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Eco-friendly synthesis and antimicrobial activity of chalcones

Ramesh C. Kamboj,[*] Rita Arora, Geeta Sharma, Dinesh Kumar, Chetan Sharma,[a] Radhika Joshi[a] and K. R Aneja[a]

Department of Chemistry, Kurukshetra University, Kurukshetra-136119, Haryana, India; [a]*Department of Microbiology, Kurukshetra University, Kurukshetra-136119, Haryana, India.*

Abstract

Chalcones are important intermediates for the synthesis of many flavones and chromones molecules. A fast and very rapid procedure is reported for the synthesis of chalcones from the ketones and aldehydes under the microwave (MW) irradiation. The chalcones were produced via the condensation reaction under microwave conditions during 20-60 seconds. Excellent yields were obtained in microwave-enhanced synthesis as compared to the conventional procedure. All these chalcones were screened for their in vitro antibacterial and antifungal activities. Most of the chalcones were found to have very promising antibacterial and antifungal activities.

Keywords: Chalcones, condensation reaction, microwave irradiation, antimicrobial activity.

INTRODUCTION

The chalcones are the important intermediates in the biosynthesis [1] of flavanoids, the substances most abundantly found in plants. Compounds having chalcone motif have been reported to exhibit a wide variety of pharmacological effects, including antioncogenic [2], anti-inflammatory [3], antiulcerative [4], analgesic [5], antiviral [6], antimalarial [7], anticancer [8], antifungal [9], and antibacterial activities [10]. The presence of a reactive α,β-unsaturated carbonyl group in chalcones is found to be responsible for their antimicrobial activity, which may be altered depending on the type and position of the substituent(s) on the aromatic rings.
Recent advances in the technology for the development of eco-friendly synthetic method have great importance and are the need of the hour [11-17]. The multi-step conventional synthesis produces considerable large amount of environmentally unfavorable wastes mainly due to a

series of complex isolation procedure involving expensive and toxic solvents after each step. The application of microwave-assisted reactions in organic synthesis has received considerable attention. Microwave provides chemical processes with special attributes such as enhanced reaction rates, higher yields of pure products, lesser by products, better selectivity and several eco-friendly advantages as compared to conventional heating [18-22]. In recent times, the synthesis of chalcones [23] and many other compounds [24] under MW irradiation has been reported. In this communication we report the synthesis of several new chalcones which are used for the preparation of many types of ether required for the synthesis of a variety of tetracyclic and spiro-compounds through H-abstractions [25-28] under photolytic conditions and may have the potential to be used as potential bioactive compounds. Our main objectives here to use the MW technology are; (i) to obtain the chalcones in shorter reaction times with high yields and with a simple work up procedure (ii) to observe the formation of cyclic products as all these synthesized chalcones contain hydroxyl group in close proximity of the enone system which may further interact to undergo cyclisation in the microwave irradiated conditions and (iii) the evaluation of these chalcones for their biological activity in quest for the discovery of new drugs.

MATERIALS AND METHODS

Reactions were carried out with a single mode domestic microwave oven (BPL-Sanyo, INDIA) in open corning glass tubes. The NaOH, KOH and $Ba(OH)_2$ were used for condensation in order to achieve the best product yields. Reactions were monitored by thin-layer chromatography. TLC plates were coated with silica gel G (suspended in $CHCl_3$-MeOH) and iodine vapours were used as visualizing agent. Melting points were determined in open capillaries and are thus uncorrected IR spectra were recorded on a Buck Scientific 500 spectrophotometer. Samples were analyzed using the KBr pellets, and frequencies are expressed in cm^{-1}. ^1H NMR spectra were recorded on a 300 MHz Bruker spectrometer using TMS as internal standard. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane. Yields were determined from isolated products.

