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# Synthesis, Characterization and Antibacterial Studies of Some New 1, 4-bis (3-Phenyl-4,5-Dihydroisoxazol-5-yl)Benzene Derivatives

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## Introduction

Chalcones, analogs of 1,3-diarylprop-2-en-1-one, form a wide class of compounds containing two aromatic rings bound with vinyl ketone fragment. They are useful in synthesis of various heterocyclic compounds.Chalcones of plant origin are known [1]. Chalcones present great interest as compounds exhibiting antimalarial [2], antibacterial [3], antifibrogenic [4], anticancer [5], antitrichomonal [6], anti-inflammatory [7], antileishmanial [8], cytotoxic and antitrypanosoma cruzi [9] activities. While the flavonoid compounds are a group of natural products found in fruits, vegetables, nuts, seeds and flowers as well as in teas and are important constituent of human diet. They have been demonstrated to possess antioxdidant [10], antihypertensive [11], antiallergic [12], antinocicepative [13], trypsin inhibitors [14], plant growth regulator [15], antibacterial and antifungal [16,17] activities.

In the last few years microwave induced organic reaction enhancement (MORE) chemistry has gained popularity as a nonconventional technique for rapid organic synthesis [18] and many researchers have described accelerated organic reactions, and a large number of papers has appeared. Proving the synthetic utility of SMORE chemistry in routine organic synthesis [19] . It has been termed as 'e-chemistry' because it is easy, effective, economical and ecofriendly and is belived to be a step toward green chemistry. In view of these observations and in continuation of our work on biologically active chalcones and their heterocycles [20], we have been planned to synthesize the new 1,4-bis(3-phenyl-4,5-dihydroisoxazol-5-yl)benzene derivatives from chalconesand also studied their antibacterial activity against esherishia coli and staphelococcas aureas .

## Experimental

**General**. Melting points were uncorrected. FT.IR-8400,SHIMADZU. NMR spectra were acquired with a Bruker Ultra Shield ($^1$H : 300 MHz) (University of AL-al-Bayt,Jordan). The chemical shifts were referenced to tetra methyl silane (TMS) as an internal standard. The elemental analysis were performed by using Euro Vector EA3000A (University of AL-al-Bayt,Jordan).

### Synthesis of 4,5-dihydroisoxazol derivatives (2a-e)

**General procedure.** To a stirred solution of chalcone (**1a–e**) ( which was prepared as mentioned in the literature) [21] (1.0 mmol) in 10 ml EtOH (96 %) was added hydroxylamine hydrochloride (2.0 mmol) and glacialaceticacid (2.5 ml) at room temperature. The reaction mixture was heated to reflux overnight. The progress of the reaction was monitored by TLC (ethyl acetate/hexane, 8:2). The EtOH was removed under reduced pressure and the residue was recrystalized from EtOH to afford the pure products (**2a–e**).

### 1,4-bis(3-phenyl-4,5-dihydroisoxazol-5-yl)benzene (2a)

was prepared from the reaction of 3,3'-(1,4-phenylene)bis(1-phenylprop-2-en-1-one) (1a) with hydrazine hydrate and gave a 70% yield with a m.p. (196-198)$^o$c. The CHN analysis for $C_{24}H_{20}N_2O_2$ ; C 78.24; H 5.47; N 7.60 Found C 78.22; H 5.46; N 7.60, FT-IR spectra (KBr pellet) υ(cm$^{-1}$) 1110 cm$^{-1}$ (N-O stretching of isoxazole ring), 3020 (C–H stretching of aromatic ring), 2880 (C–H stretching of aliphatic), 1614 (C=N stretching of isoxazoline ring), 1595 (C=C stretching of aromatic ring), 1219 (C–N stretching of pyrazoline ring), $\delta_H(CDCl_3)$ (7.529-7.941) ppm (10H,m,12,12$^/$,13,13$^/$,14,14$^/$ ,15,15$^/$ ,16,16$^/$); 7.293 ppm (4H,s, 6,7,9,10); (5.041-5.600) ppm (2H,t,4,4$^/$) ; (3.601-3.644) ppm (4H,d,3,3$^/$ ).

