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Synthesis and Free Radical Scavenging Activity of (2e,4e)-1,5-Diphenylpenta-2,4-Dien-1-One Using in Vitro Method

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ABSTRACT

Background: Chalcone synthesis is an important organic chemistry project with wide-ranging effects on drug discovery, medicinal chemistry, and material science, Chalcones serve as an intermediary product in the synthesis of a number of active substances, including aurones, isoflavonoids, and flavonoids

Objective: To learn more about the impacts of particular substituents, the relationships between the synthesized compounds' characteristics and molecular structures were examined. . The study also clarifies the mechanistic elements of the Claisen-Schmidt condensation processes that are necessary for the synthesis of chalcones

Methods: Using the Claisen-Schmidt condensation reaction, a series of chalcones (1a-1e) were effectively and synthesized by combining acetophenone as the ketone component and different aromatic aldehydes as the carbonyl donors. It was possible to create a library of chalcone derivatives with unique chemical properties by looking at the effects of various aldehydes on the final molecules. Their antioxidant activity was evaluated using the nitric oxide free radical scavenging assay in vitro. Characterization was performed using Fourier Transform Infrared (FTIR) spectroscopy and Thin Layer Chromatography (TLC).

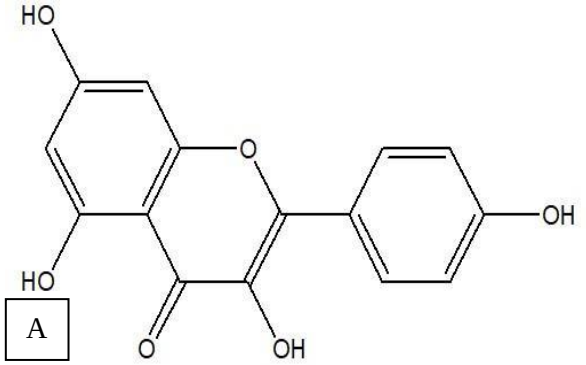
Results: Our newly synthesized compounds (a1-e1) chalcone derivatives show various degrees of antioxidant and free radical scavenging effect. The presence of electron-donating or withdrawing groups significantly affected the biological activity.

Conclusion: In this research, we have manufactured several compounds derived from chalcone by Claisen-Schmidt condensation and after testing them, they showed excellent effectiveness for antioxidant effect and free radical scavenging, However, further in vivo studies such as human pharmacological effect were needed to develop new antioxidant and free radical scavenging drugs in the future.

KAYWORDS: Chalcone, free radical, Claisen-Schmidt condensation, Nitric oxide.

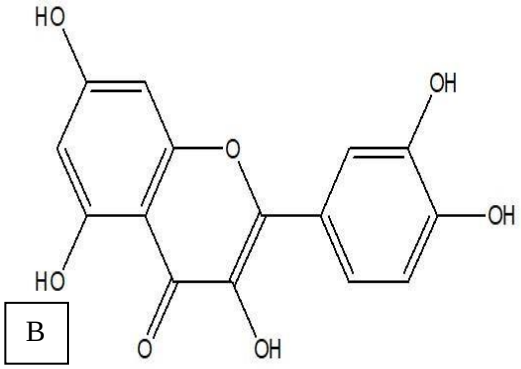
INTRODUCTION

Chalcone synthesis is an important organic chemistry project with wide-ranging effects on drug discovery, medicinal chemistry, and material science. Using the Claisen-Schmidt condensation reaction, a series of chalcones (1a-1e) were effectively and synthesized by combining acetophenone as the ketone component and different aromatic aldehydes as the carbonyl donors. The study of designing and creating pharmaceutical agents or medicines that will help people is known as medicinal chemistry. The biological characteristics and the quantitative structure-activity relationship of currently available medications are also studied [1,2] .Naturally occurring secondary metabolites of plants, chalcones are members of the flavonoid family, which is often found in inedible plants, especially fruits and vegetables. "Side-tracked" or secondary metabolites in plants are these biological products [3-6]. By reacting aryl ketones with aromatic aldehydes and adding the proper condensing agents, chalcones are created synthetically. Chalcones are chemically 1,3-diphenyl-2-propen-1-ones, which are based on two aryl (aromatic/heterocyclic) moieties connected by an α, β-unsaturated carbonyl group. For the ketone group to synthesise chalcones, at least two α hydrogens are required. Chalcones serve as an intermediary product in the synthesis of a number of active substances, including aurones, isoflavonoids, and flavonoids [6-9].



