>Acinetobacter</i>	31 mm	8mm	12 mm
<i>E.coli</i>	29mm	9 mm	2 mm
<i>Klebsiella</i>	21mm	4 mm	7 mm
<i>Staphylococcus</i>	28 mm	10 mm	18 mm
<i>Aeromonas</i>	27 mm	16 mm	11 mm

CEFUROXIME and in vitro growth of bacteria towards certain patient-isolated pathogenic bacterial strains.

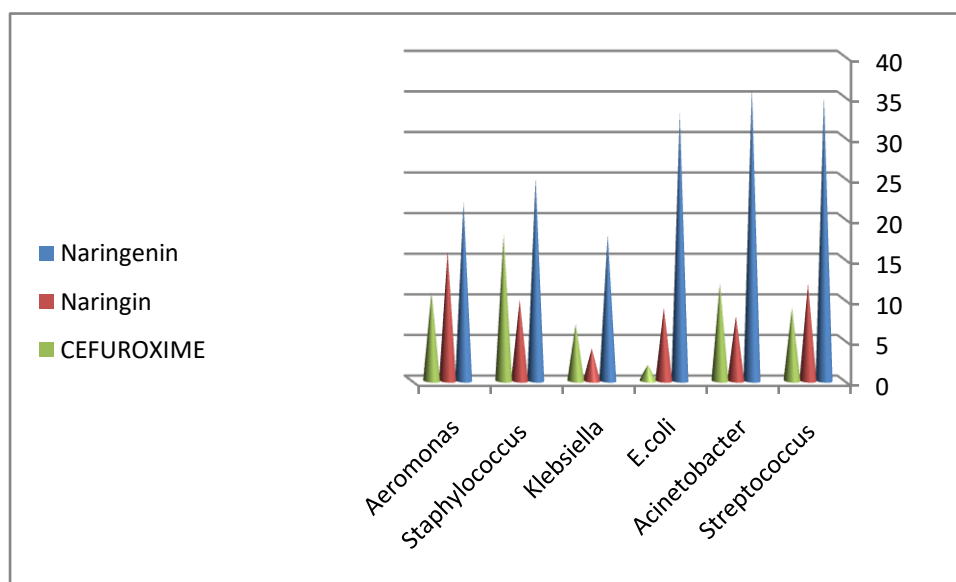


Figure 4: diagrammatic display of the growth of bacteria in vitro between Naringenin, Naringin, and CEFUROXIME towards certain pathogenic bacterial strains isolated from patients.

6. Conclusion :

As discussed earlier, the loss of saponins, coumarins, sterols, terpenoids, anthraquinones and proteins has been shown by phytochemical screening of drying grapefruit peels. Though flavonoids, alkaloids, carbohydrates, glycosides, tannins and polyphenols are present. Naringenin (flavanone) and Naringin (flavanone glycoside) were consequently isolated and extracted via drying grapefruit peels (citrus fruits) then identified by FTIR spectra, TLC, point of melting, chemical exam such as Shinoda and alkaline exam as defined in the report.

Naringenin, naringin, and cefuroxime have been screened for certain pathogens. Checked against such isolated pathogenic bacterial strains from patients such as this one as Streptococcus, Acinetobacter, E. coli, Klebsiella, Staphylococcus, Aeromonas, Naringenin flavanones were lastly isolated from grapefruit peels and characterized. The bioactivity study showed that naringenin (flavanones) had a substantial higher bioactivity than naringin and cefuroxime drugs, as tabulated in figure 4.

7. References :

- [1] inhibitory effect on the human cytochrome P450 isoform [[CYP1A2]] resulting in carcinogens of otherwise harmless substances. Fuhr U, Klittich K, Staib AH (April 1993). "Inhibitory effect of grapefruit juice and its bitter principal, naringenin, on CYP1A2 dependent metabolism of caffeine in man". Br J Clin Pharmacol. **35** (4): 431–6. doi:10.1111/j.1365-2125.1993.tb04162.x.
- [2] PMC 1381556. PMID 8485024. Ueng YF, Chang YL, Oda Y, Park SS, Liao JF, Lin MF, Chen CF (1999). "In vitro and in vivo effects of naringin on cytochrome P450-dependent monooxygenase in mouse liver". Life Sci. **65** (24): 2591–602. doi:10.1016/s0024-3205(99)00528-7. PMID 10619367.
- [3] Uday Abdul-Reda Hussein, Bassam Abdul Hussein Hasan, Hind Abedallah Salih, Ali Taher Abbas and Sahar Mezher Mtuasher (2017). Antimicrobial Activity Of Ethanolic