Microwave assisted Organic synthesis: A General procedure - The 5-chloro-2-hydroxy-acetophenone (0.170g, 0.001mol), 2-thiophenecarboxaldehyde (0.123g, 0.0011mol), finely powdered base NaOH/KOH/$Ba(OH)_2$ and few drops of dry methanol were mixed thoroughly in an open corning glass wide-mouth test tube. The amount of monoacidic bases i.e. NaOH, KOH used in the synthesis were 0.002mol while that of diacidic base S-200 $Ba(OH)_2$ was 0.001mol.This mixture was irradiated under MW in a domestic microwave oven for 25 seconds. Then, the reaction mixture was cooled, poured over crushed ice and was neutralized with dil. HCl. The crude product then filtered which was recrystalized from ethanol to obtain **2** as yellow crystals in 81-92 % yield, m. pt. 110-12 ^oC.
All other chalcones **1, 3-28** were prepared by this procedure. The spectroscopic data for the chalcones [28], [29-42] reported previously were reproduced while the structures of new ones **3**, **8**, **9**, **20**, **26, 27** and **28** were ascertained.

Conventional heating procedure [30]
To a well stirred suspension of powdered NaOH (0.08g, 0.002 mol) in ethanol (20.0ml) at 0 ^oC were added 5-chloro-2-hydroxy-acetophenone (0.170g, 0.001mol) and 2-

thiophenecarboxaldehyde(0.123g, 0.0011 mol). The reaction mixture that became deep red in colour after 30min. was stirred further for 3h. Thereafter, it was poured over ice and was neutralized with dil. HCl to obtain acrylophenone **2** that was crystallized from ethanol.
All products were characterized by ^{1}H NMR and IR. The melting points were determined to compare with those obtained by conventional methods. The ^{1}H NMR, IR spectra of chalcones produced through MW overlapped with the spectra of the chalcones obtained by traditional/conventional methods. Spectral data of the few newly synthesized chalcones are given below:

Compound **3**: ν_{max} (cm^{-1}) 1628.0 (C=O); δ_H ($CDCl_3$) 12.9 (1H, br s, OH), 8.19 (1H, d, $J_{3,2}$ = 15.0 Hz, H-3), 7.84 (1H, d, J_m = 2.4 Hz, H-6'), 7.45 (1H, dd, J_m = 2.4 Hz, J_o = 9.0 Hz, H-4'), 7.42 (1H, d, $J_{5",4"}$ = 5.4 Hz, H-5"), 7.28 (1H, d, $J_{2,3}$ = 15.0 Hz, H-2), 7.00 (1H, d, J_o = 8.7 Hz, H-3'), 6.97 (1H, d, $J_{4",5"}$ = 5.4 Hz, H-4"), 2.46(3H, s, 3"-CH_3).

Compound **8**: ν_{max} (cm^{-1}) 1628.0 (C=O); δ_H ($CDCl_3$) 13.1 (1H, br s, OH), 7.95 (1H, d, $J_{3,2}$ = 15.0 Hz, H-3), 7.84 (1H, d, J_m = 2.1 Hz, H-6'), 7.43 (1H, dd, J_m = 2.1 Hz, J_o = 9.0 Hz, H-4'), 7.29 (1H, d, $J_{2,3}$ = 15.0 Hz, H-2), 7.00-6.97 (2H, m, H-3',5"), 6.91 (1H, s, H-3"), 6.30 (1H, br s, H-4"), 3.82 (3H, s, N-CH_3).

Compound **9**: ν_{max} (cm^{-1}) 1643.0 (C=O), 3400 (OH); δ_H ($CDCl_3$) 12.9 (1H, br s, OH), 7.94 (1H, d, $J_{3,2}$ = 15.6 Hz, H-3), 7.91 (1H, d, J_m = 2.4 Hz, H-6'), 7.69 (1H, s, H-2"), 7.55-7.43 (4H, m, H-2, 4', 4", 5"), 7.05 (1H, d, J_o = 8.4 Hz, H-3'), 6.97 (1H, t, J_o = 7.5 Hz, H-5').

Compound **20**: ν_{max} (cm^{-1}) 1643.0 (C=O), 3472 (OH); δ_H ($CDCl_3$) 12.5 (1H, br s, OH), 8.34 (2H, d, J_o = 8.4 Hz, H-3",5"), 7.97 (1H, d, $J_{3,2}$ = 15.6 Hz, H-3), 7.86 (3H, m, H-2",6",6'), 7.70 (1H, d, $J_{2,3}$ = 15.6 Hz, H-2), 7.51 (1H, dd, J_m = 2.4 Hz, J_o = 9.0 Hz, H-4'), 7.05 (1H, d, J_o = 9.0 Hz, H-3").

