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SYNTHESIS AND EVALUATIVE STUDY OF CHALCONES AND THEIR DERIVATIVE FOR ANTI-OXIDANT ACTIVITY

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ABSTRACT

Background: Chalcones are among the leading categories of flavonoids across the entire kingdom of plant, The term chalcone is originated from the Greek name chalcos which means bronze. Chalcones possess a broad spectrum of biological activities including antioxidative, antibacterial, antihelmintic, amoebicidal, antiulcer, antiviral, insecticidal, antiprotozoal, anticancer, cytotoxic and immunosuppressive. Changes in their structure have offered a high degree of diversity that has proven useful for the development of new medicinal agents having improved potency and lesser toxicity and good pharmacological actions. In this study we're specifically focusing on the the anti-oxidant activity of chalcone by Synthesizing a suitable chalcone derivative moiety and evaluating for in-vitro anti-Oxidant activity by DPPH Assay and Spectral Studies such as FTIR and UV Spectroscopy.

Objectives:

- To synthesize chalcone and its derivatives
- To evaluate anti-Oxidant activity of chalcone and its derivatives
- To Conduct Spectral Studies of chalcone and its derivatives

1. INTRODUCTION

Chalcones, as compounds are the structures with multiple phenolic groups attached which based on location of attachment of certain compounds show a varied range of pharmacological activites such as anti-inflammatory, anti-oxidants, anti-bacterial etc.

Naturally, Chalcones are found in a wide variety of plants, ferns and higher plants, they're bio-precursor to a number of plant molecules and enzymes necessary for daily metabolic activities of the plant.[1]

In the following project work, we have synthesized 3 chalcone compounds viz. Chalcone, 4-Nitro chalcone and 4-Methoxy 4-Methyl chalcone. Confirmatory chemical tests and TLC were performed for each sample to confirm the presence of desired compound and carried out the testing of Anti-Oxidant activity by DPPH assay method.[2]

Following the synthesis and testing of the pharmacological activity, further spectral studies were carried out in the form of UV analysis and FTIR spectroscopy findings were reported.

There are few existing marketed formulations of chalcones in the form of methyl hesperidin chalcones mainly used as anti-oxidants and anti-inflammatory drugs. Metochalcone is such a formulation approved for its anti-choleretic activity.

Sofachalcone is approved for use in treatment of Ulcer as protective agent against H.pylori.[3]

Although many plant-based herbal medicines that are utilized in countries such as China and India have chalcones as their API and have been in use in traditional medicines for decades if not centuries. Eg. Liquochalcone is being utilized as Anti-Malarial drug in china. *Wang et al* have characterized over 300 chalcones that are used in Chinese traditional medicine.[4]

This entire project was aimed at giving us the better understanding of chalcones as pharmaceutical API's and contributing to the pre-existing knowledge about such compounds in hope of further exploration to absorb these molecules in the mainstream practice of healthcare.

1.1 *Chemistry*

The Physical Characteristics associated with Chalcones Include a Molecular Weight of 208.26 gm/mol, A Density of 1.071 g/cm3, A Melting Point that ranges from 550C to 570C, and a Boiling Point that Ranges from 345°C to 348°C. Chemical formula is Written as C15H12O. They have anti-oxidant activity which may be related to various functions and mechanism such as Free Radical Scavenging activities, Metal Ion Chelation etc. When Chalcones come in contact with a radical species they're immediately converted to stable phenoxy radicals because of hydroxyl groups of chalcones which are known to be highly reactive.[5]

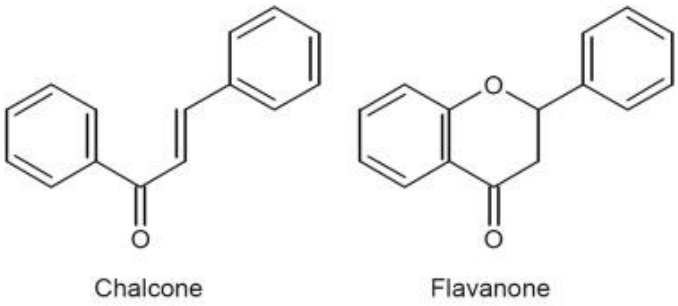
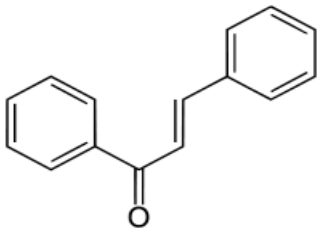
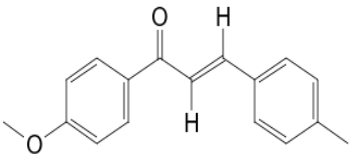


Figure 1: Structure of chalcones.

In the following project we have synthesized chalcone and its two derivatives viz. 4-Nitro Chalcone and 4-Methoxy 4-Methyl Chalcone with the properties as given in the following table.

Table 1: Chemistry of Chalcones.

