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Design, Synthesis, Characterization and Biological Evaluation of Dihydropyrazole Derivatives

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ABSTRACT

Chalcone compounds are unsaturated ketone involving the reactive keto- ethylenic group (CO-CH=CH-). To synthesis a novel Chalcone derivative by using microwave assisted synthesis, study antibacterial activity and evaluate the zone of inhibition of each concentration of the synthesized Chalcone derivative, to evaluate *in-vitro* antioxidant activity by DPPH assay method. We analysed IR and mass spectral data to confirm the structure of the synthesized compound. Synthesisis was done by means of a microwave irradiation technique and synthesized 1-(2,4-Dinitrophenyl)-3,5-diphenyl 4,5-dihydro pyrazole using 2,4-dinitrophenyl hydrazine and trans chalcone along with ethanol and few drops of conc. HCl. Antibacterial studies were carried out by using agar well plate methods. Antibacterial studies showed significant zone of inhibition against Escherichia coli and Staphylococcus aureus. In vitro antioxidant study such as DPPH assay was performed and was compared with standard ascorbic acid. The significant antimicrobial activity was recognized for the synthesized Chalcone derivative including the concentration of 50,100 and 200µg/ml. When compared to ascorbic acid (standard), the chalcone derivative is effective in antioxidant activity. The functional groups present in the chalcone derivative were determined by means of IR spectroscopy. The mass spectroscopical study was carried out to determine the molecular weight which was found out as 316 mol.

KEYWORDS: Microwave Assisted synthesis, Chalcone Derivative, Antioxidant, Antimicrobial activity, Ascorbic acid, IR, MASS.

INTRODUCTION

Chalcone compounds are unsaturated ketone involving the reactive ketoethylenic group (CO- CH=CH-) which gave colored compounds due to the presence (CO-CH=CH) the chromophore group. The name “Chalcones” was given by Kostanecki and Tambor.

Chalcones are also known as benzyl acetophenone. Chalcones are naturally abundant in medical plants including vegetables, fruits and natural foods. Chalcones are an innovative class of compounds with significant therapeutic potential against various diseases.[1-5]

MATERIALS AND METHODS

General procedure for Microwave Assisted Synthesis of1-(2,4- dinitrophenyl)-3,5-diphenyl 4,5- dihydropyrazole.

We dissolved a mixture of chalcone (4g) and different nucleophilic reagents, 2,4-dinitro phenyl hydrazine (0.2g),1mlethanol and a few drops of Con. HCl. It was irradiated under microwave for a specific time of 3-4 min at 80- 120ºC. The precipitate was filtered, dried, and recrystallized from ethanol to give the compound. An augmented yield with shorter reaction times is the added advantage of the present work.[2]

Antimicrobial activity

The synthesized compound was screened for their in vitro antimicrobial activity against some selected microorganisms, where the antibacterial activity was evaluated against one gram positive bacteria *staphylococcus aureus* and one gram negative bacteria *Escherichia coli*. The antimicrobial activity was performed by Well diffusion method at concentration 50, 100, 500 and 1000 µg/mL and reported in table 3 and 4. Muller Hinton agar &was used as solvent control for antimicrobial activity.[3,7]

Chalcone derivative was tested for anti-bacterial activity in the well diffusion method by using standard procedure. *Staphylococcus aureus* and *Escherichia coli*. All the stock cultures were obtained from MTCC lab. The microorganisms were grown overnight at 37˚C in nutrient broth (pH 7.4).[4]

Agar well plate method

Agar well plate method was used to screen the antimicrobialactivity. The MHA plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify for 5 min, inoculum suspension was spread uniformly by using sterile swab, and the inoculums were allowed to dry for 5 min. The concentration of chalcone derivative used is 100µg/ml. Fromthis, 50, 100,150 and 200 µl of the solution containing 5, 10,15 and 20

µg, respectively of chalcone derivative was loaded on 6mm well, and then allowed to diffuse for 5 min, and the plates were kept for incubation at 37^0c for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured in millimetres.