Compound **26**: ν_{max} (cm^{-1}) 1651.0 (C=O), 3418 (OH); δ_H ($CDCl_3$) 12.5 (1H, br s, OH), 8.03 (1H, d, $J_{3,2}$ = 15.9 Hz, H-3), 7.81 (1H, d, J_m = 2.4 Hz, H-6'), 7.76 (1H, d, $J_{2,3}$ = 15.9 Hz, H-2), 7.48 (1H, dd, J_m = 2.4 Hz, J_o = 9.0 Hz, H-4'), 7.44 (2H, d, J_o = 8.1 Hz, H-3", 5"), 7.27 (1H, m, H-4"), 7.03 (1H, d, J_o = 8.7 Hz, H-3').

Compound **27**: ν_{max} (cm^{-1}) 1620.0 (C=O); δ_H ($CDCl_3$) 13.2 (1H, br s, OH), 8.43 (1H, d, $J_{3,2}$ = 15.6 Hz, H-3), 7.93 (1H, d, $J_{2,3}$ = 15.3 Hz, H-2), 7.86 (1H, d, J_m = 2.4 Hz, H-6'), 7.41 (1H, dd, J_m = 2.4 Hz, J_o = 9.0 Hz, H-4'), 6.97 (1H, d, J_o = 9.0 Hz, H-3'), 6.17 (2H, s, H-3", 5"), 3.97 (6H, s, 2", 6"-OCH_3), 3.90 (3H, s, 4"-OCH_3).

Compound **28**: ν_{max} (cm^{-1}) 1637.4 (C=O); δ_H ($CDCl_3$) 12.90 (1H, br s, OH), 7.88 (1H, d, J_m = 2.4 Hz, H-6'), 7.87 (1H, d, $J_{3,2}$ = 15.3 Hz, H-3), 7.46 (1H, dd, J_m = 2.4 Hz, J_o = 9.0 Hz, H-4'), 7.43 (1H, d, $J_{2,3}$ = 15.0 Hz, H-2), 6.99 (1H, d, J_o =

9.0 Hz, H-3'), 6.90 (2H, s, H-2", 6"), 3.95 (6H, s, 3", 5"-OCH_3), 3.93 (3H, s, 4"-OCH_3).

Test microorganisms

Four bacteria, *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 121) (Gram-positive), *Escherichia coli* (MTCC 1652) and *Pseudomonas aeruginosa* (MTCC 741) (Gram-negative) procured from MTCC, Chandigarh and two fungi, *Aspergillus niger* and *A. flavus*, the ear pathogens isolated from the Kurukshetra patients, were used in the present study.

***In-vitro* antibacterial activity**

The antibacterial activity of chalcones was evaluated by the agar well diffusion method [43], [44]. All the cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5 $\times 10^8$ cfu/ml. 20ml of Mueller Hinton agar medium was poured into each Petri plate and the agar plates were swabbed with 100 µl inocula of each test bacterium and kept for 15 min for adsorption. Using sterile cork borer of 8mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 µl volume with concentration of 4.0mg/ml of each compound reconstituted in the dimethylsulphoxide (DMSO). All the plates were incubated at 37 ^oC for 24 hrs. Antibacterial activity, indicated by an inhibition zone surrounding the well containing the compounds, was recorded if the zone of inhibition was greater than 8mm. The experiments were performed in triplicate. DMSO was used as a negative control whereas ciprofloxacin was used as a positive control.

Determination of Minimum Inhibitory Concentration (MIC) of chalcones

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation**.** MIC of the chalcones active against bacterial strains was determined through a macrodilution tube method as recommended by NCCLS [45]. In this method, various test concentrations of chemically synthesized compounds were made from 128 to 0.25µg/ml in sterile tubes No.1 to10. 100 µl sterile Mueller Hinton Broth (MHB) was poured in each sterile tube followed by addition of 200 µl test chalcone in tube 1.Two fold serial dilutions were carried out from the tube 1 to the tube 10 and excess broth (100 µl) was discarded from the last tube No.10. To each tube, 100 µl of standard inoculum (1.5 $\times 10^8$ cfu/ml) was added. Ciprofloxacin was used as control. Turbidity was observed after incubating the inoculated tubes at 37 ^oC for 24 hrs.