A

3,5,7-trihydroxy-2-(4-hydroxyphenyl)chromen-4-one



B

2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one

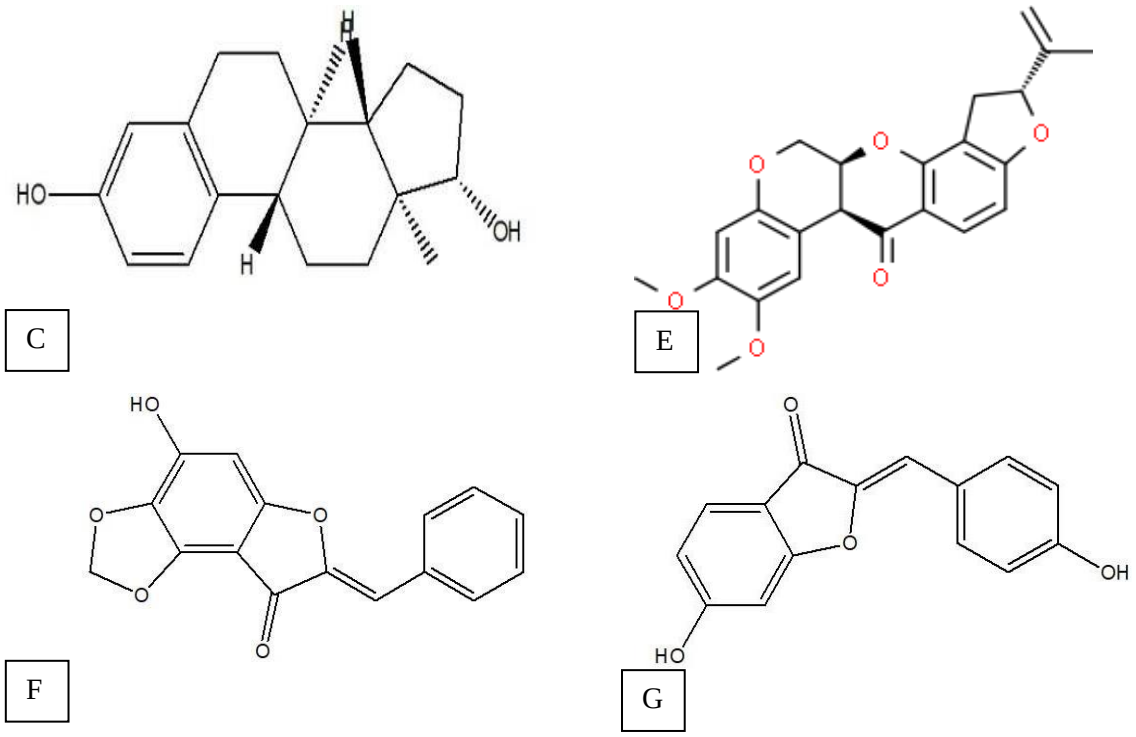


Figure 1 Example of flavonoids A)Kaempferol, B) Quercetin C) Estradiol D) Rotenoids E)Examples of aurones Cephalocerone F) Hispidol

Chalcones, in general have a variety of properties, including insecticidal, antibacterial, antifungal, anti-inflammatory, anti-cancer, anti-malarial, and antiviral properties see figure 1. Chalcones can cause irritation of the skin, eyes, and respiratory system, among other side effects. produced not only using the standard approach but also using the green route synthesis, microwave assistance, and grinding methods [10-12].

Chalcones from natural sources

Chalcones have garnered significant academic attention for decades and are the building blocks of numerous biologically intriguing chemicals derived from natural sources. To what extent have natural chalcones been isolated and their structures clarified? The extent to which the net is cast determines the response to this query [3]. Similar to several articles, the term "chalcone" broadly describes substances that have an α, β-unsaturated ketone system. Because of its great structural variability, the chalcone family can be broadly divided into two groups: hybrid chalcones, which have a core scaffold of 1, 3-diaryl2-propen-1-one, and simple/classical chalcones. Bi chalcones have two chalcone moieties in one structure, such as rhus chalcone from Rhus pyroides.

The chalcone family has garnered significant attention due to its wide range of intriguing biological functions in addition to its synthetic and biosynthetic aspects.

Chalcones have been used medicinally for thousands of years. During that time, several medical conditions like diabetes, cancer, and inflammation were treated with plants and herbs [14].

MATERIALS AND METHODS:

Chemicals Required: Acetophenone, benzaldehyde, 4-hydroxy benzaldehyde, 4-bromo benzaldehyde,4-hydroxy-3-methoxy benzaldehyde, Cinnamaldehyde, Ethanol, Methanol, Sodium hydroxide pellets, deionized water, Iodine, Benzene, Chloroform.

Instruments: Magnetic stirrer, Hot air oven, Vacuum filter, Fourier transform infrared (FTIR) spectrophotometer, U.V. chamber/cabinet, Bunsen burner, Tripod stand.