Sr No	Name	Chemical Formula	Molecular Weight	Melting Point	Structure
1	Chalcone[6]	$C_{15}H_{12}O$	208.26 gm/mol	55^0C to 57^0C	
2	4-Methoxy 4-Methyl Chalcone[7]	$C_{17}H_{16}O_2$	255.32 gm/ mol	75^oC	

2. Literature Review

Sr No	Title	Author	Year of Publication	Description
1	Antimalarial activity of newly synthesized chalcone derivatives in vitro	Yadav N, Dixit SK, et al	**2012**	The Researchers in this article have synthesized Twenty-seven novel chalcone derivatives using Claisen-Schmidt condensation and their antimalarial activity against asexual blood stages of Plasmodium falciparum was determined.
2	In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallid.	Rahman MM, Islam MB, et al	**2015**	Here, authors aimed to investigate the antioxidant and free radical scavenging properties of methanolic extracts from *Tabebuia pallida* (*T. pallida*).

3	Chalcone derivatives: Promising starting points for drug design	Gomes M,Muratov E, et al	**2017**	In this review, authors summarize current methodological developments towards the design and synthesis of new chalcone derivatives and state-of-the-art medicinal chemistry strategies.
4	Natural chalcones in Chinese materia medica: Licorice	Wang D, Liang J et al	**2020**	This review aims to summarize structures and biological activities of chalcones from licorice. Authors hope that this work can provide a theoretical basis for the further studies of chalcones from licorice.
5	A concise review on synthesis, anti-inflammatory and antioxidant activities of chalcone	Singh D, Kaundal V, et al	**2022**	The purpose of the present review is to focus on the various methods of preparation of chalcones and derivatives and their anti-inflammatory and antioxidant potencies.
6	Synthesis, characterization and evaluation of antioxidant activities of some novel chalcones analogues	Lahsasni SA, Al Korbi FH, Aljaber	**2014**	Authors ahve described facile approaches and procedures that enable the synthesis of some new chalcone fatty acid esters. Furthermore, in order to understand the structural feature that makes a compound an effective lipophilic antioxidant, Authors have measured the radical-scavenging capacity of chalcone
7	Antioxidants Explained in Simple Terms	Atli Arnarson	**2019**	In this article the author has described the benefits and mechanism of action of anti-xoidants in human body and the need to incorporate anti-oxidants in diet
8	A solid phase synthesis of chalcones by Claisen-Schmidt condensations	Cheng MS, Li RS, Kenyon G	**2000**	In this Research Article in order to accelerate the development of relatively inexpensive antimalarials that are effective against chloroquine-resistant strains of Plasmodium falclparum, a methodology for the solid phase synthesis of chalcone analogues in reasonably high yields has been developed.
9	Simple and solvent free practical	Adnan D, Singh B,et al	**2020**	In this Research article an extremely simple, expeditious

	procedure for chalcones: An expeditious, mild and greener approach			and greener synthetic method for a variety of chalcone derivatives under mild and solvent free reaction conditions has been developed.
10	Review on Chalcone	Al Zubaidi Thanaa, Et al	**2019**	This is a review article describing the various methods of synthesis and applications of chalcones.
11	Colour Reactions of Chalcones and Their Mechanism	Sharma B, Gupta ASC	**2008**	This review according to authors state that chalcones are open analogues of flavonoids and give bright red to purple colours with different reagents which can be used to distinguish them from other flavonoids such as flavanones, flavones, aurones etc.
12	Synthesis of new AIEE-active chalcones for imaging of mitochondria in living cells and zebrafish in vivo	Luo H, Li N, Liu L, et al	**2021**	On the basis of authors previous research, they have successfully designed and synthesized eleven chalcones. Through an optical performance experiment, they confirmed that compounds had obvious AIEE properties. As these AIEE molecules had excellent fluorescence properties and a large Stokes shift, they studied their application in living cell imaging.
13	Experiment Video for Synthesis of 4-methoxy-4'-methylchalcone	Lab UO	**2020**	This is an video that shows in detail the process and mechanism of synthesis the of a particular chalcone derivative viz. 4-Mehoxy 4-Methyl Chalcone
14	Thin-layer chromatography of isomeric pairs of chalkones and flavanones	Sangwan NK, Verma BS, et al	**1985**	In this article, A fundamental two-dimensional thin-layer chromatographic technique using silica gel as adsorbent for the detection and identification of isomeric pairs of chalkones and flavanones is described. The proposed method is simple, inexpensive and needs no sophisticated instruments.
15	In-vitro evaluation of selected chalcones for	Gacche RN, Dhole NA, et al	**2008**	In this research article authors assayed Synthesized Chalcones for Anti-Oxidant activity and

	antioxidant activity			the SCs were found to be reactive towards DPPH radical and had considerable reducing ability. These findings suggest that these SCs can be considered as potential antioxidant agents which might be further explored for the design of lead antioxidant drug candidates.