### 1,4-bis(3-p-tolyl-4,5-dihydroisoxazol-5-yl)benzene (2b)

was prepared from the reaction of 3,3'-(1,4-phenylene)bis(1-p-tolylprop-2-en-1-one) (1b) with hydrazine hydrate and gave a 72% yield with a m.p. (202-204)$^o$c. The CHN analysis for $C_{26}H_{24}N_2O_2$ ; C 78.76; H 6.10; N 7.07 Found C 78.75; H 6.08; N 7.05, FT-IR spectra (KBr pellet) υ(cm$^{-1}$) 1112 cm$^{-1}$ (N-O stretching of isoxazole ring), 3024 (C–H stretching of aromatic ring), 2885 (C–H stretching of aliphatic), 1610 (C=N stretching of isoxazoline ring), 1597 (C=C stretching of aromatic ring), 1218 (C–N stretching of pyrazoline ring), $\delta_H(CDCl_3)$ (7.378-7.402) ppm (4H,d, 13,13$^/$,15,15$^/$); (7.710-7.925) ppm (4H,d, 12,12$^/$,16,16$^/$); 7.293 ppm (4H,s, 6,7,9,10); (5.041-5.600) ppm (2H,t,4,4$^/$) ; (3.601-3.644) ppm (4H,d,3,3$^/$ ); 2.542 ppm (6H,s,14,14$^/$ ( $CH_3$).

**1,4- bis(3-(4-methoxyphenyl)-4,5-dihydroisoxazol-5-yl)benzene (2c)**
was prepared from the reaction of 3,3'-(1,4-phenylene)bis(1-(4-methoxyphenyl)prop-2-en-1-one) (1c) with hydrazine hydrate and gave a 77% yield with a m.p. (205-207)$^{o}$c. The CHN analysis for $C_{26}H_{24}N_2O_4$ ; C 72.88; H 5.65; N 6.54 Found C 72.87; H 5.62; N 6.52, FT-IR spectra (KBr pellet) υ($cm^{-1}$) 1115 $cm^{-1}$ (N-O stretching of isoxazole ring), 3020 (C–H stretching of aromatic ring), 2884 (C–H stretching of aliphatic), 1611 (C=N stretching of isoxazoline ring), 1596 (C=C stretching of aromatic ring), 1210 (C–N stretching of pyrazoline ring), $\delta_H(CDCl_3)$ (7.064-7.199) ppm (4H,d, 13,13$^{/}$,15,15$^{/}$); (7.910-7.944) ppm (4H,d, 12,12$^{/}$,16,16$^{/}$); 7.293 ppm (4H,s, 6,7,9,10); (5.041-5.600) ppm (2H,t,4,4$^{/}$) ; (3.601-3.644) ppm (4H,d,3,3$^{/}$ ); 3.833 ppm (6H,s,14,14$^{/}$ ( $OCH_3$).

**1,4- bis(3-(4-bromophenyl)-4,5-dihydroisoxazol-5-yl)benzene (2d)**
was prepared from the reaction of 3,3'-(1,4-phenylene)bis(1-(4-bromophenyl)prop-2-en-1-one) (1d) with hydrazine hydrate and gave a 82% yield with a m.p. (208-210)$^{o}$c. The CHN analysis for $C_{24}H_{18}Br_2N_2O_2$ ; C 54.78; H 3.45; N 5.32 Found C 54.76; H 3.44; N 5.30, FT-IR spectra (KBr pellet) υ($cm^{-1}$) 1116 $cm^{-1}$ (N-O stretching of isoxazole ring), 3022 (C–H stretching of aromatic ring), 2880 (C–H stretching of aliphatic), 1611 (C=N stretching of isoxazoline ring), 1596 (C=C stretching of aromatic ring), 1211 (C–N stretching of pyrazoline ring), $\delta_H(CDCl_3)$ (7.583-7.602) ppm (4H,d, 13,13$^{/}$,15,15$^{/}$); (7.722-7.776) ppm (4H,d, 12,12$^{/}$,16,16$^{/}$); 7.293 ppm (4H,s, 6,7,9,10); (5.041-5.600) ppm (2H,t,4,4$^{/}$) ; (3.601-3.644) ppm (4H,d,3,3$^{/}$ ).