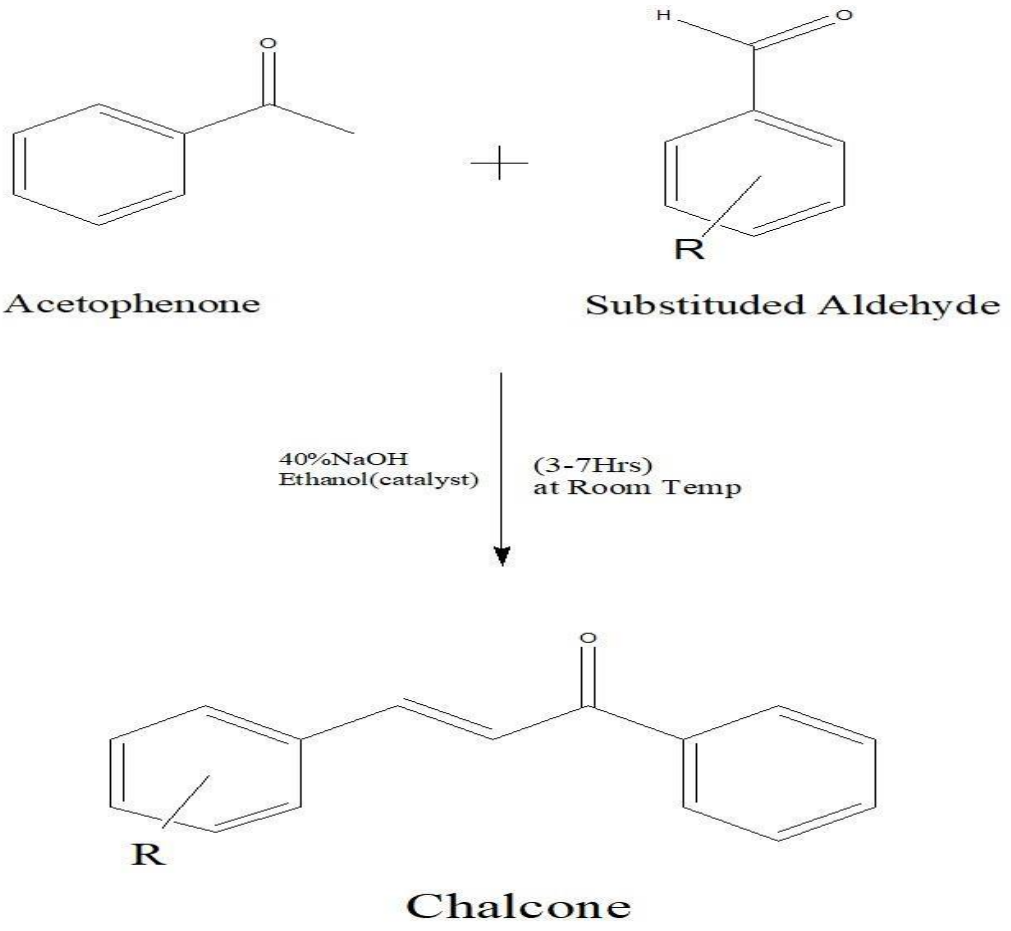
Glassware: Beaker, Round bottom flask, Test tubes, Petri plates, watch glass, glass rod, China dish, Measuring cylinder, Capillary tubes, Ignition tubes, Spatula, Thermometer and TLC plates.

Preparation of 40% NaOH Solution:

4g of sodium hydroxide pellets should be weighed and kept in a beaker. 10 ml of deionised water should be added, and the mixture should be constantly stirred until the sodium hydroxide pellets dissolve completely [15].

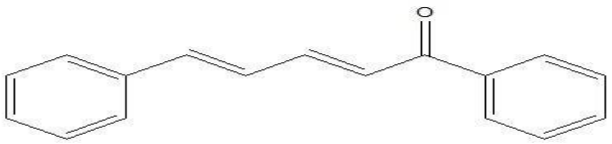
The general procedure involved in the synthesis of chalcones (1a -1e)

Equimolar amounts of ketone and aldehyde, precisely weighed, should be added to a round- bottom flask, thoroughly mixed, and then mixed with 40% NaOH solution and 15 milliliters of ethanol. Using a magnetic stirrer, mix the solution mixture at room temperature for three to seven hours. Verify the results with TLC, filter, and then recrystallize the product.



1a. R=H Benzaldehyde
1b. R=4-OH 4-hydroxybenzaldehyde
1c. R=4-br 4-bromobenzaldehyde
1d. R=4-OH , 3-OCH_3 4-hydroxy-3-methoxybenzaldehyde

1e. Cinnamaldehyde



(2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one

Figure 2 Schema of synthesis

Nitric Oxide Free Radical Scavenging Method

General Procedure:

At physiological pH, sodium nitroprusside solution produced nitric oxide radical. Two millilitres (ml) of sodium nitroprusside (10 mm) combined with one millilitre (ml) each of phosphate buffer saline (PH 7.4) and test/standard. Then for 150 minutes, the mixture was

incubated at 25°C.

