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Microwave Assisted Efficient Synthesis of Some Flavones for Antimicrobial and ADMET Studies

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Abstract

Microwave (MW) assisted synthetic technique was applied for the preparation of chalcone derivatives **5-7** employing Claisen-Schmidt condensation between 2-hydroxyacetophenone and aromatic aldehydes. These chalcones were further subjected to oxidative cyclization *via* MW irradiation and furnished the related flavones **8-10** which were characterized by FT-IR, ^{1}H and ^{13}CNMR spectra. The use of these MW assisted reactions provided higher productivity (92-98%) in shorter reaction time (2-6 min) with eco-friendly mild reaction conditions and hence found to be a convenient method as compared to conventional synthesis. These chalcones **5-7**, and flavones **8-10** were screened for *in vitro* antimicrobial activities against five bacterial and three fungal pathogens. The study indicated that they were more active against fungal pathogens than that of bacterial organisms and comparable to the standard antifungal antibiotic nystatin. Interestingly, the prediction of activity spectra for substances (PASS) was also found in agreement with the *in vitro* results. Some of the compounds were found to have good ADMET properties.

Keywords: Microwave assisted synthesis; Chalcones; Flavones; PASS; Antimicrobial activity; MIC.

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1. Introduction

Chalcones and flavones are the major classes of low molecular weight natural compounds abundantly distributed throughout herbs, fruits and vegetables, often responsible for the therapeutic efficacy of these natural compounds in the treatment or prevention of cancer [1], and bacterial infection [2]. More promising therapeutic research results, including pharmaceutical [3,4], antioxidant [5,6], anti-inflammatory [7], antimutagenic, antiallergic, and several enzymes inhibitory activities are well documented [6,8,9]. Due to biological

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importance, in addition to isolation from natural sources, several strategies for the synthesis of chalcones and flavones have so far been reported [1,7]. In a similar context, we have also reported the synthesis and antimicrobial effects of several heterocycles and flavonoids containing various groups in their ring system [10-14]. Among the synthetic strategies, Claisen–Schmidt condensation [7], oxidative cyclization of 2-hydroxychalcones [15,16], three-step Baker-Venkataraman rearrangement [7], and the cyclodehydration of 1-(2-hydroxyphenyl-3-phenyl-1,3-propanedione) [17] were commonly used. However, most of these conventional methods suffered from harsh reaction conditions, toxic reagents, prolonged reaction time, lower yields, and less selectivity.

Recently, the microwave radiation (MWI) technique has gained attention due to its unique advantages, such as shorter reaction times, cleaner reaction products, higher yields, and better selectivity [18]. It offers a valuable alternative tool to efficiently accomplish various organic reactions maintaining environment friendly milder reaction conditions. A combination of MWI with the solvent-free approach provides a further advantage to conduct organic reactions in open vessels [18-20]. Thus, there is a promising opportunity to apply this excellent combination strategy for the synthesis of biologically important flavonoid compounds such as flavones **8-10**. These flavones **8-10** were although synthesized previously *via* conventional methods which took unnecessary longer reaction time with moderate yields [21,22]. These observations, along with our continuous efforts towards the synthesis of medicinally important biomolecules [23,24], led us to apply and validate this combinatorial solvent-free MWI approach for the synthesis of flavones **8-10** and their precursor chalcones **5-7**. Also, their PASS predication, *in vitro* antimicrobial efficacy, and ADMET properties are discussed herein.

2. Experimental

2.1. *Materials and methods*

All the reagents and chemicals used in this study were of reagent grade (Merck, Germany) and used without purification unless mentioned. Melting points (mp) were recorded with electrothermal melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed on Kieselgel $GF_{254,}$ and visualization was accomplished by iodine vapor or UV light. The infrared (IR) spectra were measured by the FT-IR spectrophotometer (Model-8900, Shimadzu, Japan) using the KBr matrix in the range 4000-200 cm^{-1}. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on JEOL GS×400, GEOL JNM-AL 400 spectrometer with Tetramethylsilane (TMS) as internal standard by using $CDCl_3$ as a solvent and expressed in δ ppm. All the reactions were performed in a commercially available LG microwave oven (MB-3947C, China), having a maximum power output of 800 W operating at 2450 MHz.