**1,4-bis(3-(4-nitrophenyl)-4,5-dihydroisoxazol-5-yl)benzene (2e)**
Was prepared from the reaction of 3,3'-(1,4-phenylene)bis(1-(4-nitrophenyl)prop-2-en-1-one) (1f) with hydrazine hydrate and gave a 85% yield with a m.p. (210-212)$^{o}$c. The CHN analysis for $C_{24}H_{18}N_4O_6$ ; C 62.88; H 3.96; N 12.22 Found C 62.85; H 3.94; N 12.20, FT-IR spectra (KBr pellet) υ($cm^{-1}$) 1114 $cm^{-1}$ (N-O stretching of isoxazole ring), 3023 (C–H stretching of aromatic ring), 2881 (C–H stretching of aliphatic), 1613 (C=N stretching of isoxazoline ring), 1597 (C=C stretching of aromatic ring), 1213 (C–N stretching of pyrazoline ring), $\delta_H(CDCl_3)$ (8.332-8.426) ppm (4H,d, 13,13$^{/}$,15,15$^{/}$); (8.009-8.039) ppm (4H,d, 12,12$^{/}$,16,16$^{/}$); 7.293 ppm (4H,s, 6,7,9,10); (5.041-5.600) ppm (2H,t,4,4$^{/}$) ; (3.601-3.644) ppm (4H,d,3,3$^{/}$ ).

**Biological activity**
Kirby Bauer method was carried out by using discs (6 mm diameter) of paper chromatography which were sterilized by the autoclave apparatus at 121 $^{o}$C for 15 minutes under 1 atm and kept in clean sterilized glass screw plugs.

The primary screening of the antibacterial activity of the synthesized pyrazoline compounds was investigated by the method of plate agar diffusion with slight modification by using Muller-Hinton agar medium which was sterilized by autoclave apparatus at 121 $^{o}$C for 15 minutes under 1 atm and then cooled and poured into sterilized Petri dishes.

Two bacterial species were used, Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli*. The isolated bacteria were cultured by strecking on nutrient agar (N. A.) in order to obtain youth colonies of 24 hours age.

One colony from the fresh agar culture of each organism was inoculated into 5 ml sterile nutrient broth (N. B) and was incubated at 37 $^{o}$C for 6 hrs. In order to obtain germ growth of optical density = 0.1 (equivalent to $10^6$ cell/ml), 0.1 ml of each growing organism was spread on the Muller-Hinton Agar (M. H. A.) surface by sterilized swabs in three directions to get homogenous growth. The discs saturated with the prepared nitrone and isoxazolidine compounds (concentration 1000 g/ml) were added to (M.H.A.) medium by clean forceps, then incubated at 37 $^{o}$C for 24 hours.

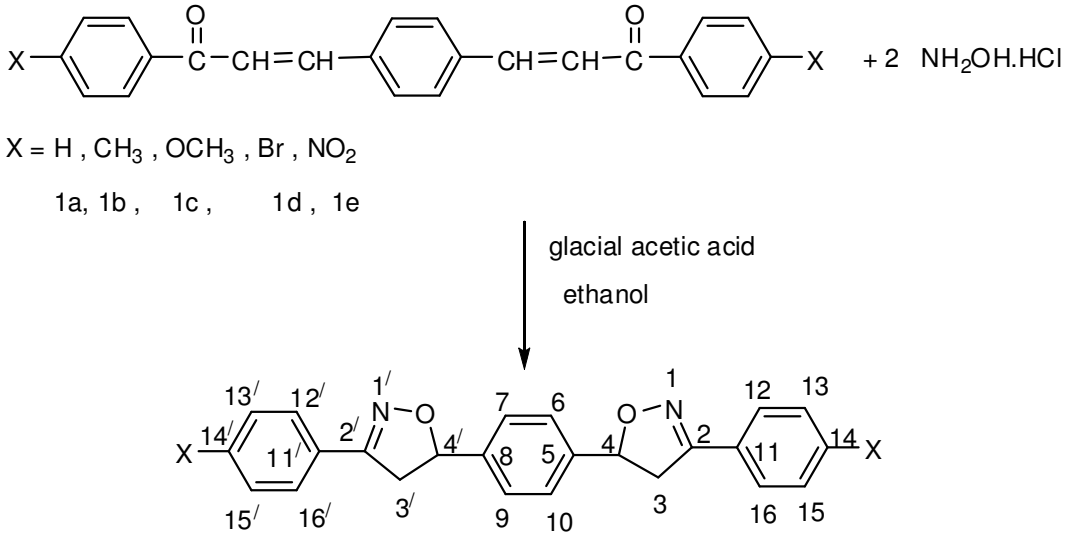
The dishes were examined for the presence or absence of bacterial growth and the averages of inhibition zone diameters around each disc in millimeters were measured (the zone where there is no bacterial growth) [22] ,as shown in table (1).