3. NEED AND OBJECTIVE

Chalcones have been under study since more than a hundred years and have been evaluated for various possible pharmacological benefits however the its anti-oxidant activity however has been under special attention due to its effect. Previous Literature has shown that many chalcone derivates have excellent free-radical scavenging mechanism.[8]

Anti-oxidant activity was especially chosen as it is a gateway to a variety of different disorders which may be prevented by anti-oxidant drugs. Free radicals which are produced in body due to oxidative stress are linked to a variety of diseases. Therefore anti-oxidant supplements are necessary as they prevent cell degradation which happens due to oxidative stress, including cancer.[9]

Therefore anti-oxidant activity evaluation was chosen as measure of chalcones benefits as it may be a compatible opportunity in further expanding the study regarding chalcone compounds.

4. Project Plan of Work

Goal 1: To Synthesize Chalcones and its Derivatives				
Key Action Steps	**Timeline**	**Expected Outcome**	**Data Source and Evaluation Methodology**	**Comments**
Collection and selection of data and synthetic process.	***15 Days***	Having a finalized process to follow with understanding of materials required for further steps	Collection of research papers, journals, books, articles mentioning the synthesis of chalcones and its derivatives in detail.	Claisen-Schimdt condensation process was selected based on reliability and availability of resources
Collection of raw materials, chemicals, equipments for synthesis	20 Days	Having all the precursors and equipments ready to perform synthesis in a	Based on all the previous information collected, a list of all the chemicals and equipments was made	A collection of all equipments or their sources was obtained in given time.

		timely manner	and cross checked for their availability, few chemicals and analytical processes were outsourced as per their availability	
Synthesis of chalcones and its derivatives	5 Days	Successful synthesis of chalcone and its derivative with good yield	Experimental lab work carried out on basis of above collected information, with a step by step laid out process under supervision	Products were obtained with good yield

Goal 2 : To Perform Confirmatory Analysis of Obtained Products				
Key action steps	**Timeline**	**Expected outcome**	**Data source and evaluation methodology**	**Comments**
Thin layer chromatography	***1 Day***	Obtain a chromatograph in accordance with researched source material	A process was carried out by sourcing information through various articles, journals and E-books	A chromatograph with close values to the source material was obtained
Melting point determination	1 Day	Obtain Melting point in accordance to the researched source material	Previously reported melting point were found through help of articles, journals and E-Books, and carried out process via Melting point determination.	Melting point values with closeness to source material was found
UV analysis	2 Days	To obtain absorbance bands close to the source material	UV analysis was performed in the institute by comparing to the source material and absorbance was noted down	Standard absorbance values were obtained.
FTIR analysis	8 Days	To obtain FTIR graph depicting content of the product	FTIR spectroscopy was outsourced to an analysis lab and compared to data in various articles, journals and E-books.	A graph confirming the product was obtained.
Goal 3: To Check Anti-Oxidant Activity				
Key Action Steps	**Timeline**	**Expected Outcome**	**Data Source and Evaluation Methodology**	**Comments**
Collection and selection of data and process.	***3 Days***	Having a finalized process to follow with understanding of materials required for further steps	Collection of research papers, journals, books, articles mentioning the anti-oxidant activity of Chalcones	DPPH assay was finalized as a standard method to check anti-oxidant activity of chalcones
Collection of raw	3 Days	Having all the	DPPH, Methanol and	A collection of all

Goal 2 : To Perform Confirmatory Analysis of Obtained Products				
materials, chemicals, equipments for DPPH assay		precursors and equipments ready to perform DPPH assay in a timely manner	other samples were sourced and prepared.	equipments, Materials or their sources was obtained in given time.
DPPH assay	1 Day	Successful DPPH assay of chalcone and its derivative with good results	Experimental lab work carried out on basis of above collected information, with a step by step laid out process under supervision.	Good Values were obtained after final calculation

Goal 4: Preparation of Report				
Key Action Steps	**Timeline**	**Expected Outcome**	**Data Source and Evaluation Methodology**	**Comments**
Preparation of Report	***10 Days***	Have a detailed report of all the findings and processes	Typing of all data was done in accordance to the format provided	All the findings and processes were reported in a document.

5. MATERIALS AND METHODS

5.1 *Chemicals Required*

Table 2: Chemicals Required.

Sr.No	Chemical	Quantity
1	Distilled Water	2000 ml
2	Acetophenone	250 ml
3	Benzaldehyde	250 ml
4	NaOH soln	500 ml
5	4-Methyl benzaldehyde	50 gm
6	4-Methoxy Acetophenone	50 gm
7	DPPH	2mg
8	Methanol	1000 ml
9	Ice	250 gm

Figure 2: Chemicals used in synthesis.

5.2 *Glassware and Instrumentation Required*

Table 3: Glasswares/ Instruments used in project.