2.2. *Syntheses: General procedure for the preparation of chalcones*

A mixture of 2-hydroxyacetophenone and aromatic aldehydes (1:1 mol) was dissolved in a minimum amount of rectified spirit and KOH (10 %) and placed in a porcelain dish. The porcelain dish was covered with a glass, and then the dish was transferred into a domestic microwave oven. The mixture was irradiated below 320 Watt microwave irradiation for 100-140 sec only (lit [21,22] reaction time 72-75 h]. The development of the reaction was monitored by TLC (*n*-hexane-ethyl acetate, 6:1) after every 30 sec. After completing the reaction, the reaction mixture was cooled and diluted with ice-cold water, acidified with dil. HCl and extracted with diethyl ether. The organic layer was cleaned with water and dried over anhydrous Na_2SO_4. Moreover, the solvent was evaporated under reduced pressure. The obtained product was solid and recrystallized from ethyl acetate and *n*-hexane solvent mixture.

*2'-Hydroxy-4-methylchalcone (**5**):* Yellow crystals, mp 111-112 °C (lit [21] mp 39-40 °C); Yield: 96.7 %, FT-IR (KBr) υ_{max} (cm^{-1}): 3433 (OH), 2916, 1635 (C=O), 1592 (C=C), 1485 (C=C, Ph), 1199, 1026, 748; ^{1}HNMR (400 MHz, $CDCl_3$) δ_H ppm: 2.43 (s, 3H, 4-C*H*$_3$), 6.96 (m, 1H, H-4'), 7.05 (d, 2H, *J*=8 Hz, C3-H and H-5), 7.27 (d, 1H, *J*=8 Hz, H-3'), 7.50 (m, 1H, H-5'), 7.59 (d, 2H, *J*=8 Hz, H-2 and H-6), 7.65 (d, 1H, *J*=16 Hz, H-α), 7.94 (d, 1H, *J*=16 Hz, H-β), 7.96 (d, 1H, *J*=8 Hz, H-6'), 12.88 (s, 1H, 2'-OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm: 21.9 (4-CH_3), 117.2 (C-3'), 119.6 (C-5'), 121.9 (C-1'), 124.2 (C-α), 126.8 (C-2 and C-6), 129.9 (C-3 and C-5), 130.1 (C-1), 132.1 (C-6'), 136.2 (C-4'), 137.3 (C-4), 143.9 (C-β), 158.7 (C-2'), 186.4 (CO).

*2'-Hydroxy-3,4-methylenedioxychalcone (**6**):* Yellow crystal, mp 128-130 °C (lit [22] mp 124-127 °C), Yield: 97.1 %; FT-IR (KBr) ν_{max} (cm^{-1}): 3468 (C-OH), 3066, 2904, 1639 (C=O), 1573 (C=C), 1489 (C=C, Ph), 1242 (C-O), 1037, 759; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 6.07 (s, 2H,-OCH_2O-), 6.89 (d, 1H, *J*=8Hz, H-5), 6.95(t, 1H, *J*=8Hz, H-5'), 7.05 (d, 1H, *J*=8Hz, H-6), 7.18 (d, 1H, *J*=8Hz, H-3'), 7.20 (s, 1H, H-2), 7.52 (d, 1H, *J*=15.2Hz, H-α), 7.51 (m, 1H, H-4'), 7.88 (d, 1H, *J*=15.2Hz, H-β), 7.94(d, 1H, *J*=8Hz, H-6'), 12.90 (s, 1H, 2'-OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm: 91.6 (-O*C*H_2O-), 112.6 (C-2), 115.3 (C-5), 117.1 (C-3'), 120.2 (C-6), 121.9 (C-5'), 124.1 (C-α), 124.3(C-1'), 128.6 (C-1), 130.2 (C-6'), 137.2 (C-4'), 142.6 (C-β), 146.2 (C-4), 147.8 (C-3), 158.3 (C-2'), 186.6 (CO).

*2'-Hydroxy-2,4,5-trimethoxychalcone (**7**):* Orange needles, mp 124-125 °C (lit [21] mp 114-116 °C); Yield: 97.3 %; FT-IR (KBr) υ_{max}(cm^{-1}): 3433 (OH), 2958, 1627 (C=O), 1546 (C=C), 1516 (C=C, Ph), 1338 (C-O), 1234, 1026, 817, 775; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 3.95 (s, 3H, 2-OCH_3), 3.96 (s, 3H, 5-OCH_3), 3.99 (s, 3H, 4-OCH_3), 6.56 (s, 1H, H-3), 6.96 (t, 1H, *J*=8 Hz, H-5'), 7.04 (d, 1H, *J*=7.6 Hz, H-3'), 7.16 (s, 1H, H-6), 7.49 (dd, 1H, *J*=8 and *J*=4 Hz, H-4'), 7.64 (d, 1H, *J*=15.6 Hz, H-α), 7.95 (d, 1H, *J*=8.4 Hz, H-6'), 8.24 (d, 1H, *J*=15.6 Hz, H-β), 13.08 (s, 1H, 2'-OH); ^{13}C NMR (100 MHz, $CDCl_3$)