To complete diazotization, add 1 ml of sulphuric acid reagent to 0.5 ml of the incubated solution, stir thoroughly, and let stand for 5 minutes.

After that, 1 millilitre of NEDD was added, stirred, and allowed to stand in diffused light for 30 minutes to generate a pink chromophore. At 546 nm, the absorbance of the control, test, and standard solutions was measured compared to the blank solutions [16].

Physiological pH:

In the absence of pathological states, the PH of the human body ranges between 7.35-7.45, Average is 7.4.

Diazotization:

The chemical process used in converting a primary aromatic amine into the corresponding diazonium has of the amine.

A. **Preparation of solutions:**

Sodium nitro prusside solution preparation: Dissolve 0.13gm of sodium nitroprusside in 50ml of distilled water.

Preparation 1ml of sulphanilic acid: Dissolve 20ml of glacial acetic acid in 100ml of distilled water & then add 0.33gm of sulphanilic acid to the solution. Stir it continuously until dissolved.

Preparation of NEDD solution: Dissolve 0.05gm of NEDD reagent in 50ml distilled water.

Dissolve 0.05gm of sulphanilic acid in 50ml of distilled water.

Dissolve 2.5 ml of orthophosphoric acid in 50 ml of distilled water. Mix all of those 3 solutions.

Preparation of phosphate buffer solution: Dissolve 3.1gm of sodium dihydrogen phosphate & 10.9 gm of sodium hydrogen phosphate in 1liter of distilled water.

Control preparation:

2ml of sodium nitroprusside solution + 0.5ml of 7.4ph buffer solution.

Kept it in the incubator for 150 minutes at 25ºC. Take 1ml of sulphonic acid reagent. Kept it aside for 5 minutes. Again, add 1 ml of NEDD solutions. Kept it aside for 30 minutes.

Sample/ standard preparation

•To take 2ml of 10mm sodium nitroprusside, 1ml of saline phosphate buffer and 1ml of different concentrations of sample/standard solution kept

incubated at 25°C for 150 minutes.

- After incubation 0.5ml of reaction mixture was mixed with 1ml of sulfonilic acid reagent and allowed to stand for 5 minutes to complete diazotization.
- After this a further 1ml of NEDD solution was added and mixed was allowed to stand for 30 minutes at 25°C.
- The concentration of nitrite was assayed at 546 nm and it was calculated with the control absorbance of the standard nitrite solution.

Synthesis method

Synthesis of (E)-3-(phenyl)-1-phenyl prop-2-en-1-one (compound 1a)

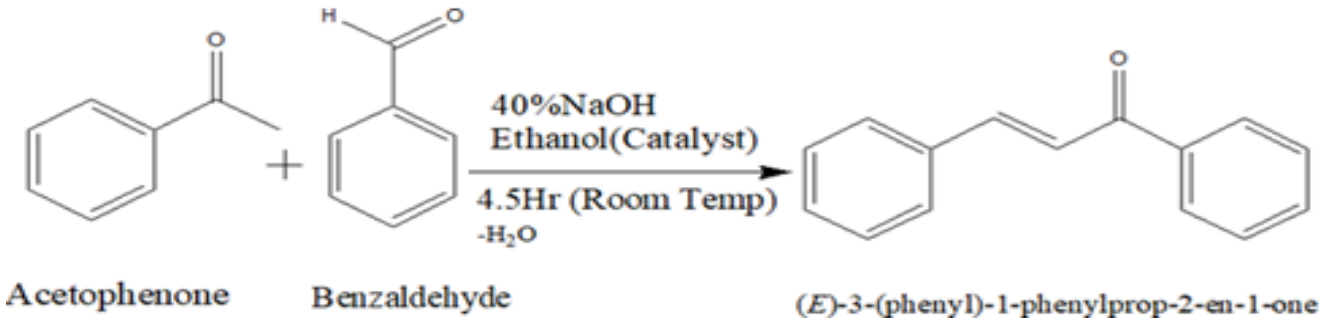


Figure 3 synthesis scheme :Molecular formula of benzaldehyde : C6H6CHO; Molecular formula of acetophenone : C8H8O; Molecular formula of 1a : C15H14O; Molecular weight of benzaldehyde : 106.12 g/mol; Molecular weight of acetophenone : 120.4 g/mol; Molecular weight of 1a : 210 g/mol ; Melting point (MP) of 1a chalcone : 95°C; Solubility : Benzene

Procedure for synthesis compound 1a:

Accurately weigh 0.1 moles of acetophenone and 0.1 moles of benzaldehyde. Transfer the mixture into a round-bottom flask, mix well, and add 15 millilitres of ethanol and 4 grams of 40% NaOH solution (10 millilitres in 10 millilitres of distilled water). Using a magnetic stirrer, mix the solution mixture at room temperature for four and a half hours. Verify the results with TLC, filter, and then recrystallize the product [10].