Sr No	Glassware/Instrument
1	Beakers
2	Measuring Cylinder (250 ml,100 ml,10 ml)
3	Volumetric Flasks (500 ml, 250 ml)
4	Vacuum Filtration Apparatus
5	Melting Point Determination Machine
6	Magnetic Stirrer
7	UV Spectrophotometer
8	FTIR Spectrophotometer
9	Miscellaneous (Filter Paper, Thermometer, Burner, Stand, Test tube, Test tube holder, Wire Gauze, Tripod Stand, Etc)

6. EXPERIMENTAL WORK

6.1 *Synthesis*

Two compounds viz Chalcone and 4-Methoxy 4-Methyl Chalcone were prepared by claisen-schimdt condensation reaction also known as cross-aldol condensation reaction.

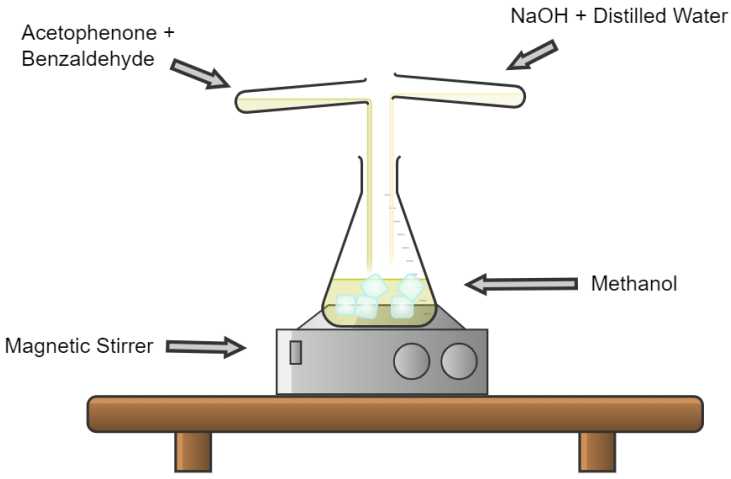


Figure 3: Synthesis Procedure.

6.1.1 *Chalcone*

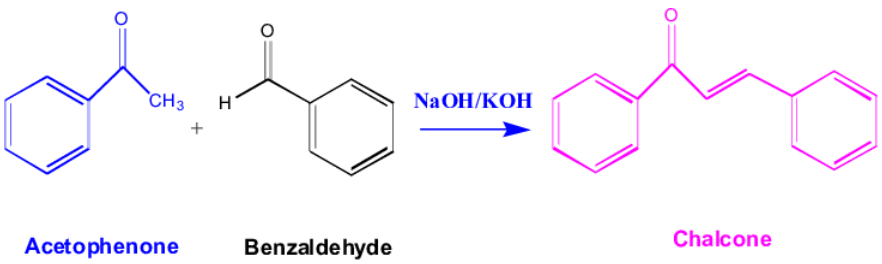


Figure 4: Synthesis of chalcone.

Process:- A compilation of latest work related to synthesis of chalcones were collected[10,11,12] and a clear and simple process was obtained from the information referred based on that information the following process was followed for Synthesis of Chalcone.

1. A solution of NaOH and distilled water was prepared.
2. A mixture of Acetophenone and Benzaldehyade in Methanol was added drop by drop in presence of ice to the previously mentioned solution.
3. The mixture was stirred on mechanical stirrer for 2 hours till solid residue was obtained
4. The Mixture was kept still at room temp for 24 hours, filtered and then recrystallized from methanol

The Yellow Residue obtained was dried and purified for further confirmation.

Theoretical Yield was calculated to be 22.5 gm. Practical yield was 12.3 gm 54% Yield was obtained.

Figure 5: Obtained Chalcone product with characteristic yellow color.

Confirmation was done via specified chemical tests for chalcones[13]

i.e Red coloration by addition of Sulphuric acid and subsequent discoloration by addition of Nitric acid.

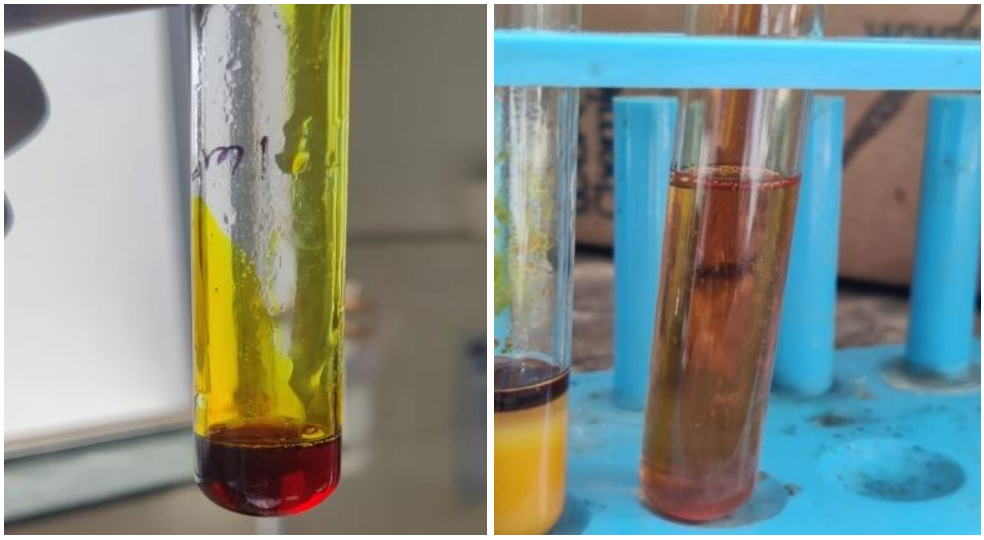


Figure 6: Chemical tests for chalcones.