**Results and discussion**
Treatment of chalcones derivatives **(1a-e)** with hydroxylamine hydrochloride in boiling ethanol gave 4,5-dihydroisoxazole derivatives compounds, after purification by recrystallization from ethanol, pure 4,5-dihydroisoxazole derivatives compounds as shown in (scheme 1) in (70-85)% yield were obtained. The structures of these products were established from their elemental analysis, FT-IR,C.H.N and $^{1}$H NMR spectra. The FT-IR spectra of 4,5-dihydroisoxazole compounds were characterized by the disappearance of the absorption band that was attributed to the (C=O) stretching which appeared at (1672-1710) $cm^{-1}$. These fact confirmed the correct expected chemical structure of these compounds. All the IR spectra of 4,5-dihydroisoxazole derivatives showed a peak at (1610-1614) $cm^{-1}$ which related to (C=N) stretching of 4,5-

dihydroisoxazole ring , a peak at (1210-1219) $cm^{-1}$ which appeared due to (C-N) stretching of pyrazoline ring and a peak at (1595-1597) $cm^{-1}$ which appeared due to (C=C stretching of aromatic ring). While, the C-H stretching aromatic rings showed a peak within the range (3020-3024) $cm^{-1}$ and the C-H stretching aliphatic showed a peak within the range (2880-2885) $cm^{-1}$. The N-O stretching showed a peak within the range (1110-116) $cm^{-1}$.

All the $^{1}H$ NMR spectra of 4,5-dihydroisoxazole ring were characterized [23-25] by the presence protons (3,3$^{/}$ ) of 4,5-dihydroisoxazole ring showed doublet signals within the range (3.601-3.644) ppm because interaction with protons in (4,4$^{/}$ ) position and showed triplet signals within the range (5.041-5.600) ppm which appeared to protons in (4,4$^{/}$ ) position because interaction with two protons in (3 and 3$^{\backslash}$) position , while The proton in position (1) of furan ring showed doublet signals at (7.900-7.921) ppm. The protons of aromatic rings in compound (2a) showed singlet signal within the range 7.293 ppm which appeared to four protons in (6,7,9,10), while the other protons in (12,12$^{/}$,13,13$^{/}$,14,14$^{/}$,15,15$^{/}$,16,16$^{/}$ ) showed multiplet signals within the range (7.529-7.941) ppm. The aromatic protons in compounds (2b,2c,2d and 2e) showed doublet signals within the range (7.064-8.426) ppm which appeared to the two protons in (13 and 15) positions. The other two protons in positions (12 and 16) showed doublet signals within the range (7.710-8.039) ppm. The $CH_3$ protons showed singlet signal for six protons in the region 2.542 ppm. The $OCH_3$ protons showed singlet signal for six protons at 3.833 ppm.




**Scheme (1)**

**The biological activity**

The antibacterial activities of the synthesized 4,5-dihydroisoxazole compounds against the tested organisms; *Staphylococcus aureus* and *Escherichia coli* using Hahn method[26], were summarized in table (1) and figures (1-4). This method was based on the disc diffusion for testing chemical agents and antimicrobial effectiveness by measuring and determining the agents zones of inhibitions which sizes are proportional to how sensitive the organism is to the particular antibiotic in the disc [27].

Generally, it is clear that Gram negative bacteria (*E. coli*) are more affected than Gram positive bacteria (*S. aureus*). It has been postulated that the cell membrane of *E. coli* contains many condensed fat layers as compared to *S. aureus*. Accordingly, chemicals, antibiotics or antiseptics face difficulty in penetrating these membranes and therefore their effectiveness is diminished [28].

Table (1) shows the values of inhibition zones in (mm) of the 4,5-dihydroisoxazole compounds against the two tested microorganisms.