***In-vitro* antifungal activity**

The antifungal activity of the chalcones was evaluated by poison food technique [46]. The molds were grown on Sabouraud dextrose agar (SDA) at 25^oC for 7 days and used as inocula.The 15ml of molten SDA (45^oC) was poisoned by the addition of 100 µl volume of each compound having concentration of 4.0mg/ml, reconstituted in the DMSO, poured into a sterile Petri plate and allowed to

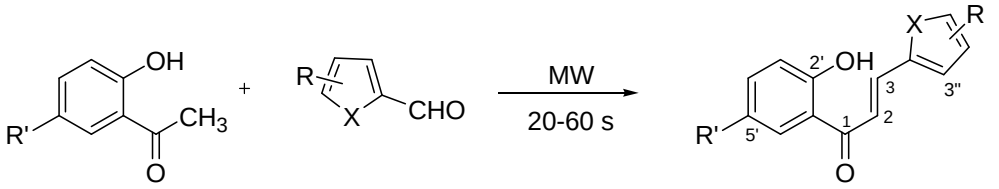
solidify at room temperature. The solidified poisoned agar plates were inoculated at the centre with fungal plugs (8mm diameter), obtained from the actively growing colony and incubated at 25 ^{o}C for 7 days. DMSO was used as the negative control whereas fluconazole was used as the positive control. The experiments were performed in triplicates. Diameter of the fungal colonies was measured and expressed as percent mycelial inhibition determined by applying the formula.

Inhibition of mycelial growth % = (dc-dt) / dc × 100
where,
dc = average diameter of fungal colony in negative control plates
dt = average diameter of fungal colony in experimental plates

RESULTS AND DISCUSSION

o-Hydroxyacetophenones were submitted to react to several aldehydes separately in dry methanol as solvent under microwave irradiation, as depicted in schemes 1-3 for the synthesis of chalcones **1-28** (Table 1). These chalcones were also synthesized by the usual conventional procedure for the comparison purpose. The structures of all the chalcones **1-28** were ascertained by their spectral parameters (IR and ^{1}H NMR, vide experimental). For example, the compound **3** in its IR spectrum has C=O stretch located at 1628 cm^{-1}. In the ^{1}H NMR spectrum (300MHz, $CDCl_3$) the peak due to 2'-OH proton was a broad singlet at δ 12.9. The absorption at δ 8.19 (1H, d) and 7.28 (1H, d) were due to H-3 and H-2 protons respectively. The coupling constant (J= 15.0 Hz) indicated the *trans*- relationship of the H-3 and H-2 protons. The protons H-3', H-4' and H-6' were revealed at δ 7.00 (1H, d, J_o = 8.7 Hz), 7.45 (1H, dd, J_m = 2.4 Hz, J_o = 9.0 Hz) and 7.84 (1H, d, J_m = 2.4 Hz) respectively. The protons due to thiophene ring were placed as two doublets at δ 7.42 (H-5"), 6.97 (H-4") respectively with coupling constant *J* = 5.4 Hz, The methyl group protons showed a sharp singlet at δ 2.46. Likewise the structures of other hydroxychalcones were ascertained.

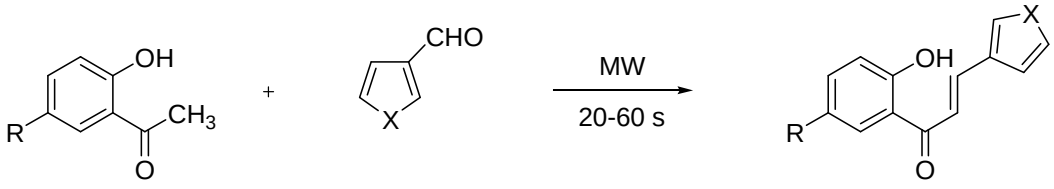
Scheme 1.



1- 8

1. R' = R = H, X = S
2. R' = Cl, R = H, X = S
3. R' = Cl, R = 3-CH_3, X = S
4. R' = Cl, R = 5-CH_3, X = S
5. R' = R = H, X = O
6. R' = Cl, R = H, X = O
7. R' = Cl, R = 5-CH_3, X = O
8. R' = Cl, R = H, X = N-CH_3

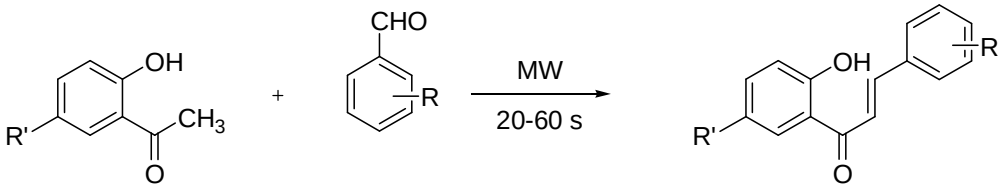
Scheme 2.