δ_C ppm: 56.4 (5-O*C*H$_3$), 56.6 (4-O*C*H$_3$), 56.7 (2-O*C*H$_3$), 103.1 (C-3), 113.9 (C-6), 114.3 (C-1), 117.2 (C-3'), 121.5 (C-5'), 123.6 (C-α), 124.2 (C-1'), 130.3 (C-6'), 139.3 (C-5), 137.6 (C-4'), 142.6 (C-β), 148.3 (C-4), 154.6 (C-2), 158.3 (C-2'), 186.5 (CO).

2.3. *General procedure for the synthesis of flavones 8-10*

2'-Hydroxychalcones (1 mmol) were suspended in (DMSO, 2 mL) and placed in a porcelain dish followed by the addition of iodine (0.02 mmol). The porcelain dish containing reaction mixture was placed in the microwave oven. The mixture was irradiated within 320 Watt microwave irradiation for 300-325 sec (lit [22] reaction time 2.5 h under reflux). The progress of the reaction was observed by TLC (*n*-hexane-ethyl acetate, 4:1). The reaction mixture was diluted with water (excess) and extracted with diethyl ether (2×15 mL). The ether layer was washed with aqueous 20 % sodium thiosulphate, water and dried over anhydrous Na_2SO_4, and the solvent was evaporated. The solid thus obtained was further purified by recrystallization from ethyl alcohol to give flavones which produced blue fluorescence in the UV light.

*4'-Methylflavone (**8**):* Light brown needles mp 75-76 °C (lit [21] mp 75-76 °C); Yield: 98 %; FT-IR (KBr) υ_{max} (cm^{-1}): 3035, 2920, 1639 (C=O), 1566 (C=C), 1465 (C=C, Ph), 1373, 1122 (C-O), 1199, 1026, 748; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 2.46 (s, 3H, 4'-C*H*$_3$), 6.82 (s, 1H, H-3), 7.34 (d, 2H, *J*=8 Hz, H-3' and H-5'), 7.43 (t, 1H, *J*=8 Hz, H-6), 7.58 (d, 1H, *J*=8 Hz, H-8), 7.71 (m, 1H, H-7), 7.84 (d, 2H, *J*=8 Hz, H-2' and H-6'), 8.25 (d, 1H, *J*=8 Hz, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm: 23.1(4'-*C*H$_3$), 97.8 (C-3), 117.5 (C-8), 123.3 (C-6), 124.6 (C-4a), 127.8 (C-2' & C6'), 129.6 (C-5), 130.2 (C-3' and C-5'), 132.3 (C-1'), 135.9 (C-7), 137.8 (C-4'), 158.3 (C-8a), 168.9 (C-2), 178.3 (CO).

*3',4'-Methylenedioxyflavone (**9**):* Pale yellow crystal, mp 175-176 °C (lit [22] mp 131-132 °C); Yield: 92.7 %; FT-IR (KBr) ν_{max} (cm^{-1}): 3105, 3016, 2920, 1647 (C=O), 1600, 1504 (C=C), 1446 (C=C, Ph), 1346, 1242 (C-O), 1126, 1022, 848, 779; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 6.113 (s, 2H, -OC*H*$_2$O-), 6.83 (s, 1H, H-3), 6.97 (d, 1H, *J*=8Hz, H-5'), 7.43(m, 2H, H2' and H-6'), 7.56 (q, 2H, *J*=6.4Hz, H-6 and H-8), 7.73 (dd, 1H, *J*=8.8 and 1.6 Hz, H-7), 8.26(dd, 1H, *J*=8 and 1.6 Hz, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm: 101.95 (-O*C*H$_2$O-) 106.28 (C-3), 106.53 (C-2'), 108.75 (C-5'), 117.94 (C-8), 121.45 (C-6'), 123.86 (C-6), 125.15 (C-4a), 125.64 (C-1'), 125.67 (C-5), 133.66 (C-7), 148.48 (C-4'), 150.64 (C-3'), 156.11 (C-8a), 163.05(C-2), 178.3 (C=O).