**Table (1): Inhibition zones (mm) of the synthesized 4,5-dihydroisoxazole derivatives against – standard microorganisms**

| Compounds | IZ (*E. coli* ) mm | IZ (*S. aureus*) mm |
|---|---|---|
| 2a | **14** | **11** |
| 2b | **9** | **10** |
| 2c | **8** | **16** |
| 2d | **10** | **10** |
| 2e | **7** | **10** |

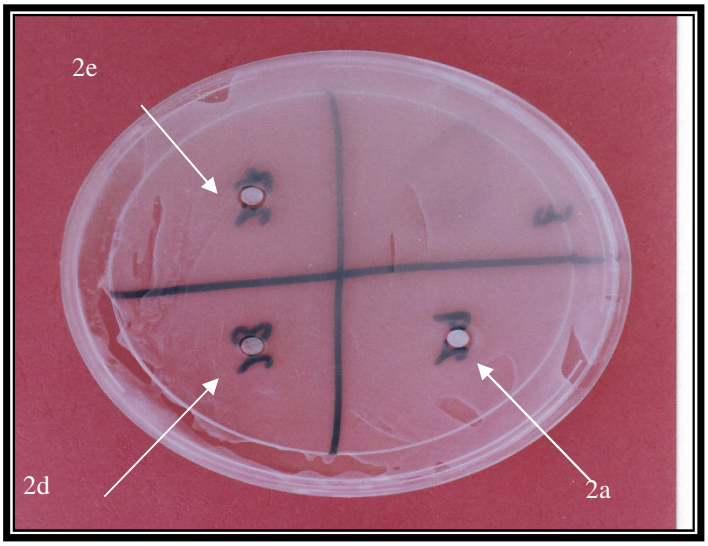



**Figure 1 : inhibition zone for compounds 2a,2d,2e against E.Coli**

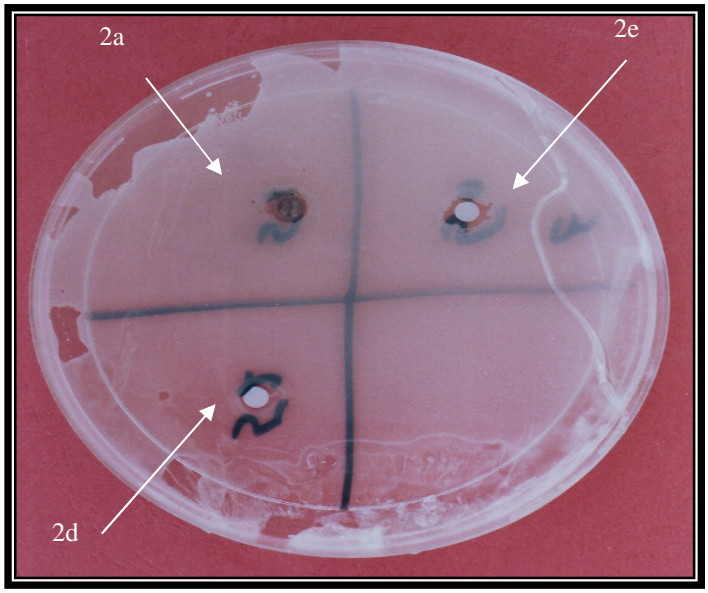



**Figure 2 : inhibition zone for compounds 2a,2d,2e against S.areous**

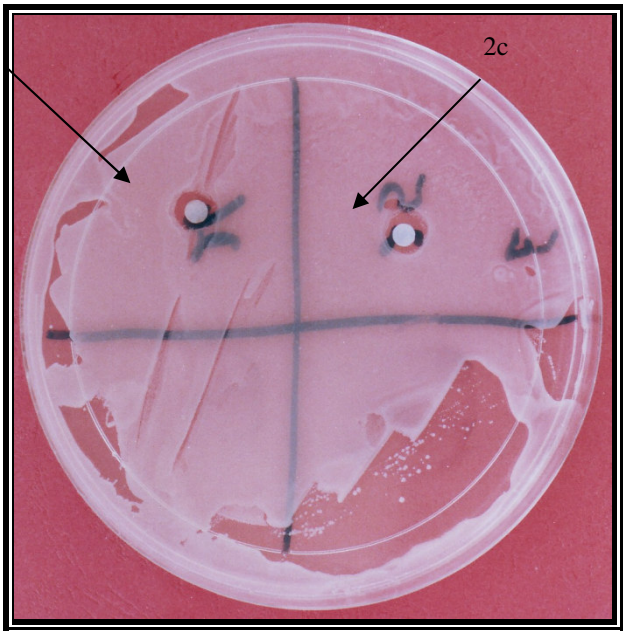



**Figure 3 : inhibition zone for compounds 2b,2c against E.Coli**

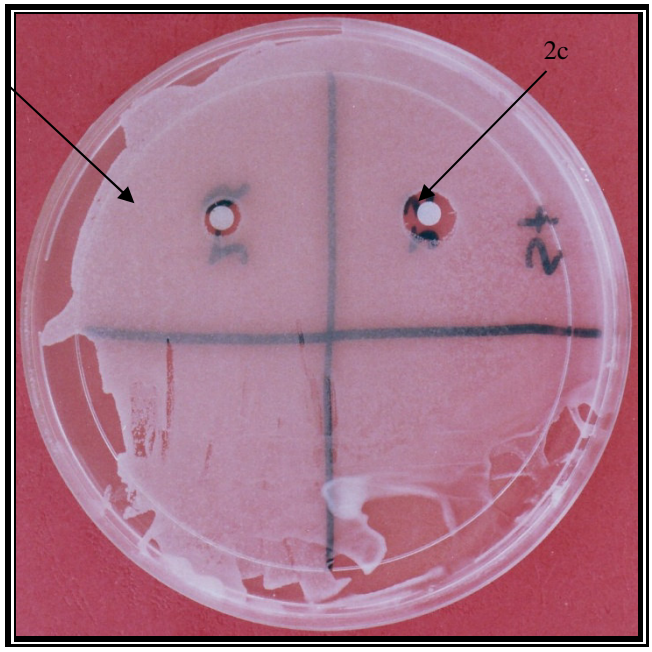



**Figure 4 : inhibition zone for compounds 2b,2c against *S. aureus***

**References**

1.Hijova E, Bioavailability of Chalcones, Bratisl Lek Listy,107(3): 80-84,(2006).