9-11

9. R = H, X = S
10. R = Cl, X = S
11. R = H, X = O

Scheme 3.



12-28

12. R' = R = H; **13.** R' = H, R = 2-OH;**14.** R' = H, R = 3-OH
15. R' = H, R = 4-OH;**16.** R' = H, R = 4-OCH_3; **17.** R' = H, R = 4-Cl
18. R' = Cl, R = H; **19.** R' = Cl, R = 4-CH_3; **20.** R' = Cl, R = 4-NO_2
21. R' = Cl, R = 2-OH; **22.** R' = Cl, R = 3-OH; **23.** R' = Cl, R = 4-OH
24. R' = Cl, R = 4-OCH_3; **25.** R' = R = Cl; **26.** R' = Cl, Rs = 2-, 6-Cl
27. R' = Cl, Rs = 2-, 4-, 6-OCH_3; **28.** R' = Cl, Rs = 3-, 4-, 5-OCH_3

Schemes 1- 3: Synthesis of Chalcones under MW

Table 1: Chalcones synthesized by microwave irradiation

Chalcone	Mp (^{o}C)	Yield(MW) %			Yield (CM) %	Time (MW) sec	Time (CM) min	color
		NaOH	KOH	$Ba(OH)_2$				
1	98-100(obs.) 99-100(lit.)[29]	73.39	82.60	94.80	64	25	240	Yellow orange
2	110-112(obs.) 110-112(lit.)[30]	91.30	82.25	92.30	80	25	240	Yellow
3	108-110(obs.)	75.53	71.90	68.30	78	25	240	Yellow
4	134-136(obs.) 138(lit.)[31]	77.00	86.33	86.46	81	25	240	Yellow orange
5	104-106(obs.) 103-104(lit.)[32]	62.50	72.90	93.75	61	25	240	Yellow orange
6	90-91(obs.) 90-91(lit.)[33]	88.70	82.25	89.70	85	25	240	Yellow
7	156-157(obs.) 156-157(lit.)[34]	92.85	95.05	91.30	85	25	360	Yellow
8	134-136(obs.)	72.51	76.34	87.78	72	25	240	Orange
9	66-68(obs.)	69.36	76.34	72.51	79	25	240	Yellow orange
10	63-65(obs.) 65(lit.)[33]	92.30	88.46	92.30	86	25	240	Yellow
11	116(obs.) 110-113(lit.)[28]	64.54	69.25	70.00	70	25	240	Yellow
12	88-90(obs.) 88-89(lit.)[35]	91.30	86.70	78.00	78	25	240	Yellow
13	158-160(obs.) 160-161(lit.)[36]	34.20	47.44	32.84	50	40*	7200	Yellow orange
14	165-167(obs.) 167-168(lit.)[36]	65.00	72.00	74.50	52	40*	240	Yellow orange
15	150-151(obs.) 157-158(lit.)[36]	69.00	71.40	72.31	57	40*	7200	Yellow orange
16	51-52(obs.) 54(lit.)[37]	93.05	94.15	93.75	68	25	240	Yellow
17	149-150(obs.) 150(lit.)[37]	88.00	80.88	87.45	75	25	240	Yellow orange
18	104-106(obs.) 108-109(lit.)[36]	89.14	73.64	74.67	80	25	240	Yellow
19	106-111(obs.) 131-133(lit.)[38]	88.00	80.88	87.67	74	25	240	Yellow
20	212-214(obs.)	72.70	65.45	80.00	79	25	240	Orange

Table 1 contd.