Synthesis of (E)-3-(4-hydroxyphenyl)-1-phenylprop-2-en-one (compound 1b)

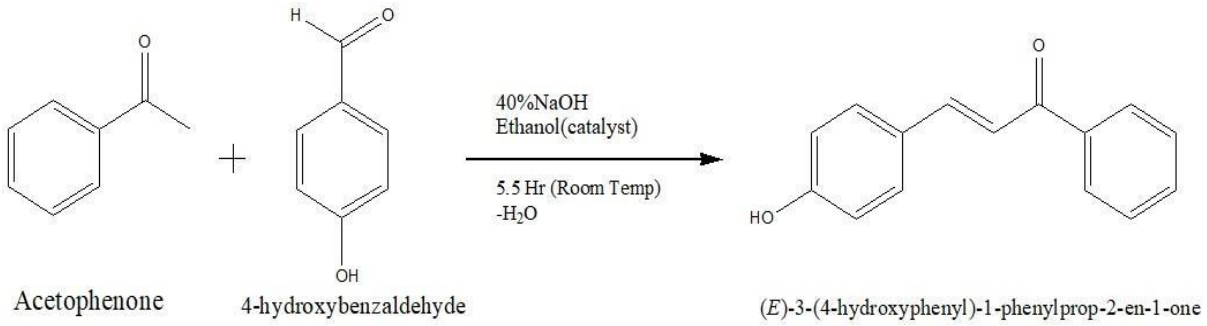


Figure 4 synthesis scheme: Molecular formula of 4-hydroxybenzaldehyde : C7H6O2; Molecular weight of 4-hdroxybenzaldehyde : 122.123 g/mol; Molecular weight of acetophenone : 120.4 g/mol; Molecular formula of 1b : C15H15O2 Molecular weight of 1b : 357 g/mol; Melting point of 1b chalcone : 110 0C; Solubility : Benzene

Procedure for compound synthesis 1b:

Accurately weigh 0.1 moles of acetophenone and 0.1 moles of 4-hydroxy benzaldehyde. Transfer both mixtures into a round-bottom flask, mix well, and add 15 millilitres of ethanol and 4 grammes of 40% NaOH solution (10 millilitres in 10 millilitres of distilled water). Using a magnetic stirrer, mix the solution mixture at room temperature for five and a half hours. Verify the results with TLC, filter, and then recrystallise the product [16, 17].

Synthesis of (E)-3-(4-bromophenyl)-1-phenylprop-2-en-1-one (compound 1c)

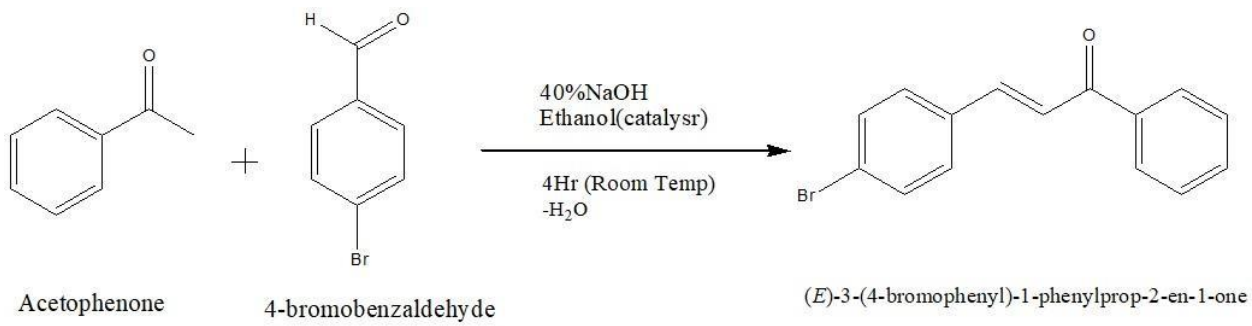


Figure 5 synthesis scheme: Molecular formula of 4-bromobenzaldehyde : C7H5BrO; Molecular weight of 4-bromobenzaldehyde : 185.02 g/mol; Molecular weight of acetophenone : 20.4 g/mol; Molecular formula of 1c : C15H14OBr;Molecular weight of 1c : 289 g/mol; Melting point of 1c chalcone : 110 0C; Solubility : Benzene

Procedure for compound (1c)

Accurately weigh 0.1 mole of acetophenone and 0.1 mole of 4-bromobenzaldehyde. Transfer the mixture into a round-bottom flask, stir well, and add 15ml of ethanol and 4g of 40% NaOH solution. After four hours of room temperature stirring with a magnetic stirrer, TLC confirmation is obtained, followed by filtering and product recrystallization [18].