2. Liu M, Wilairat P and Mei-Lin G, Antimalarial Alkoxylated and Hydroxylated Chalcones: Structure-Activity Relationship Analysis, J.Med.Chem.,44 (25): 4443-4452, (2001).
3. Opletalova V, Chalcones and Their Heterocyclic Analogues as Potential Therapeutic Agents of Bacterial Diseases, Cesk.Slov.Farm.49:278-284, (2000).
4.Lee SH, Nan JX, Zhao YZ, Woo SW, Park EJ, Kang TH, Seo GS, Kim YC and Sohn DH, The Chalcone Butein from Rhus verniciflua shows antifibrogenic activity, Planta. Med.69: 990-994, (2003).
5. Konieczny MT, Konieczny W, Sabisz M, Skladanowski A, Wakiec R, Augustynowicz- Kopec E and Zwolska Z, Synthesis of Isomeric, Oxathiolone fused Chalcones, and Comparison Their Activity towards various Microorganisms and Human Cancer Cells Line, Chem. Pharm. Bull.55: 817-820, (2007).
6. Oyedapo AO, Mankanju VO, Adewunmi CO, Iwalewa EO and Adenowo TK, Antitrichomonal Activity of 1,3-Diaryl-2-propen-1-ones on Trichomonas Gallinae, Afr.J.Trad. CAM 1:55-62, (2004).