21	150(obs.) 148-150(lit.)[39]	32.84	36.45	47.44	50	60*	7200	Yellow
22	149-150(obs.) 149-150(lit.)[40]	68.00	68.00	72.00	70	60*	240	Yellow
23	165(obs.) 166-168(lit.)[36]	69.00	68.56	70.00	75	60*	7200	Yellow orange
24	102(obs.) 102-104(lit.)[41]	68.49	95.89	-	89	25	240	Pale yellow
25	182(obs.) 181-182(lit.)[42]	92.89	94.30	89.04	79	25	240	Bright yellow
26	134-136(obs.)	82.60	73.39	94.80	72	25	240	Yellow
27	184-187(obs.)	69.36	86.70	89.78	76	25	240	Orange
28	161-163(obs.)	91.95	80.45	86.20	90	25	240	Orange

CM = Conventional method; MW = Microwave;

- The beaker (500 ml) containing H_2O was used as a trap.

The formation of chalcones upon MW irradiation occurred within few seconds (Table 1), which otherwise were usually formed after 3-4 hours stirring at room temperature by the conventional method. There were improved yields to a large extent as compared to the conventional procedure.

Three most commonly employed bases viz., NaOH, KOH and $Ba(OH)_2$ were used for the synthesis of chalcones to test their efficacy. And the $Ba(OH)_2$ was proved to be the best for the MW synthesis of all these chalcones. As is amply clear from the chemical structures, all these chalcones contain hydroxyl group attached to the phenyl ring in the close proximity of their enone system which warrants the possibility of their cyclisation to yield flavones but in our studies no such cyclised products were realized. The discussion on the so-called microwave effect is of course now an old one. Microwave irradiation is a rapid way of achieving a desired temperature [47]. The reductions on reaction times are attributed to be a result of both temperature and pressure effects and possible modifications [48] of activation parameters $\Delta H^{\neq}$ and $\Delta S^{\neq}$.

All these chalcones **1-28** were screened for their antibacterial and antifungal activities. These chalcones possessed variable antibacterial activity against both Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa)* bacteria. On the basis of zone of inhibition produced against the test bacterium, the chalcones **4**, **6** and **10** were found to be the most effective against *S. aureus* showing the maximum zone of inhibition of 25.6mm, 25.0mm and 26.6mm while against *B. subtilis*, the chalcones **5**, **10** and **11** produced 23.3mm, 25.3mm and 23.6mm, zone of inhibition respectively. However, in the case of Gram-negative bacteria, the compounds **9** and **13,** and **14** were found to be the most active against *E. coli* with maximum zone of inhibition of 19.3mm and 18.6mm and the compounds **4**, and **9** and **13** showed the maximum zone of inhibition of 18.3mm and 18.6mm, respectively against *P. aeruginosa*. Seven chalcones **18**, **19**, **24**, **25**, **26**, **27** and **28** did not show any inhibitory activity against any tested bacteria (Table 2). A carefull analysis of the data for the antibacterial

activity of all these chalcones (Table 2) demonstrates an interesting finding that the incorporation of –OCH_3 group (except in **16**) and more than one chlorine atom in the chalcones skeleton separately causes the complete loss of antibacterial activity.

Table 2: *In vitro* antibacterial activity of chalcones determined by agar well diffusion method

Chalcone	Diameter of growth of inhibition zone (mm)[a]			
	Staphylococcus aureus	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***
1	22.3[a]±0.58 [b]	20.6±0.58	15±0	16.6±0.58
2	24± 0	22.6±0.58	15.6±0.58	16.3±0.58
3	23.6±0.58	18.6±0.58	-	-
4	25.6±0.58	21±0	17.6±0.58	18.3±0.58
5	21.6±0.58	23.3±0.58	16.6±0.58	15±0
6	25±0	22.6±0.58	17.6±0.58	16.3±0.58
7	23.3±0.58	20±0	14±0	15±0
8	22.6±0.58	18.3±0.58	-	-
9	22.3±0.58	21.6±0.58	19.3±0.58	18.6±0.58
10	26.6±0.58	25.3±0.58	18.3±0.58	17±0
11	24.6±0.58	23.6±0.58	18±0	14.6±0.58
12	20.6±0.58	22.3±0.58	17.3±0.58	15.6±0.58
13	21±0	21±0	19.3±0.58	18.6±0.58
14	18.6±0.58	20.3±0.58	18.6±0.58	17±0
15	21.6±0.58	18.3±0.58	15±0	15.6±0.58
16	19.3±0.58	18±0	15.6±0.58	17±0
17	18.6±0.58	20.3±0.58	15.6±0.58	16.3±0.58
18	-	-	-	-
19	-	-	-	-
20	22.3±0.58	19.3±0.58	15±0	14.3±0.58
21	22±0	21.6±0.58	-	-
22	21.3±0.58	22.6±0.58	17.6±0.58	16.6±0.58
23	24.6±0.58	17±0	-	-
24	-	-	-	-
25	-	-	-	-
26	-	-	-	-
27	-	-	-	-
28	-	-	-	-
Ciprofloxacin	26±0	24±0	25±0	22±0

- No activity; [a] Values, including diameter of the well (8mm), are means of three replicates; [b] Standard deviation.