***Synthesis of (E)-3-(4-hydroxy-3-methoxyphenyl)-1-phenylprop-2-en-1-one* (Compound 1d)**

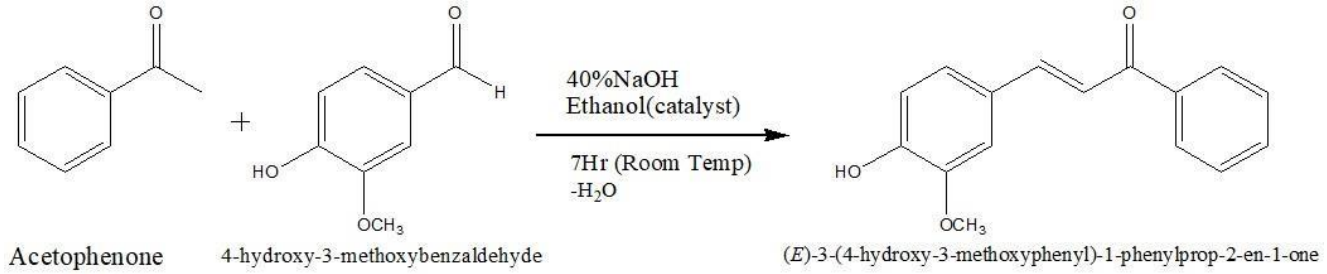


Figure 6 synthesis scheme: molecular formula of 4-hydroxy-3-methoxybenzaldehyde **:** C8H8O3; Molecular weight of 4-hydroxy-3-methoxybenzaldehyde **:** 152.15 g/mol; Molecular weight of acetophenone **:** 120.4 g/mol; Molecular formula of 1d **:** C16H12O3; Molecular weight of 1d **:** 252 g/mol; Melting point of 1d chalcone **:** 90 ^{0}C ; Solubility **:** Benzene

Procedure for synthesis compound 1d::

Accurately weigh 0.1 mole of acetophenone and 0.1 mole of 4-hydroxy, 3-methoxy benzaldehyde. Transfer both mixtures into a round-bottom flask, stir well, and add 15ml of ethanol and 4g of 40% NaOH solution. Using a magnetic stirrer, mix the solution combination at room temperature for seven hours. Verify the results with TLC, filter, and then recrystallise the product [19].

Synthesis of (2E,4E)-1,5-diphenylpenta-2,4-dien-1-one (1e)

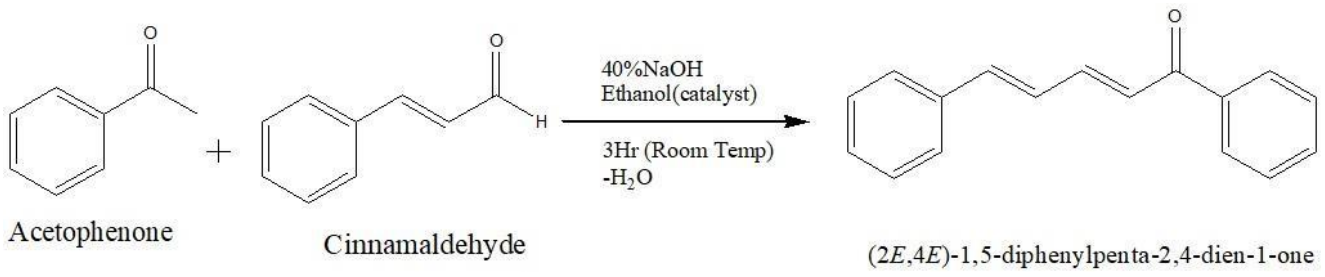


Figure 7 synthesis scheme: molecular formula of cinnamaldehyde **:** C_9H_8O Molecular weight of cinnamaldehyde **:** 132.16 g/mol; Molecular formula of 1e **:** $C_{17}H_{16}O$; Molecular weight of 1e **:** 236 g/mol Melting point of 1e chalcone **:** 120 ^{0}C; Solubility **:** Benzene

Procedure for synthesis compound 1e:

Precisely weigh out 0.1 moles of acetophenone and 0.1 moles of cinnamaldehyde. Transfer them into a round-bottom flask, properly mix, and then add 15ml of ethanol and 4g of 40% NaOH solution (10ml in 10ml of distilled water). Using a magnetic stirrer, mix the solution combination at room temperature for three hours. Verify the results with TLC, filter, and then recrystallise the product [20].

RESULT

Synthesis of Chalcones (1a-1e): The Claisen-Schmidt condensation reaction successfully yielded a series of chalcones (1a-1e) using acetophenone as the ketone component and various substituted aromatic aldehydes as the carbonyl donors. The reaction was carried out under alkaline conditions using 40% NaOH in ethanol as the reaction medium. The completion of the reaction was monitored using Thin Layer Chromatography (TLC), and the obtained crude products were purified through

recrystallization. The percentage yields of the synthesized chalcones ranged from 65% to 85% Figure 8 and table 4 show TLC result.