Antioxidant activity

The antioxidant activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH radical. 1.5 ml solution of DPPH in methanol was added to 1.5mL of various concentrations (150, 300, 450, 600, 750 µg/ml) of test (chalcone derivative) and the standard (Ascorbic acid), mixed and left to stand in the dark at room temperature for 30 min and then absorbance was measured at 517 nm against a blank. A control reaction was carried out without the test sample. All the tests were performed in triplicate in order to get the mean values. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. Antiradical activity was expressed as percentage inhibition (I %) and calculated using the following equation.

$$\text{Percentage inhibition (I \%)} = \frac{\text{Abs control - Abs sample}}{\text{Abs control}}$$

Where „ Abscontrol ‟ was the absorbance of the control reaction and „Atest‟ was the absorbance in the presence of the sample/ standard. The antioxidant activity of the extract was expressed as IC50. (IC50 –concentration required to obtain a 50% radical scavenging activity). Where „ Abscontrol ‟ was the absorbance of the control reaction and „Atest‟ was the absorbance in the presence of the sample/ standard. The antioxidant activity of the extract was expressed as IC50. (IC50 - concentration required to obtain a 50% radical scavenging activity).[5,20]

IR Spectroscopy

IR spectroscopy has established itself as a valuable tool for the determination of organic, and to a lesser extent, inorganic structure. The utilization of IR data in conjunction with other physical measurements, such as those obtained from nuclear magnetic resonance and mass spectroscopy, has elucidated many facts about the structure and properties of organic compounds, facts which were heretofore accepted solely on a theoretical basis. Bruker FTIR Spectrometer ALPHA II was the instrument used to determine the IR spectra of compound.

Mass Spectroscopy

The synthesized Chalcone derivative was subjected to enter the electron ionization (mass spectroscopic) detector. There, they are bombarded with a stream of electrons causing them to break apart into fragments. These fragments can be large or small pieces of the original molecules. The fragments are charged ions with a certain mass. The mass of the fragment divided by the charge is called the mass to charge ratio (m/z). Since most fragments have a charge of +1, the m/z usually represents the molecular weight of the fragment. In the mass spectrum signal intensities are proportional to the levels of the individual charged species produced by electron impact. The signal of greatest intensity is referred to as the base peak and is assigned an arbitrary intensity of 100%. All other signals are expressed as a percentage of this. The mass spectroscopy used to determine the molecular weights. It is used to establish the distribution of substituents. The mass spectrum was recorded on QTOF-micro mass UK electron spray Ionization mass spectrometer. Waters triple core was the instrument used to determine the IR spectra of compound.

RESULTS

Antimicrobial activity

Table 1: Zone of inhibition in diameter on human pathogens.

Sl no.	**Concentration of (*µg/ml)***	***Zone of inhibition(mm)***	
		Staphylococcusaureus (gram +ve)	***Escherichia coli (gram – ve)***
1	**500**	**20nm**	**20nm**
2	**1000**	**24nm**	**30nm**
3	**2000**	**30nm**	**34nm**
4	control	_	_

Figure* 1: Zone of inhibition A) *Escherichia coli* B) *Staphylococcus aureus.

Antioxidant Activity

DPPH Scavenging assay of the synthesized chalcone derivative exhibit a significant percentage inhibition with respect to the standard Ascorbic acid. TheIC_{50}Value shows that**:**

Table 2: *In vitro* antioxidant study by DPPH assay.

Sample	Concentration (µg/ml)	Absorbance	Percentage inhibition (%)	IC 50
Standard (Ascorbic acid)	10	1.432±0.12	26.75	**13.11**
	20	0.502±0.08	74.32	
	30	0.226±0.05	88.43	
	40	0.099±0.01	94.93	
	50	0.102±0.01	94.78	
Test (MR)	10	1.268±0.029	43.297	**27.06**
	20	1.179±0.008	47.32	
	30	0.8497±0.06	62.02	
	40	0.76+0.0265	66.041	
	50	**0.6343+0.01**	**71.671**	