*2',4',5'-Trimethoxyflavone (**10**):* Colorless needles, mp 174-175 °C (lit [21] mp 131-132 °C); Yield: 92.5 %; FT-IR (KBr) υ_{max} (cm^{-1}): 2935, 2839, 1639 (C=O), 1612, 1562 (C=C), 1465 (C=C, Ph), 1388, 1258 (C-O), 1203, 1022, 852, 756; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 3.97 (s, 3H, -OC*H*$_3$), 3.98 (s, 3H, -OC*H*$_3$), 4.0 (s, 3H, -OC*H*$_3$), 6.63 (s, 1H, H-3'), 7.30 (d, 1H, *J*=13.6 Hz, H-3), 7.43 (dd, 1H, *J*=8 Hz and 1.6 Hz, H-6), 7.45 (s, 1H, H-6'), 7.57 (d, 1H, *J*=8.4 Hz, H-8), 7.71 (dd, 1H, *J*=7.2 and 1.6 Hz, H-7), 8.26 (d, 1H, *J*=6.4

Hz, H-5); ^{13}CNMR (100 MHz, $CDCl_3$) δ_C ppm: 56.12 (5'-O*C*H_3), 56.34 (4'-O*C*H_3), 56.76 (2'-O*C*H_3), 97.31 (C-3), 111.57 (C-3'), 111.90 (C-1'), 112.05 (C-6'), 117.88 (C-8), 123.81 (C-6), 124.82 (C-4a), 125.58 (C-5), 133.34 (C-7), 143.18 (C-5'), 152.59 (C-4'), 153.94 (C-2'), 156.29 (C-8a), 160.30 (C-2), 178.85 (CO).

2.4. *PASS predication of 5-10*

Web-based PASS (prediction of activity spectra for substances; http://www.pharmaexpert.ru/PASSonline/index.php) was used for the prediction of the biological spectrum of compounds **5-10** [25]. This program is designed to anticipate a plethora of biological activities with 90 % accuracy. PASS result is designated as Pa (probability for active compound) and Pi (probability for inactive compound). Only activities with Pa>Pi are considered as possible for a particular compound. Initially, structures of the compounds were drawn and then converted into their SMILE format and used to predict the biological spectrum using the PASS online version.

2.5. *Evaluation of antimicrobial activities*

2.5.1. *Antibacterial screening*

The antibacterial activity of the synthesized compounds **5-10** were studied against five human pathogenic bacteria. Of them two were Gram-positive viz., *Bacillus cereus* (BTCC 19) and *Staphylococcus aureus* (ATCC 6538), and three were Gram-negative viz. *Vibrio cholera* (ICDDR, B), *Pseudomonas aeruginosa* (CRL, ICDDR, B), and *Salmonella typhi* (AE 14612).The disc diffusion method [26,27] was employed for the detection of antibacterial activity. Ampicillin was used as the standard antibiotic for the antibacterial test. Nutrient agar (NA) was used as the basal medium for test bacteria. These agar media were vaccinated with 0.5 mL of the 24 h liquid cultures containing 10^7 micro-organisms/mL. The diffusion time was 24 h at 5 °C, and the incubation time was 12 h at 37 °C for bacteria. Discs with only dimethyl sulfoxide (DMSO) were used as control. The diameter (in mm) of the observed inhibition zones was taken as a measure of inhibitory activity.

2.4.2. *Determination of MIC and MBC*

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are used by commercial laboratories mainly to confirm resistance, but most frequently as a research tool to determine the new antimicrobial *in vitro* activities. In the present study, the MIC and MBC values of the synthesized compounds were determined against five human pathogenic bacteria. The MIC and MBC of these tested compounds compared to ampicillin were determined against those selected bacteria by the broth micro-dilution method [28]. The main advantage of the micro-dilution method for the

MIC determination lies in the fact that it can readily be assigned to determine the MBC, as well. The media used in this respect were nutrient broth. Dilution series were setup with 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1024 µg/mL of nutrient broth medium. To each well, 100 µL of standardized suspension of the testing bacteria (10^7 cell/mL) was added and incubated at 30 °C for 24 h.