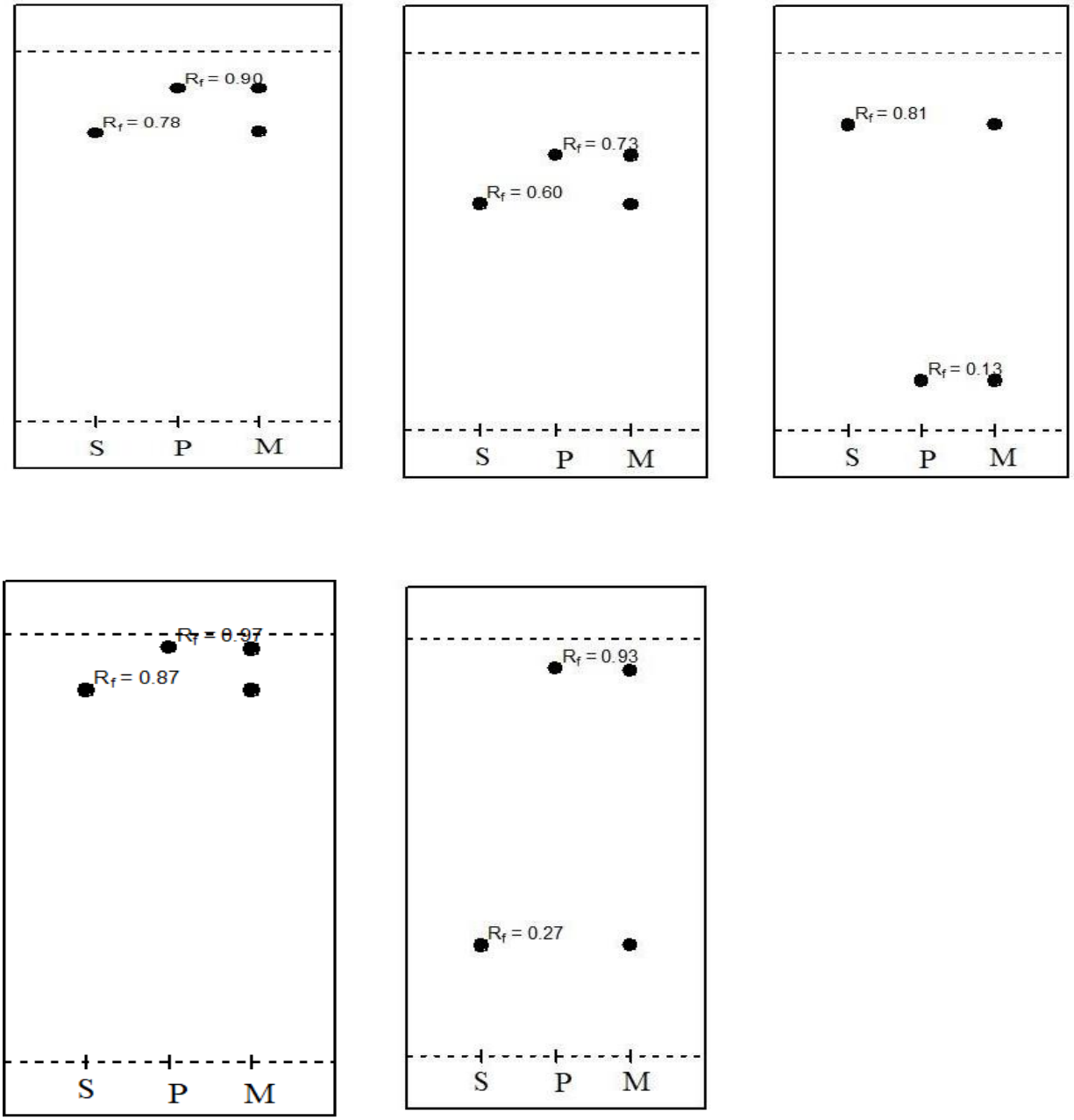


Figure 8 TLC plate A) TLC of compound Benzaldehyde Chalcone 1a , B) TLC of 4-hydroxybenzaldehyde chalcon , C) TLC of 4-bromobenzaldehyde chalcone, D) TLC of 4-hydroxy3- methoxybenzaldhyde, E) TLC of cinnamaldehyde chalcone

Table 1 TLC Values for compounds					
compound	compound a 1	1B	1C	1D	1 E
Distance traveled by the solvent	4.2 cm	3cm	3.7cm	4.1cm	4.8cm
Distance traveled by sample (solute)	3.3 cm	2cm	3cm	3.6cm	1.3cm
Distance traveled by product (solute)	3.8cm	2cm	0.5cm	4cm	4.5cm
Rf value of the sample	0.78	0.6	0.81	0.0.87	0.27
Rf value of the product	0.90	0.73	0.13	0.97	0.93
Practical yield		7.56	12.48	12.84	4.31
% yield		63.6	86.18	76.5	49.42

Spectral Characterization:

Fourier Transform Infrared (FTIR) spectroscopy confirmed the structural identity of the synthesized chalcones. The characteristic absorption bands were observed for the conjugated carbonyl (C=O) stretching at ~1650 cm-1 and C=C stretching at ~1580 cm-1. Additional bands corresponding to hydroxyl (-OH), methoxy (-OCH3), and bromo (-Br) functional groups were present in the spectra of substituted chalcones.