6.1.2. *4-Methoxy 4-Methyl Chalcone*

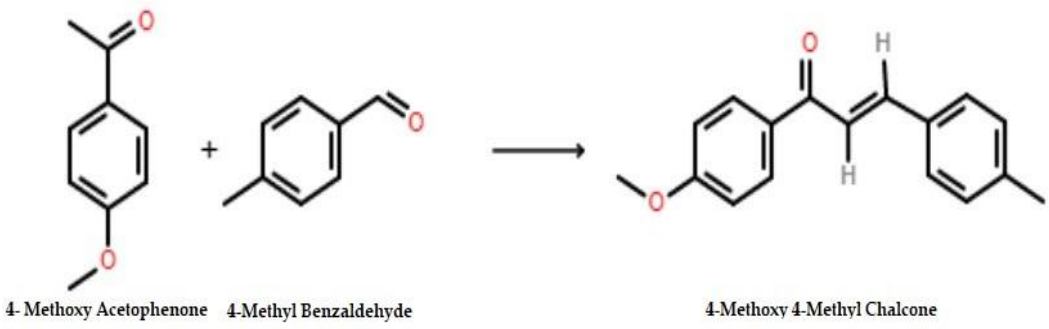


Figure 7: Synthesis of 4-Methoxy 4-Methyl Chalcone.

Process:- A compilation of work[14,15] related to the synthesis of 4-Methoxy 4-Methyl Chalcone was collected and a detailed step by step process was drawn based on which the synthesis was carried out.

1. A solution of NaOH with Distilled water was prepared
2. A solution of 4-Methoxy Acetophenone and 4-Methyl Benzaldehyde was prepared with Methanol in a volumetric flask
3. NaOH solution was added to the latter drop by drop in presence of ice and stirred on mechanical stirrer for two hours
4. The obtained mixture was kept still at room temperature for 24 hours, filtered and recrystallized from methanol.

Figure 8: Obtained Chalcone Derivative Product.

Theoretical yield obtained was calculated to be 1.26 Gm. Practical yield obtained was 0.75 Gm Percentage Yield was found to be 59%.

6.2 Thin Layer Chromatography (TLC) of Chalcones

For assessment of purity of the samples, TLC of the obtained products of chalcones were done by referring to the collected source material[16,17] by using Chloroform as an solvent and Benzene as mobile Phase the process was performed, Iodine chamber was prepared to be used as a visualizing agent.

Figure 9: TLC of Chalcones.

The Process is more clarified in the figure below.

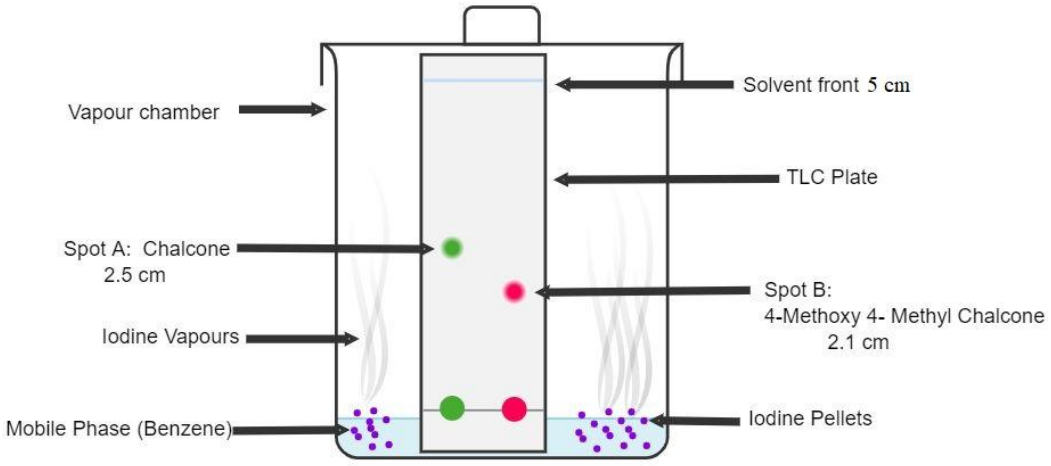


Figure 10: Process of TLC of Chalcones.