2.4.3. *Antifungal screening*

The antifungal activity of compounds **5-10** was evaluated towards threeplant pathogenic and mold fungi viz. *Aspergillus flavus* (ATCC 9643), *Aspergillus ochraceus* (ATCC 18500) and *Rhizopus spp.* (ATCC 52369). The antifungal activities of synthesized compounds were assessed by the food poison technique [29]. For comparison, nystatin (50 µg/disc) was used as the standard reference antifungal antibiotic. Potato dextrose agar (PDA) was used as a basal medium for test fungi. The test chemicals (100 µg) were mixed with sterilized PDA medium (42 °C) at the rate of 100 mg/mL PDA. The medium was poured in sterilized Petri-plates. Inoculation was done by just touching the sample with the needle and then touching it at the middle of the plate containing solidified PDA media and then incubated at 27 °C for five days. The fungal colony's radial development was measured in mm after 3-5 days of incubation at (25±2) °C. A control set was maintained in each experimental using only PDA with DMSO as the growth medium. The inhibition activity of mycelial growth of the test fungi was calculated in percentage.

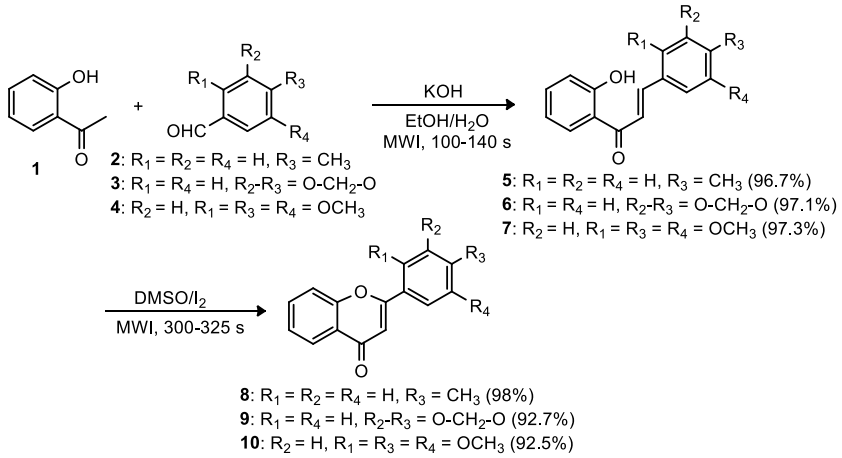
3. Results and Discussion

3.1. *Synthesis of flavone derivatives*

Initially, we applied the microwave radiation (MWI) technique for the synthesis of some chalcone derivatives employing Claisen-Schmidt condensation. Thus, a mixture of 2-hydroxyacetophenone (**1**) and aromatic aldehydes **2** in the presence of 10 % KOH (alc) upon MWI for just 130 sec undergoes condensation which after recrystallization furnished yellow crystals, mp111-112 °C in excellent yield (96.7 %)(Scheme 1).

In its FT-IR spectrum, a band at 3433 cm^{-1} indicated the presence of the hydroxyl group, which was further confirmed as phenolic by positive ferric chloride test. Also, a band at 1635 cm^{-1} in FT-IR indicated the presence of a conjugated carbonyl group (C=O). The presence of a three-proton singlet at δ 2.43 in its ^{1}H NMR spectrum confirmed the presence of a methyl group in the ring B of the molecule. Also, it showed the presence of a total of ten protons in the aromatic region. However, the appearance of two distinct one-proton doublets at δ7.65 and 7.94 with a high coupling constant (*J*=16 Hz) indicated the presence of *trans*-olefinic protons i.e., the compound had two protons conjugated with a carbonyl group. Hence, the remaining eight protons must be due to the two aromatic rings which must be substituted. This fact was further confirmed by the appearance of a carbonyl carbon signal at δ 186.4, a methyl carbon at δ 21.9, two olefinic carbon signals at

δ 124.2 and 143.9, and eight aromatic carbon signals in its ^{13}C NMR spectrum. Thus, based on its FT-IR, ^{1}H, and ^{13}C NMR spectral analyses, the compound was assigned as 2'-hydroxy-4-methylchalcone (**5**). It should be noted that the conventional condensation of the same reaction gave 79 % yield after 72-75 h heating [21].



Scheme 1. MW assisted synthesis of chalcones **5**-**7** and flavones **8**-**10**.

Having success in MW assisted preparation of **5**, we employed two more aromatic aldehydes such as **3** and **4** for similar a type of Claisen-Schmidt condensation with **1**, and obtained 2'-hydroxy-3,4-methylenedioxychalcone (**6**, 97.1 %, lit [22] yield 68 %) and 2'-hydroxy-2,4,5-trimethoxychalcone (**7**, 97.3 %, lit [21] yield 72 %), respectively. Hence, MWI condensation produced chalcones **5**-**7** very effectively with higher yields and within a couple of minutes.

