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# SYNTHESIS AND EVALUATION STUDY OF ANTIOXIDANT ACTIVITIES OF CHALCONES ANDTHEIR DERIVATIVE

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## ABSTRACT

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. Furthermore, the role and contribution of different functional groups on the antioxidant activity of the synthesized chalcone derivatives are also probed and rationalized in terms of their electronic and structural effect.

**KEYWORDS:** Chalcones, anti-oxidant activity, DPPH Assay.

## 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 activities 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 outthe 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 ofhealthcare.

**Physical Characteristics**

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]

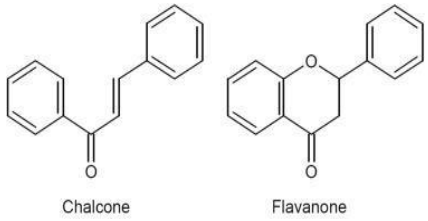
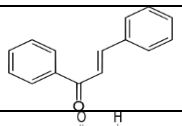
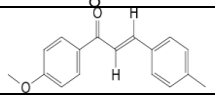



**Fig. 1: Structure of chalcones.**

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] | $C_{15}H_{12}O$ | 210.16 gm/mol | $56^0C$ |  |
| 2 | 4-Methoxy 4-Methyl Chalcone[7] | $C_{17}H_{16}O_2$ | 260.22 gm/ mol | $75^oC$ |  |

## 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 derivatives have excellent free-radical scavenging mechanism.[6,7,8] Anti-oxidant activities 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.

## MATERIALS AND METHODS

### 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 |

**Table 3: Glassware's / Instruments used in project.**

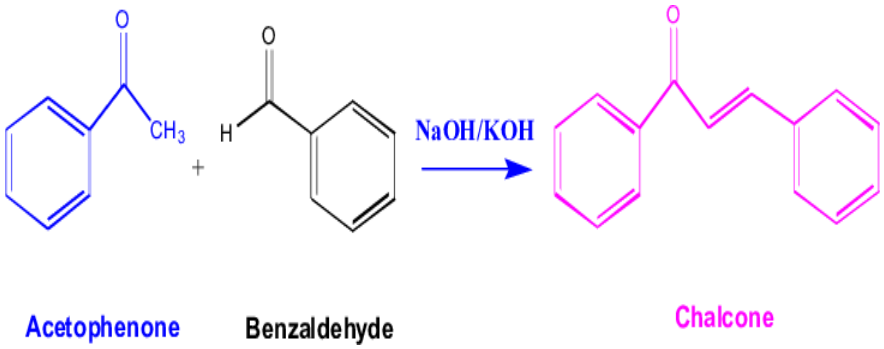
| Sr. No. | Glassware/Instrument |
|---|---|
| **1** | Beakers |
| **2** | Measuring Cylinder (250 ml,100 ml,10 ml) |
| **3** | Volumetric Flasks ( 500 ml, 250 ml) |
| **4** | Vacuum Filtration Apparatus |
| **5** | Melting Point Determination Machine |
| **6** | Magnetic Stirrer |
| **7** | UV Spectrophotometer |
| **8** | FTIR Spectrophotometer |

## EXPERIMENTAL WORK

### 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.

### Chalcone




**Synthesis of chalcone.**

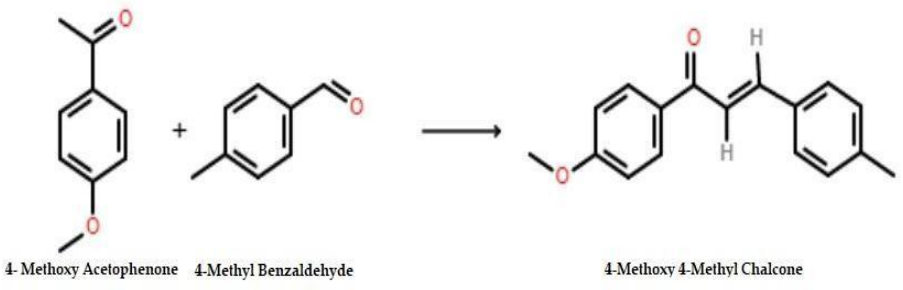
### Process

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.

- A solution of NaOH and distilled water was prepared.
- A mixture of Acetophenone and Benzaldehyade in Methanol was added drop by drop in presence of ice to the previously mentioned solution.
- The mixture was stirred on mechanical stirrer for 2 hours till solid residue was obtained

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. 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.