6.3 UV Analysis of Chalcones

UV Spectroscopy measurement of Chalcone and its derivative was done in order to characterize their absorbance in the UV region of 200-400 nm and check the wavelength of absorption to confirm the presence of chalcones and its derivative in the synthesized compound. The process was carried out by using methanol as baseline in the Shimadzu The observations are listed in the table below.

Table 4: UV Analysis of Chalcones.

Name	Band	Absorbance	Wavelenght
Chalcone	I	0.785	245
Chalcone	II	0.837	370
4-Methoxy 4-Methyl Chalcone	I	0.892	345
4-Methoxy 4-Methyl Chalcone	II	0.98	390

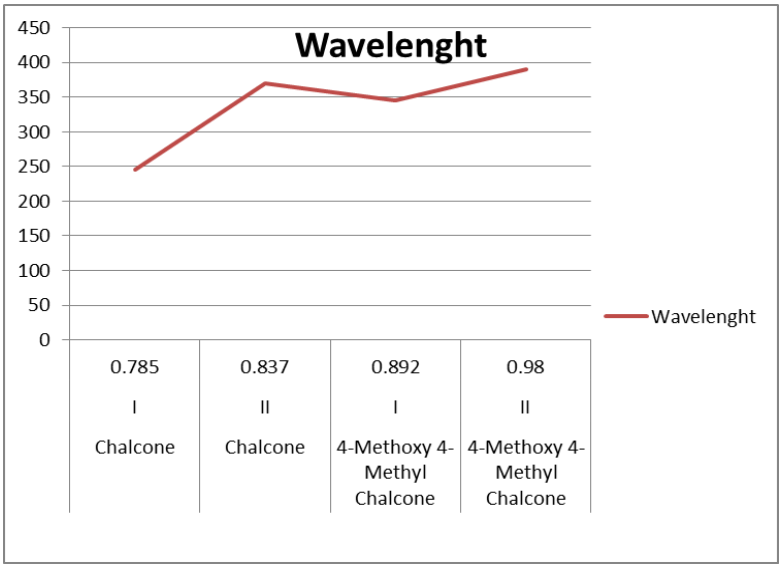


Figure 11: Graph – UV analysis of Chalcone.

6.4 FTIR Spectroscopy Analysis of Chalcones

FTIR spectrum of the synthesized chalcone was firstly carried out to confirm the functional group of the chalcone. The FTIR spectra's of chalcone derivative were recorded using Shimadzu FTIR spectrophotometer using ATR mode of operation and scanning was carried in the range of wavelength 4000-400cm^{-1} at room temperature.

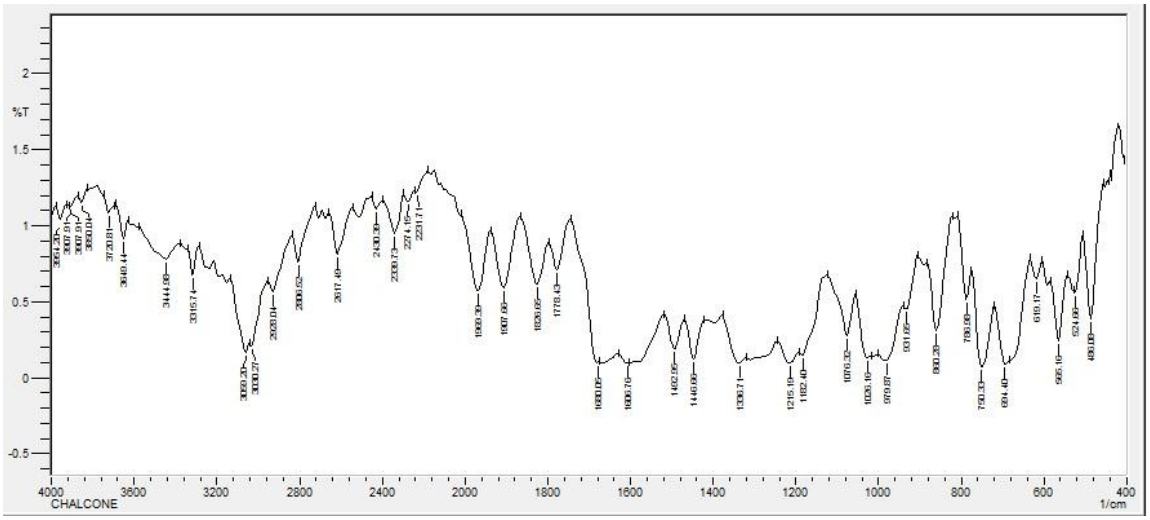


Figure 12: Graph – FTIR Analysis of Chalcone.