Spectral values compound synthesis (1 b) FTIR spectra: -C=O stretching value is 1895.15, -CH=CH- stretching value is 3229.68

Spectral values for compound (1c) FTIR spectra: -C=O stretching value is 1912.33, -CH=CH- stretching value is 3053.78

Spectral values compound *(1d* FTIR spectra: -C=O stretching value is 1907.05, -CH=CH- stretching value is 3088.99.

Spectral values for compound (1e) FTIR spectra: -C=O stretching value is 1970.74, -CH=CH- stretching value is 3061.97.

Nitric Oxide Free Radical Scavenging Activity: The antioxidant potential of the synthesized chalcones (1a-1e) was evaluated using the nitric oxide (NO) free radical scavenging assay. The results were compared with ascorbic acid, which served as the standard antioxidant see table 2

TABLE 2 Nitric oxide free radical scavenging activity results of chalcones (1a-1e) and Standard

S. No.	Concentration (µg/ml)	Percentage of inhibition (%)					
		1a	1b	1c	1d	1e	std
1	2	37.25	39.45	38.23	33.56	28.89	48.74
2	4	41.56	42.43	40.45	36.54	30.56	52.74
3	6	47.67	45.54	43.56	39.07	32.78	58.81
4	8	50.76	48.63	46.23	42.45	35.34	62.84
5	10	54.84	50.34	48.56	44.46	38.78	66.86

The nitric oxide scavenging activity of the synthesized compounds increased in a dose-dependent manner. Among the tested chalcones, compound 1d (4-hydroxy-3-methoxy substituted chalcone) exhibited the highest radical scavenging activity, with an IC50 value of 58.2 µg/mL. Compound 1b (4-hydroxy substituted chalcone) also showed significant scavenging activity with an IC50 value of 67.4 µg/mL. The remaining chalcones displayed moderate activity, with IC50 values ranging between 70.5 µg/mL and 88.3 µg/mL. Ascorbic acid, the reference standard, exhibited an IC50 value of 42.6 µg/mL

DISCUSSION:

Chalcones are effectively synthesised with the help of acetophenone and a

number of aldehydes, including vanillin, 4-hydroxybenzaldehyde, 4-bromobenzaldehyde, and benzaldehyde. 4-Bromo Benzaldehyde is soluble in ethanol and has an Rf value of 0.13. Its melting point is 110°C and its practical yield is 86.18%. The ethylene range in the FTIR spectrum is 3053.79, while the ketone range is 1912.33. Additionally, the highest concentration exhibits the best free radical activity.

The melting point of vanillin is 82°C. It dissolves in benzene, ethanol, and methanol. It has a yield percentage of 76.5%. The Rf value is 0.97 in it. The ethylene range in FTIR spectra is 3088.99, whereas the ketone range is 1907.05. Only at the absolute lowest concentrations does it exhibit the strongest free radical scavenging action. The melting point of hydroxy-benzaldehyde is 110°C. The Rf value is 0.73 in it. It has a yield percentage of 63.6%. The ethylene range in the FTIR spectra is 3229.68, while the ketone range is 1895.15 .

The melting point of cinnamon aldehyde is 120°C, its percentage yield is 49.48%, and its product's Rf value is 0.93. Ethylene is found in the FTIR spectral range of 3061.97, whereas ketone is found in the range of 1970.74. In ethanol, it is soluble.

Therefore, the high practical yield is provided by the electron withdrawing group linked to the benzene. The results demonstrate these chalcone compounds' strong antioxidant action. Only at the lowest doses can 4-Bromo Benzaldehyde and vanillin exhibit strong free radical scavenging action. The inhibitory concentration of the chemicals determines it. Our result is in line with other researchs to synthesis or use chalcone derivateves as antioxidant and free radical scavenger effect [21,22].

CONCLUSION:

In the current work, we principally synthesized chalcones (1a-1e) using a standard approach, and we then used an in vitro method to report their IC50 for free radical scavenging activity. It's noteworthy to notice that the chalcone works as an intermediary to allow for further structural alterations and has a privileged template with an α, β-

unsaturated carbonyl system. Because of this, scientists are putting a lot of emphasis on modifying the skeleton of chalcones to create innovative materials with a wide range of uses. Chalcone is therefore a novel scaffold that is important to the drug discovery process.

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Author contribution

All authors contributed equally in this research

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