7. Jin F, Jin XY, Jin YL, Sohn DW, Kim S-A, Sohn DH, Kim YC and Kim HS, Structural Requirements of 2',4',6'-Tris (methoxymethoxy) chalcone Derivatives for Anti-inflammatory Activity: The Importance of a 2'-Hydroxy Moiety, Arch.Pharm.Res.30(11): 1359-1367, (2007).
8. Narender T, Khaliq T, Shweta, Nishi, Goyal N and Gupta S, Synthesis of Chromenchalcones and Evaluation of their in vitro Antileishmanial Activity, Bioorg.Med.Chem.13: 6543-6550, (2005).
9. Aponte JC, Verastegui M, Malaga E, Zimic M, Quiliano M, Vaisberg AJ, Gilman RH and Hammond GB, Synthesis, Cytotoxicity and Anti- Trypanosoma cruzi Activity of New Chalcones, J.Med.Chem.51: 6230-6234, (2008).
10.Yoo H, Kim SH, Lee J, Kim HJ, Seo SH, Chung BY, Jin C and Lee YS, Synthesis and Antioxidant Activity of 3-Methoxyflavones, Bull.Korean Chem.Soc.26 (12):2057-2060, (2005).
11. Li JX, Xub B, Chai Q, Liu ZX, Zhao AP and Chan LB, Antihypertansive Effect of Total Flavonoid fraction of Astragalus complanatus in Hypertensive Rats, Chin.J.Physiol.48: 101-106, (2005).
12. Inoue T, Sugimoto Y, Masuda H and Kamei C, Antiallergic Effect of Flavonoid Glycosides obtained from Mentha piperita L., Biol.Pharm.Bull.25: 256-259, (2002).
13.Umamaheswari S, Viswanathan S, Sathiyasekaran BWC, Parvathavarthini S and Ramaswamy S, Antinociceptive Activity of Certain Dihydroxy Flavones, Indian J.Pharm.Sci.68 (6): 749-753,S (2006).
14.Maliar T, Jedinak A, Kadrabova J and Sturdik E, Structural Aspects of Flavonoids as Trypsin Inhibitors, Eur.J.Med.Chem.39: 241-248, (2004).
15.Keriko JM, Nakajima S, Baba N, Isozaki Y and Iwas J, Plant Growth Regulators from Kenyan Plant, Psiadia punctulata, Sci.Rep.Agr.OKAYAMA, 84: 7-11, (1995).
16.Mostahar S, Katun P and Islam A, Synthesis of Two Vanillin Ring Containing Flavones by Different Methods and Studies of Their Antibacterial and Antifungal Activities, J.Biol.Sci.,7 (3): 514-519, (2007).
17.Katade S, Phalgune U, Biswas S, Wakharkar R and Deshpande N, Microwave studies on synthesis of biologically active chalcone derivatives, Indian J.Chem., 47B: 927-931, (2008).
18.Verma RS, Solvent-free organic syntheses using supported reagents and microwave irradiation, Green Chemistry,: 43-55, (1999).
19.Kidwai M and Misra P, Ring closure reactions of chalcones using microwave technology, Synth.Commun., 29 (18) : 3237-3250, (1999).
20. a) Vibhute YB and Basser MA, Synthesis of 6-Hydroxy-3-naphthylidene-5,6'-Benzoflavanone, J.Indian Chem.Soc.,78: 319, (2001).
b)Vibhute YB and Basser MA, Synthesis and Activity of a new series of Chalcones as Antibacterial Agents, Indian J.Chem.,42B: 202-205, (2003).
c) Mokle SS, Sayyed MA, Kothawar, and Chopde. Studies on Synthesis and Antimicrobial Activity of some new Iodochalcones, Flavones and Flavonols, Int. J. Chem. Sci., 2(1): 96-100, (2004).
d) Mokle SS, Sayyed MA, Bhusare SR, Pawar RP and Vibhute YB, Synthesis and Antibacterial Activity of new Pyrazoline and Benzothiazepines derivatives, Chemistry: An Indian Journal,2(9): 302-305, (2005).
e) Navale VA, Mokle SS, Vibhute AY, Karamunge KG, Junne SB and Vibhute YB. Microwave-Assisted Synthesis and antibacterial activity of some new flavones and 1,5-benzothiazepines, Asian J.Research Chem.( In press).
21. I. Karamana, H. Gezegenb, M. B. G_rdereb, A. Dingilb, Ceylan (2010) , " screening of biological activities of a series of chalcones derivatives against human pathogenic microorganisms", J. chemistry & biodiversity , 7 ,400-408
22. A. Bauer, W. Kinby and M. Turck, *Amer. J. Clin. Pathol.*, 45, 493 (1966).
23. Silverstien R.M., Webster F.X., Kiemle D.J., Spectrometric Identification of Organic Compounds, sixth ed., John Wiley and Sons, 2005, New Yourk, USA.
24. Cooper J.W., Spectroscopic Techniques for Organic Chemistry, John Wiley and Sons,1980, New Yourk, USA.
25. Shriner R.L., Hermann C.K., Spectroscopic Techniques for Organic Chemistry, John Wiley and Sons, 2004,New Yourk, USA
26- F. E. Hahn, "Mechanism of Action of Antibacterial Agent Springer", *Velary, Berlin*,VOL. 5, 67-80 (1979).
27- V. Gautam, N. K. Gupta, V. Chaudhary and D. R. Arora, *The Brazillian Journal of Infections Disease,* 6, 281-287 (2002).
28- J. T. Staley, "Microbiology", *Saunders College Publishing*, New York, USA, (1997).

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