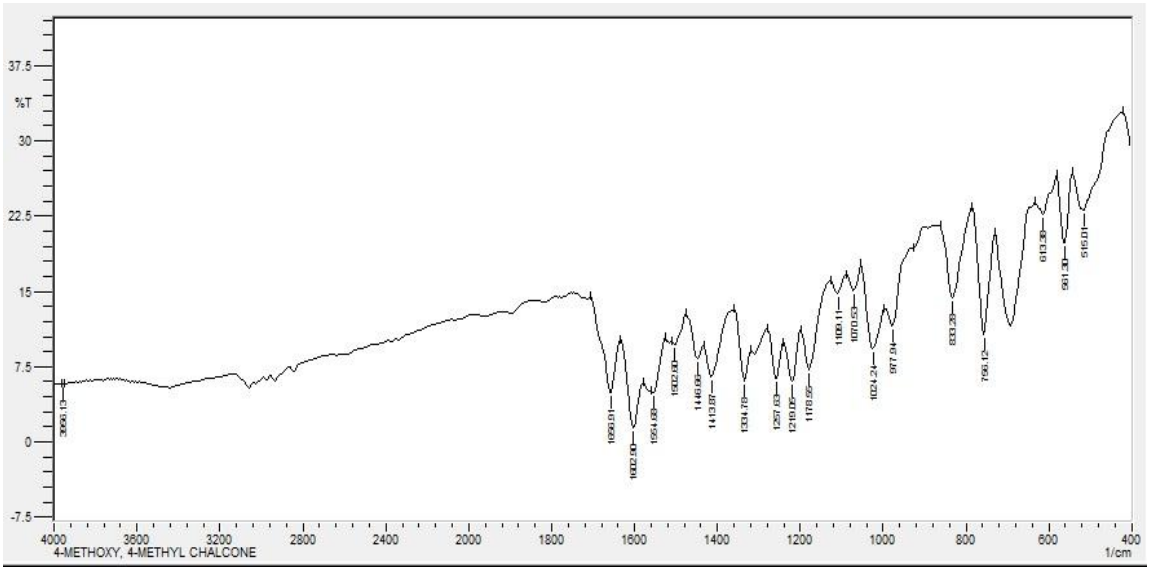


Figure 13: Graph – FTIR Analysis of 4-Methoxy 4-Methyl Chalcone.

7. DPPH assay of chalcones for anti-oxidant activity

Chalcone and obtained derivative was checked for anti-oxidant activity in-vitro by DPPH free-radical scavenging assay.[18]

Methanol + DPPH was used as Blank Control and Ascorbic Acid in the concentration of 500ug/ml and 1 mg/ml were used as Standard Chalcone and 4-Methoxy 4-Methyl Chalcone were assayed in the concentration of 2mg/ml and 4 mg/ml each.

Table 5: DPPH Assay of Chalcones.

Sample Name	Concerntration	Absorbance	% Inhibition
Ascorbic Acid	500 ug/ml	0.1	75%
Ascorbic Acid	1 mg/ml	0.4	54%
Blank		0.87	
Chalcone	2 mg/ml	0.343	47%
Chalcone	4 mg/ml	0.32	50%
4-Methoxy 4 -Methyl Chalcone	2 mg/ml	0.602	15.30%
4-Methoxy 4 -Methyl Chalcone	4 mg/ml	0.349	46.80%
		Std Deviation	0.160243149
		P Value	0.003659016

Basic chalcones showed good Anti-Oxidant Activity at 47% and 50% at 2mg/ml and 4 mg/ml concentrations respectively although it was less than that of standard. Chalcone at 4mg/ml showed the Best Anti-Oxidant activity compared to the rest 4-Methoxy 4 -Methyl Chalcone also showed good Anti-oxidant activity at 4 mg/ml concentration at 46.80% but poor activity at 15.30% concentration.

The results are represented in a more comprehensible manner graphically below.