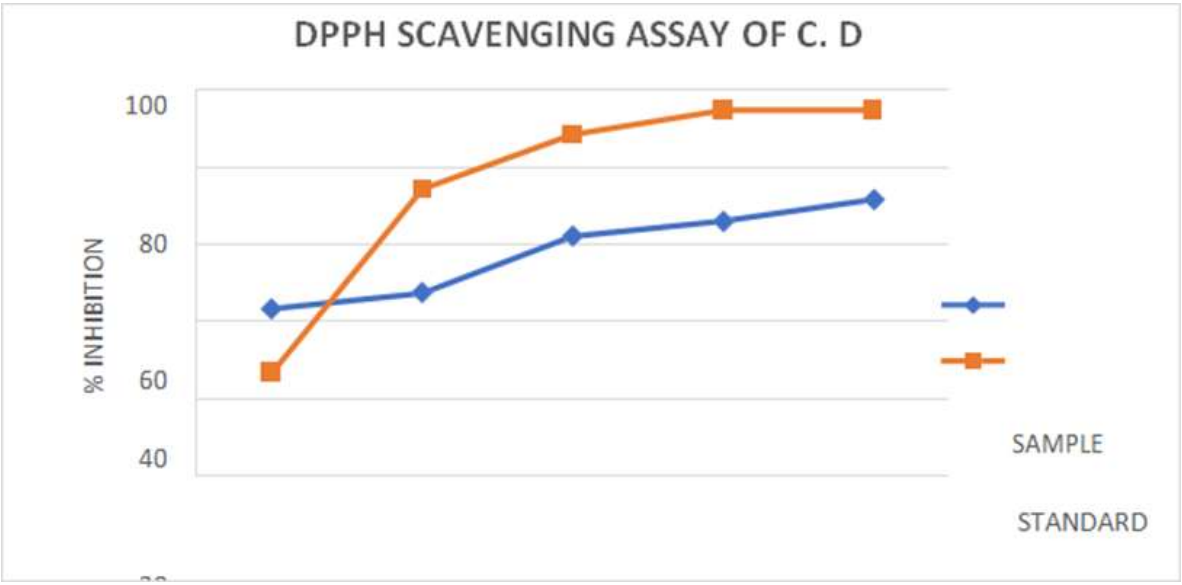

Figure 2: DPPH Scavenging Assay of Chalcone Derivative.

Spectral analysis Infrared Spectroscopy

Infraredspectroscopyisoneofthemostpowerfultechniqueswhichoffersthe possibility of chemical identification. C-H bending vibration in alkenes appeared in the region 1485- 1340 cm^{-1}. In aromatic hydrocarbons available C-H stretching absorption occur in the region 3050-3000 cm^{-1}. Carbon-carbon double bond stretching at 1650-1450cm^{-1} and C-H bending vibration at 900- 700 cm^{-1}. For aromatic compound, the most characteristic C=C stretching bands are of 1660 cm^{-1}, 1601 cm^{-1}, 1592 cm^{-1}. In the Nitro group attached with the aromatic ring, N-O stretching band shows in the region 1550-1475 cm^{-1}. N-H/C-H stretching bands show in the region between 3400-2600 cm^{-1} for Pyrazole derivatives. IR spectrum was recorded on Bruker, ATR, ALPHA II- FTIR using methanol as the solvent.

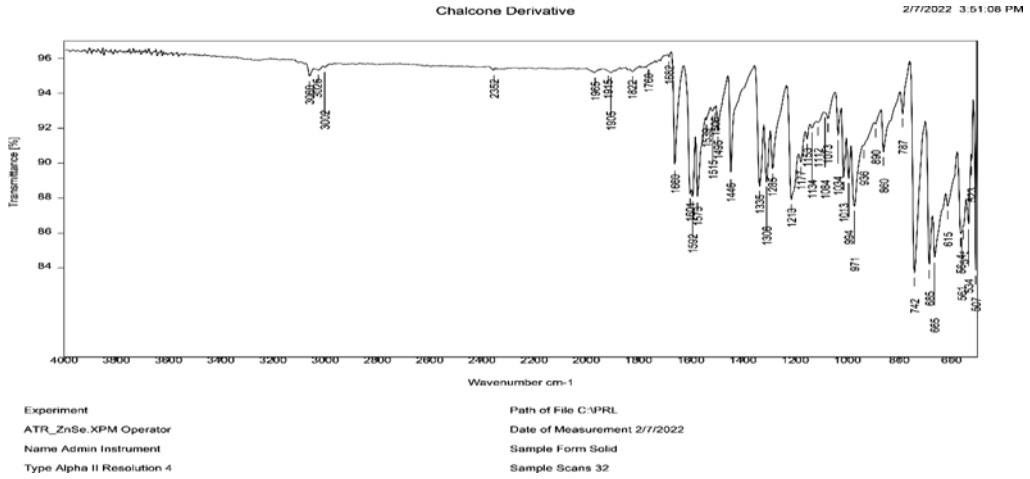

Figure 3: IR Spectroscopy of Chalcone derivative.