**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.

- A solution of NaOH with Distilled water was prepared.
- A solution of 4-Methoxy Acetophenone and 4-Methyl Benzaldehyde was prepared with Methanol in a volumetric flask.
- NaOH solution was added to the latter drop by drop in presence of ice and stirred on mechanical stirrer for two hours.
- The obtained mixture was kept still at room temperature for 24 hours, filtered and recrystallized from methanol. Theoretical yield obtained was calculated to be 1.26 gm. Practical yield obtained was 0.75 gm. Percentage Yield was found to be 59%.

**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.

**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 | 0.885 | 250 |
| Chalcone | II | 0.937 | 380 |
| 4-Methoxy 4-Methyl Chalcone | I | 0.992 | 350 |
| 4-Methoxy 4-Methyl Chalcone | II | 0.95 | 410 |

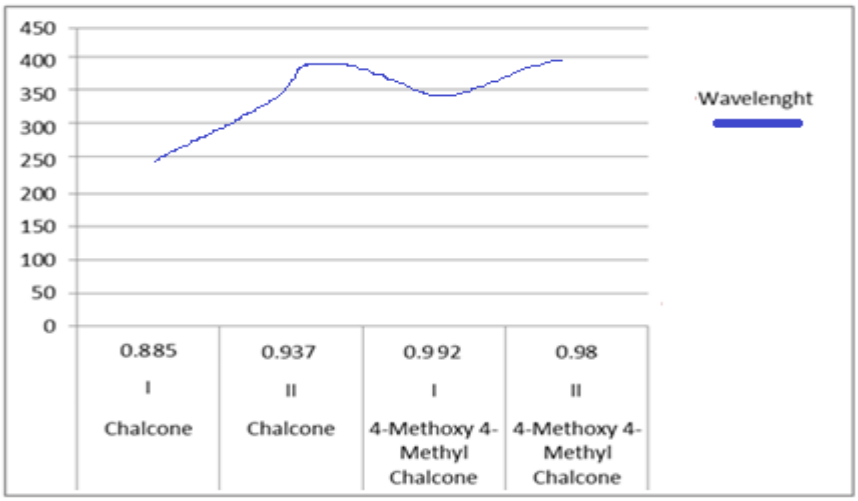



**Fig. 2: UV analysis of Chalcone.**

**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.

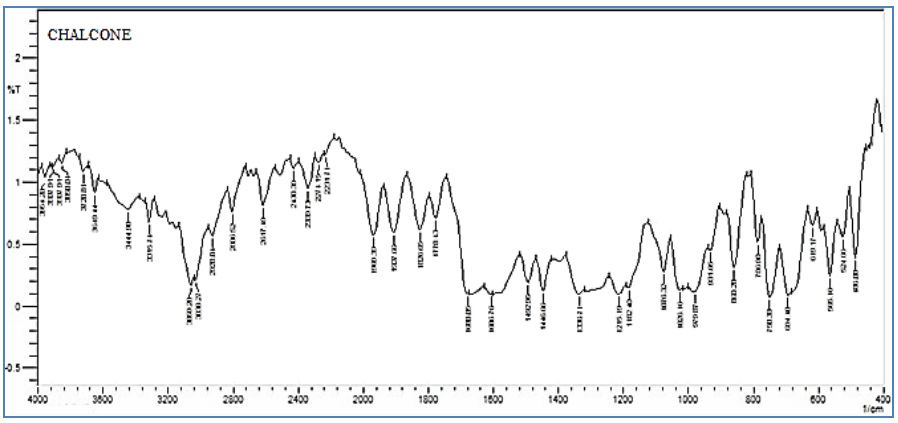



**Fig. 3: FTIR Analysis of Chalcone.**

**DPPH assay of chalcones for anti-oxidant activity[18]**

Chalcone and obtained derivative was checked for anti-oxidant activity in-vitro by DPPH free-radical scavenging assay. 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 | Concentration | 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 | 17.20% |
| 4-Methoxy 4 -Methyl Chalcone | 4 mg/ml | 0.349 | 42.65% |
| | | Std deviation | 0.160243149 |
| | | P Value | 0.003659016 |

Basic chalcones showed good Anti-Oxidant Activity at 45% 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 42.65 % but poor activity at 17.20% concentration. From the above experiment it could be concluded that chalcone and the synthesized derivative have good anti-oxidant activity.

## 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 17 % to 50%.

## 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, Antiviral, Analgesic, Antipyretic, 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.

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