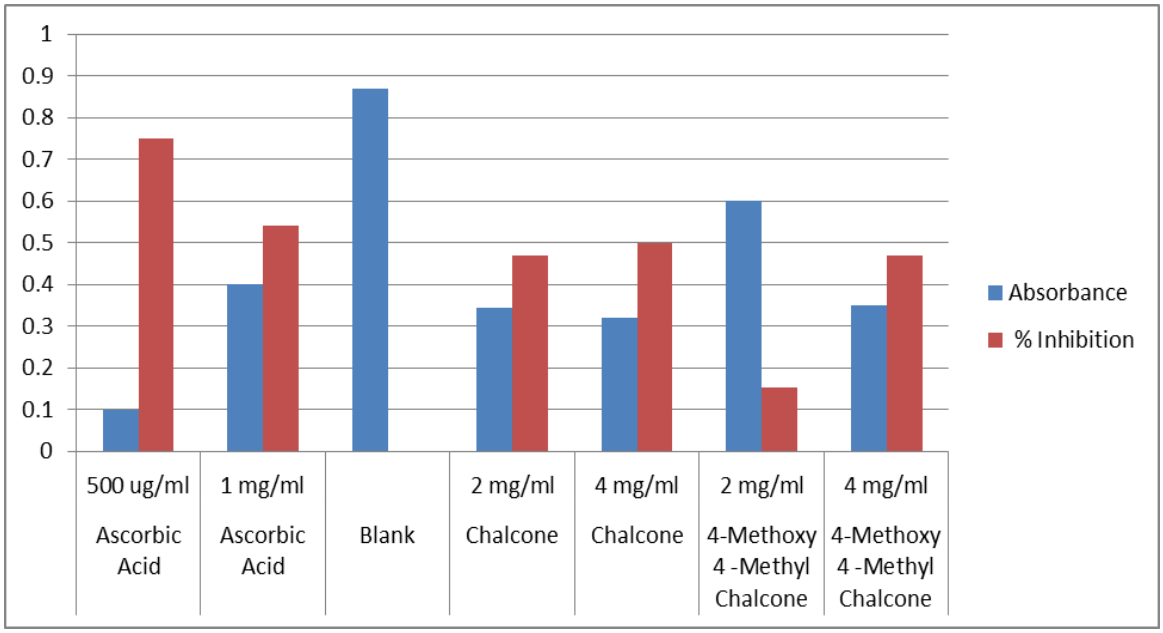


Figure 14: Graph – DPPH assay of Chalcones.

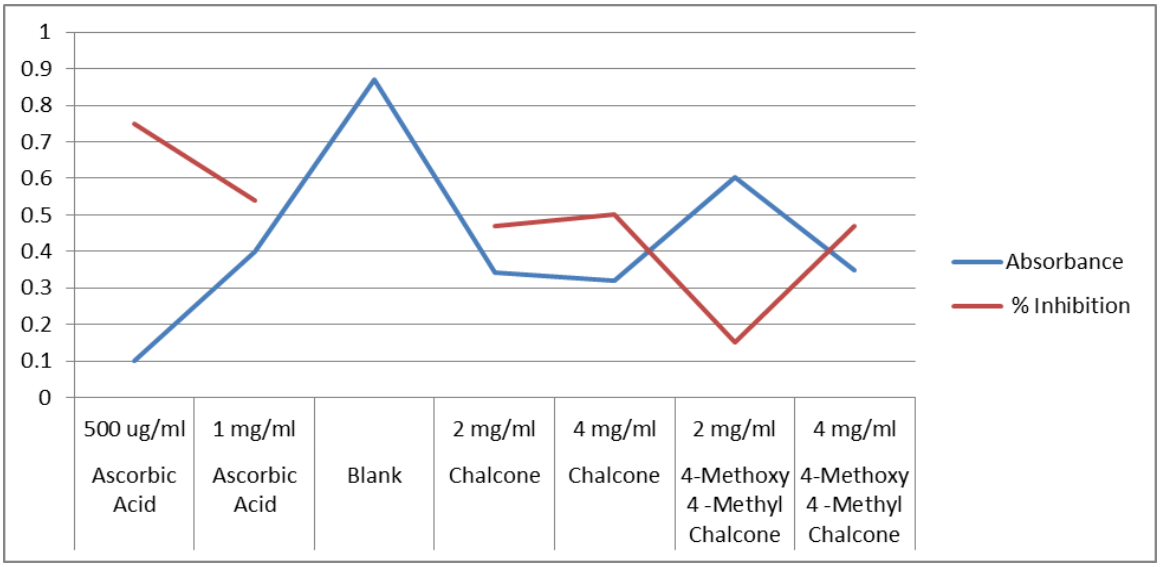


Figure 15: Graph – DPPH Assay of Chalcones.

From the above experiment it could be concluded that chalcone and the synthesized derivative have good anti-oxidant activity.

8. RESULT AND DISCUSSION

The compounds viz. chalcone and 4-methoxy 4-methyl chalcone were successfully synthesized by claisen-schimdt condensation reaction and characterized by Melting point determination, TLC, UV and FTIR spectroscopy. Further their Anti-Oxidant Activity was determined using DPPH free-radical scavenging assay and they were shown to have good anti-oxidant activity ranging from 15% to 50%.

9. CONCLUSION

From the above report it was concluded that chalcone and 4-methoxy 4-methyl chalcone have a number of pharmacological activities such as Anti-Inflammatory, Anti-Bacterial, Anti-Malarial, Anti-Viral, Anti-Leshminal, Analgesic, Anti-Pyretic, Insecticidal etc but in this project they were evaluated for their Anti-Oxidant activity which was characterized by DPPH free-radical scavenging assay.

Their characterization was also carried out by a number of methods. A few marketed formulations were also discussed. Going forward chalcones may prove to be a major API in many pharmaceutical formulations but still there is much research and study to be done in proper application of the knowledge gained about them.

LIST OF ABBREVIATIONS

Abbreviation	Meaning
TLC	THIN LAYER CHROMATOGRAPHY
DPPH	2,2-DIPHENYL-1-1PICRYL-HYDRAZYL HYDRATE
UV	ULTRA-VIOLET
FTIR	FOURIER TRANSFORM INFRARED
API	ACTIVE PHARMCEUTICAL INGREDIENT

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