- And Aqueous Extracts Of Pomegranate Peel Against Extended Spectrum Beta-Lactamase Producing Bacteria. Journal Of Thi-Qar University., 12(4).
- [4] Bahare Salehi 1 , Patrick Valere Tsouh Fokou 2 , Mehdi Sharifi-Rad 3,* , Paolo Zucca 4,* , Raffaele Pezzani 5,6,* , Natália Martins 7,8,* and Javad Sharifi-Rad 9,10,*(2019)The Therapeutic Potential of Naringenin: A Review of Clinical Trials
Pharmaceuticals 2019, 12, 11; doi:10.3390/ph12010011
www.mdpi.com/journal/pharmaceuticals
- [5]Chede PS () Phytochemical analysis of Citrus sinensis peel. Int J Pharm Biol Sci 2013; 4(1): 339-343.
- [6] Maitham M Abdulridha, Haider S Abdulhussein, Firas Fadhil Alyaseen and Bassam A Hassan (2019) Phytochemical and antibacterial activity of the pegnum harmalaseeds and its alkaloids. Plant Archives 19(1), 1439-1444.
- [7]FADIL M. HAMED1 , SEMMAH. SHALAAL2 , AFRAH T. HLAIL3 AND BASSAM A. HASSAN4 (2019) Phytochemical and Antimicrobial Study of Acetone Extracts of Menthe and Olive Leaves . The Lepidoptera Research Foundation. June ,Volume 50 (2): 51-56
- [8] Semma H. Shalaal1 , Afrah T. Halail2 , Fadil M. Hamed 3 and Bassam A. Hassan4 (2019) MACERATION TECHNIQUES EXTRACTION OF THYMUS VULGARIS AND LAUREL (LAURUS NOBILIS) LEAVES WITH ANTIBACTERIAL STUDY . Plant Archives Vol. 19 No. 2, 2019 pp. 4041-4044
- [9] Lawal D, Bala JA, Aliyu SY, Huguma MA () Phytochemical screening and in vitro antibacterial studies of the ethanolic extract of Citrus sinensis (Linn.) Peel against some clinical bacterial isolates. 2013: Int J Innov Appl Stud 2(2): 138-145.
- [10] Suja D, et al. Phytochemical Screening, Antioxidant, Antibacterial Activities of Citrus Limon and Citrus Sinensis Peel Extracts. Int J Pharmacogn Chinese Med 2017, 1(2): 000108.
- [12] Hesperidin and hesperitin preparation and purification from Citrus sinensis peels, N. Lahmer1 N. Belboukhari1*, A. Cheriti2 and K. Sekkoum1, 2015:Der Pharma Chemica, 7(2):1-4.
- [13] Bassam A. Hassan, Fadil Mohsen Hamed, & Firas F. Alyaseen. (2018). Phytochemical screened, characterization and antibacterial activity of hesperetin and hesperidin extracted and isolated from dried oranges peels. International Journal of Research in Pharmaceutical Sciences, 9(4), 1362-1367. Retrieved from <https://pharmascope.org/ijrps/article/view/477>
- [14] Bassam A Hassan, Hameedi N Nasera and Maitham M Abdulridha (2019) Synthesis and antimicrobial evaluation of fused heterocyclic compound [1,2,4] triazolo [4,3-b][1,2,4,5] tetra zine. Int. J. Research in Pharmaceut. Sci. 10(2), 1254-1258. <https://doi.org/10.26452/ijrps.v10i2.417>.
- [15] Bassam A.Hasan, Maitham M.Abdulridha (2014) PREPARATION AND CHARACTERISATION OF SOMETRANSATION METAL COMPLEXES OF NEW [BUTANAL (5-ETHYL-1, 3, 4-OXADIAZOL-2-YL) HYDRAZONE]
- [16] Bassam A.Hasan, Maitham M.Abdulridha (2014) PREPARATION AND CHARACTERISATION OF SOMETRANSATION METAL COMPLEXES OF NEW

[BUTANAL (5-ETHYL-1, 3, 4-OXADIAZOL-2-YL) HYDRAZONE] The Swedish Journal of Scientific Research (sjsr), Volume 1. Issue 5. October 2014

- [17] Najim Abbas Jabir Al Awwadi, Bassam Abdulhussein Hasan Alsafel and Maitham Mohamed Abdulridha (2016) Synthesis And Characterization Of Cu(II) And Fe(II) Metal Complexes of Oxazepine Derivative Via Schiff Base [Fe(HPOHBOT)Cl₂] And [Cu(HPOHBOT)Cl₂]. Afr. J. Pharm. Pharmacol. 10, 728-736.
- [18] Firas F Alyaseen, Bassam A Hassan and Haider S Abdulhussein (2018). Extraction, isolation and chemical identification of piperine alkaloid from black pepper seeds and its antibacterial activity. Plant Archives 18(2), 2171-2176.