At this stage, we were interested in investigating the oxidative cyclization of chalcones using MWI. Thus, a well-mixed 2'-hydroxychalcone **5** and DMSO/I_2 upon MWI for 320 sec followed recrystallization furnished a brownish solid, mp 75-76 °C in 98 % (Scheme 1). The presence of a carbonyl band at 1639 cm^{-1} and disappearance of hydroxyl stretching bands in its FT-IR spectrum indicated the cyclization of the molecule. This fact was further supported by its ^{1}H NMR, which showed the absence of OH peaks, the presence of one olefinic, and eight aromatic protons. The disappearance of one olefinic proton as compared to its precursor **5** clearly confirmed the cyclization of the molecule. Complete analysis of its ^{1}H and ^{13}C NMR, as well as comparison with literature data [21-22], led us to assign the compound as 4'-methylflavone (**8**). In this case, the MWI gave a higher yield than the conventional reflux method (69 %) in a shorter time (2.5 h [21]).

Finally, application of similar oxidative cyclization for chalcone **6** and 7 furnished 3',4'-methylenedioxyflavone (**9**, 92.7 %, lit. [22] 61.5 %) and 2',4',5'-trimethoxyflavone (**10**, 92.5 %, lit. [21] 64 %), respectively.

3.2. *Computational biological activities evaluation: Prediction of activity spectra for substances (PASS)*

The web-based PASS program is used to predict a plethora of pharmacological and toxicological activities of a compound with 90 % accuracy [25]. PASS result is designated as *Pa* (probability for active) and *Pi* (probability for inactive). Being probabilities, the *Pa* and *Pi* values vary from 0.000 to 1.000. Only activities with *Pa*>*Pi* are considered as possible for a particular compound. Biological activities were obtained by PASS online version (http://www.pharmaexpert.ru/PASSonline/index.php) using SMILES of compound **5-10** and predicted antimicrobial, anticancer, and antioxidant activities are mentioned in Table 1.

PASS predication (Table 1) of the compounds **5-10** were found 0.22<*Pa*<0.36 in antibacterial and 0.23<*Pa*<0.45 in antifungal. This indicated that these chalcones and flavones were more potent against phytopathogenic fungi as compared to that of bacterial pathogens. We have extended our studies for anticarcinogenic and antioxidant evaluation also (Table 1). Thus, PASS predication indicated 0.35<*Pa*<0.45 for anticarcinogenic and 0.38<*Pa*<0.48 for antioxidant, which refers that these compounds were equally potent as anticarcinogenic agents and as antioxidant agents although in moderate level.

Table 1. Predicted biological activities of **5-10** using PASS software.

Biological activity							
Antibacterial		Antifungal		Anticarcinogenic		Antioxidant	
Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi
0.351	0.043	0.464	0.037	0.422	0.028	0.451	0.009
0.283	0.066	0.311	0.076	0.357	0.039	0.407	0.011
0.321	0.053	0.449	0.040	0.431	0.026	0.480	0.007
0.272	0.071	0.376	0.055	0.409	0.029	0.397	0.012
0.230	0.095	0.239	0.112	0.403	0.030	0.388	0.013
0.226	0.097	0.316	0.075	0.445	0.025	0.421	0.010

Pa = Probability 'to be active'; Pi = Probability 'to be inactive'

3.3. *Antimicrobial efficacy*

Knowing the PASS predicted potentiality, we have conducted *in vitro* antimicrobial screening of the chacone and flavone derivatives.

3.3.1. *Antibacterial activities*

The antibacterial activities of compounds **5-10** were assayed against two Gram-positive and three Gram-negative pathogenic bacteria as summarized in Table 2 (Fig. 1). The diameter of zone of inhibition is measured three times and the average value is shown to the nearest whole number in mm. The results indicated that these compounds were weak to moderate antibacterial inhibitors. Among them, flavone **8** was found comparatively better inhibitor against both Gram-positive and Gram-negative pathogens.

Table 2. Antibacterial screening for the compounds **5-10**.