Mass Spectroscopy

Values-m/z ratio-316.13 and 316.01–chemical abundance 100% showing the molecular formula of the compound.

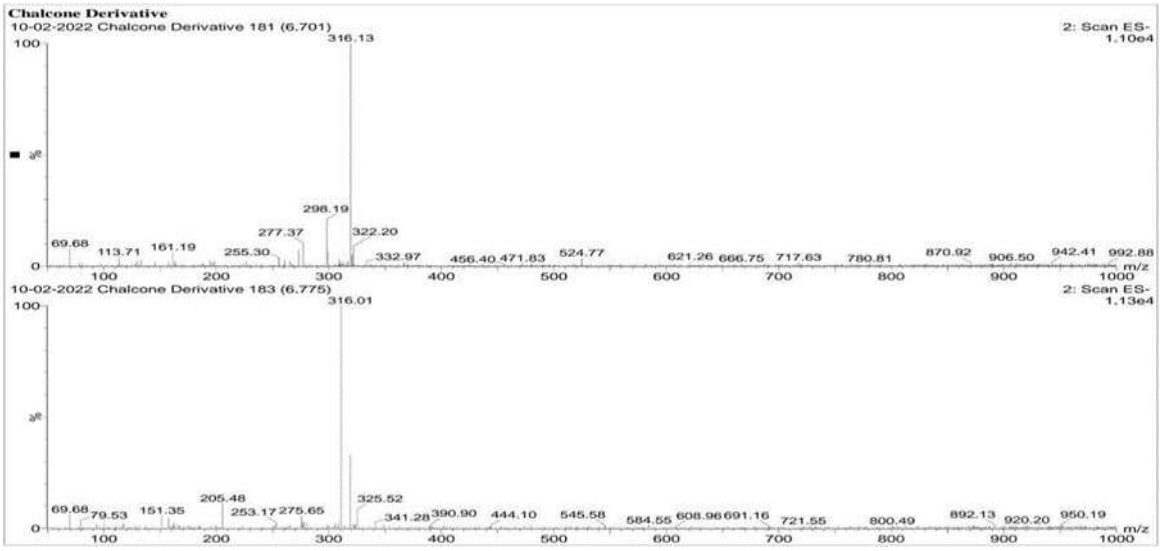

Figure 4: Mass Spectroscopy of Chalcone derivative.

DISCUSSION

Chalcones are attractive to synthetic chemists because they are easy to prepare, have a large number of replaceable hydrogens, there by having significant biological potential. Chalcones and their derivatives (carbocyclic as well as heterocyclic) exhibit a range of biological properties including anticancer, antimalarial, antioxidant, anti-inflammatory, and anti-tubercular activities. Their promising biological profile, along with their ease of synthetic manipulations, have triggered the design and development of new chalcone derivatives as well as their conjugates with active pharmacophore affording therapeutic templates targeting various diseases.

This review focuses on chalcones derivative of 1-(2,4-Dinitrophenyl)- 3,5-diphenyl 4,5- dihydro pyrazole synthesized by microwave assisted synthesis. Melting points were determined on electrothermal apparatus using open capillaries. The compound was tested for antibacterial activity in the agar plate method, the bacterial strains *Staphylococcus aureus and Escherichia coli* were used in this study. Antioxidant activity of the compound determined by DPPH scavenging assay using ascorbic acid as standard. IR spectroscopy has done with the instrument Bruker ATR, model Alpha II, sample directly placed on the crystal plate on the instrument and run in the FTIR finger print region. The mass spectroscopy used to determine the molecular weights with water triple core.

Although a great number of extra ctscontainingchal cones have been published in bibliographic sources, the potential of this scaffold could be deeply studied in many different areas in which almost nothing has been described. Among various classes of organic molecules, chalcones are suitable candidates for the design of new chemo sensors for targeted ones.

CONCLUSION

We used microwave-assisted synthesis to formulate the Chalcone derivative1-[2,4- Dinitrophenyl4,5-dihydropyrazole]. Dimethyl sulfoxide (DMSO) was used as the solvent to study the antimicrobial properties of Chalcone derivatives, using bacterial strains of Staphylococcus aureus and Escherichia coli. DPPH scavenging assay, the compound was determined to possess antioxidant activity. Using spectroscopic data (Ultraviolet/visible, infrared and mass spectroscopy), we were able to confirm the composition of the compound.

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