The minimum inhibitory concentration (MIC) of various tested chalcones ranged between 8 and 128 µg/ml against Gram- positive bacteria. The chalcone **10** was found to be best which showed the lowest MIC of 8µg/ml. In case of Gram-negative bacteria, the MIC of these tested

compounds ranged between 64 and 128 µg/ml. The enones **9**, **13** and **14** showed lowest MIC of 64 µg/ml against *E. coli* while the enones **4**, **9** and **13** had lowest MIC of 64 µg/ml against *P. aeruginosa* (Table 3)**.**

Table 3: Minimum inhibitory concentration (MIC) (in µg/ml) of chalcones evaluated by macrodilution method

Chalcone	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
1	64	64	>128	128
2	32	64	>128	128
3	32	128	nt	nt
4	16	64	128	64
5	64	32	128	>128
6	16	64	128	128
7	32	64	>128	>128
8	64	128	nt	nt
9	64	64	64	64
10	8	8	128	128
11	32	32	128	>128
12	64	64	128	>128
13	64	64	64	64
14	128	64	64	128
15	64	128	>128	>128
16	128	128	>128	128
17	128	64	>128	>128
20	64	128	>128	>128
21	64	64	nt	nt
22	64	64	128	128
23	32	128	nt	nt
Ciprofloxacin	5	5	5	5

nt- Not tested

Of all the 28 chalcones screened, the chalcones **10** was found to be highly effective in inhibiting the growth of both the Gram-positive bacteria producing the zone of inhibition of 26.6 mm against *S. aureus* and 25.3mm against *B. subtilis* and with an MIC of 8µg/ml. It also showed good inhibition of both the Gram-negative bacteria i.e. *E. coli* and *P. aeruginosa* with zone of inhibition of 18.3mm and 17.3mm, respectively with an MIC value of 128 µg/ml.
Amongest the synthesized chalcones tested for their antifungal activity, the two chalcones, **4** and **22** showed the best activity against Aspergillus *niger* and *A. flavus* respectively. In case of *A. flavus*, the percent inhibition of mycelial growth was found to be equal to that of standard drug (fluconazole) used. This showed that these chalcones were as potent as that of a fluconazole, the standard antifungal antibiotic used commercially for treating various fungal infections (Table 4)**.**

Table 4: *In vitro* antifungal activity of chalcones determined by poisoned food method

Chalcone	Mycelial growth inhibition (%)	
	Aspergillus niger	***Aspergillus flavus***
1	55.5	44.4
2	55.5	44.4
3	44.4	61.1
4	77.7	66.6
5	55.5	50
6	55.5	66.6
7	44.4	55.5
8	61.1	61.1
9	66.6	61.1
10	55.5	61.1
11	55.5	66.6
12	55.5	61.1
13	66.6	61.1
14	55.5	50
15	50	55.5
16	50	55.5
17	38.8	50
18	-	38.8
19	44.4	55.5
20	66.6	55.5
21	55.5	55.5
22	66.6	**77.7**
23	55.5	44.4
24	61.1	44.4
25	-	55.5
26	50	44.4
27	-	50
28	55.5	44.4
Fluconazole	81.1	77.7

CONCLUSION

The present microwave (MW) methodology used is an excellent, eco-friendly and safe approach for the synthesis of hydroxychalcones and shows many advantages including shorter reaction times and higher product yields as compared to conventional methods. Also, the present method is an important addition to microwave-assisted synthetic methodologies. No cyclised products were realized from these chalcones even though they contain hydroxyl group in close proximity of the enone system. Many of these chalcones displayed excellent broad-spectrum, antibacterial and antifungal activities. Interestingly, some chalcones from this series

have been found to be equipotent to that of commercial antibiotics (ciprofloxacin and fluconazole).

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