Diameter of zone of inhibition in mm (100 µgdw/disc)				
Gram-positive		Gram-negative		
B. cereus	*S. aureus*	*P. aerugnosa*	*S. typhi*	*V. cholerae*
NI	8	NI	NI	10
10	10	NI	NI	NI
NI	NI	9	9	8
13	12	12	10	10
10	10	11	NI	NI
NI	NI	10	8	8
18	*20	18	14	*20

Significant inhibition values are marked with * sign and, ** sign for reference antibiotic ampicillin (APC). dw = Dry weight; NI = No inhibition. NI was observed for control DMSO.

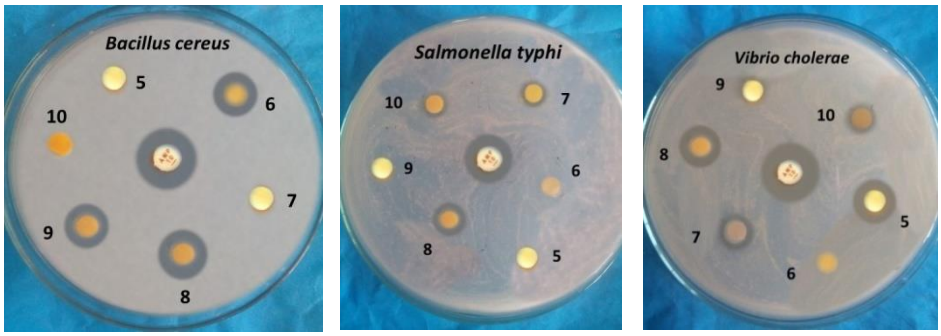


Fig. 1. Inhibition zones against bacterial pathogens by 5-10 and ampicillin.

3.3.2. *Minimum inhibitory activity*

The MIC and MBC of compounds **5-10** along with ampicillin (APC) were determined [28] for bacterial pathogens and presented in Tables 3 and 4, respectively. It was evident from the Tables that MIC and MBC for chalcone and flavone derivatives **5-10** were higher than that of the standard antibacterial antibiotic ampicillin. These observations were in complete agreement with the weak antibacterial susceptibility of compounds **5-10** as discussed in the previous Section (Table 2).

Table 3. MIC values of **5-10** for bacterial organisms.

Organisms	MIC (minimum inhibitory concentration) of compounds in µg mL^{-1}						
	5	**6**	**7**	**8**	**9**	**10**	**APC**
B. cereus	128	64	256	64	64	128	4
S. aureus	128	128	128	64	128	128	4
P. aeruginosa	128	128	128	64	128	128	8
Salmonella typhi	64	32	128	32	64	128	4
Vibrio cholerae	128	128	128	64	64	128	4

Table 4. MBC values of **5-10** for bacterial organisms.

Organisms	MBC (minimum bactericidal concentration) of compounds in μg mL^{-1}						
	5	**6**	**7**	**8**	**9**	**10**	**APC**
B. cereus	256	128	>256	128	128	256	8
S. aureus	256	256	256	128	256	256	8
P. aeruginosa	256	128	256	64	128	256	16
Salmonella typhi	128	64	128	64	128	128	8
Vibrio cholerae	256	256	256	128	128	256	8

3.3.3. *Antifungal activity*

The antifungal activity of compounds **5-10** has been assayed *in vitro* against three fungi, and the results are calculated as the percentage of the zone of inhibition. As shown in Table 5, both chalcones and flavones were found to be more active against fungal pathogens than that of bacterial organisms which was in consistent with our PASS predication (Table 1). Again, flavones **8-10** were found to be more active than chalcone derivatives **5-7**. Of the flavones, compound **8** was comparatively more prone against fungi (Fig. 2) and showed maximum percentage of the inhibition against *Rhizopus spp.* (*76 %) which was a better than that of the nystatin (68 %). Overall, chalcones and flavones were found to be better inhibitor of *A. ochraceus* and *Rhizopus spp*. than *A. flavus* (Table 5).

Table 5. *In vitro* antifungal screening for the compounds **5-10**.

Drugs	Percentage of inhibition of mycelial growth (100 μgdw/mL PDA		
	A. flavus	*A. ochraceus*	*Rhizopus spp.*
5	41	54	*60
6	56	*66	53
7	59	*63	*63
8	*68	*63	*74
9	50	*77	*63
10	41	*77	*60
****Nystatin**	53	*66	*68

* = Significant inhibition values; ** =Standard antibiotic nystatin (50 μg/disc); dw = Dry weight; NI = No inhibition. NI was observed for control DMSO.

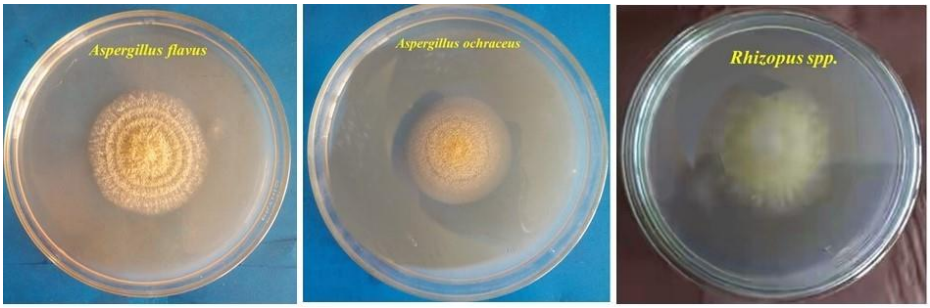


Fig. 2. Inhibition of zone of fungal growth by the flavones **8**.

3.4. *ADMET analysis*

Having encouraging antifungal potentiality of **5**-**10** we thought of calculating their absorption, distribution, metabolism, excretion and toxicity (ADMET) properties. ADMET properties, as derived from two online servers (http://lmmd.ecust.edu.cn and http://biosig.unimelb.edu.au), revealed that (Table 6) the synthesized compounds have positive human intestinal absorption (HIA) value although nystatin has negative HIA. These compounds (except **5**, **6** and **8**) are P-glycoprotein inhibitor and can pass through blood brain barrier (except **5**, **10** and nystatin).

Table 6. Admet SAR calculation of compound **5**-**10**.

Drug	Absorpt-ion	Distribution		Metabo-lism	Excretion	Toxicity		
	HIA	PG inhibitor	BBB	CYP3A4 substrate	TRC (ml/mg/kg)	hERG inhibitor	Acute oral toxicity	Carci-nogen
5	+0.9875	-0.9137	-0.2429	Yes	+0.094	WI,0.8783	III	NC
6	+0.9713	-0.7949	+0.8786	Yes	-0.057	WI,0.9732	III	NC
7	+0.9814	+0.7239	+0.9348	Yes	+0.313	WI,0.9678	III	NC
8	+0.9924	-0.4306	+0.8143	Yes	+0.322	WI,0.8714	III	NC
9	+0.9787	+0.7679	+0.8909	Yes	+0.191	WI,0.9855	III	NC
10	+0.9928	+0.9667	-0.2867	Yes	+0.408	NI,0.8758	II	NC
NST	-0.9664	+0.7242	-0.9930	No	-1.211	WI,0.9777	III	NC

NST = Nystatin; HIA = Human intestinal absorption; PG = P-glycoprotein; BBB = Blood brain barrier; TRC = Total renal clearance; hERG = Human ether-a-go-go-related gene; NI = Non-inhibitor, I = Inhibitor, WI = Weak inhibitor, NC = Non-carcinogenic, C = Carcinogenic.

Cytochromes P450's (CYP450) are responsible for the metabolism of many drugs. Its inhibitors dramatically alter pharmacokinetic properties, and hence we checked the metabolism property by calculating CYP3A4 substrate activity. All the compounds could be metabolized by CYP450 although standard antifungal was found to possess non-metabolic property. As the compounds are metabolized they can pass through kidney as observed from total renal clearance result (except **6**).

All the compounds (except **10**) including nystatin showed weak inhibitory feature for human ether-a-go-go-related gene (hERG). They are non-carcinogenic and showed III category (except **10**) acute oral toxicity (Table 6) which suggests that these compounds are relatively harmless for oral administration.

4. Conclusion

In the present study, the application of MW assisted Claisen-Schmidt condensation, and the oxidative cyclization was successfully investigated for the synthesis of some chalcones and flavones. It is worth to mention that this MWI technique was found highly convenient over the conventional heating method with respect to enhanced productivity, shorter reaction time, low cost, and green approach. The technique mentioned here could

be useful for further synthesis of new flavone derivatives and related drug design. The synthesized chalcones **5-7**, and flavones **8-10** were found more prone against fungal pathogens than that of bacterial organisms tested. Interestingly, the *in vitro* results were in complete accord with the PASS predications. Flavone **8** was found to be the most potential antimicrobial agent, and hence further study is expected in the near future.These compounds are non-carcinogenic and showed mostly less acute oral toxicity which indicated that they are relatively harmless for oral administration.

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