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ORIGINAL ARTICLE **OPEN ACCESS**

Green Synthesis Approach of Cinnamic Acid by Knoevenagel Condensation and It's Antibacterial Activity

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ABSTRACT
Cinnamic acids have been discovered to be intriguing substances with cytotoxic and antioxidant effects. In the current study, Knoevenagel condensation processes and Perkin reactions were used to create simple cinnamic acids, which were then examined for biological activity. In contrast to styrene or benzoic acid, The double bond (C=C) in between phenyl ring as well as the carboxyl group in cinnamic acid derivatives interferes with the pi electron system of the molecule and prevents electron delocalization. The chemical compound known as cinnamic acid has the formula C6H5CH = CHCOOH. It is a bright white chemical that readily dissolves in many live solvents and just barely dissolves in water. It is a naturally occurring carboxylic acid that is classified as unsaturated and can be found in a wide range of plants. It is both the cis isomer and the Tran's isomer. Green approach of synthesis of cinnamic acid has numerous advantages over conventional methods.
Keywords: *Cinnamic acid, Green synthesis, Knoevenagel reaction*

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INTRODUCTION

In an effort to lessen human and environmental exposure to chemicals, the phrase "green chemistry," which was introduced to the scientific community in 1991, was created to eliminate or minimize dangerous molecules [1]. Currently, a variety of green processes are utilized to produce a variety of products, including electronics, medications, plastics, insecticides, and power [2]. Any chemical compound, as a general rule, has potentially dangerous regions because of their malleable internal structure (cells). The many kinds of dangers include toxicity (deathly, a source of cancer or other ailments), physical hazards (flammability, explosive qualities), and worldwide dangers such as warming, climate change, and ozone layer loss [3].

For these ideas to be effective, they must be used concurrently. There is no such thing as an entirely green synthesis; instead, the notion of a greener synthesis is much more accurate [4]. The primary classic book in the this field as well as online sites [5] both describe these green chemistry ideas. These guidelines are helpful and straightforward, but they do not cover all aspect of chemical processes, particularly when it comes to scale - up and cost evaluation [6]. While these procedures can lessen environmental harm, they cannot entirely stop the interaction of dangerous chemicals also with environment. It is possible to carry out our energy-efficient processes to avoid the formation of wastes, provide matter and energy economy, use safer solvents and renewable chemicals, and yield less-hazardous materials with low toxicity, as well as to carry out smart catalysis with fewer side products under degradable process design [7]. Several methods of control, including the use of alternative, carefully chosen precursors, solvents, catalysts, and reagents, modification of the target product, real-time process monitoring, and shorter syntheses, can be used to reduce environmental risks in the various steps of a chemical process in the fabrication of materials. [8]. For evaluating the amount of waste produced during a synthesis process, the so-called E-factor (example of environmental, mass of waste per mass of product, in kilogrammes; the range 25-100 belong to the pharmaceuticals production and is considered as highest value) is utilized. The formula

weight of the intended final product is correlated with the total formula weights of all precursors (starting reagents) by another factor called "atom economy" [9].

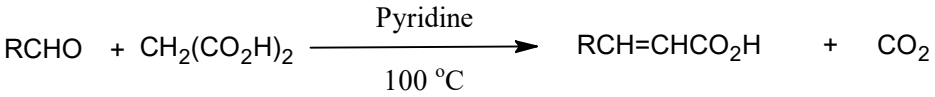
Green acts enable avoiding issues with numerous environmental laws and regulations. Greener reactions are particularly crucial in organic chemistry, where use of harmful chemicals and solvents in numerous industrial processes causes serious environmental damage [10]. Green methods in organic chemistry include C-H bond activation reactions, fluorous, solid-supported, bio- and asymmetric catalysis as well as synthesis, use of water and other green solvents (particularly ionic liquids (ILs)), or no solvent at all, microwave-, ultrasonic-, and UV light assisted reactions, and use of flow reactors[11]. The development of catalyst-aided reactions and proper solvent selection are two key components of green approaches in organic chemistry. Due to their volatility, toxicity, and ability to deplete the ozone layer, aromatic chlorinated solvents are not advised for use. Particularly ionic liquids can be utilized as an alternative since they are non-volatile, non-aqueous, and polar. The catalyst might stay in such a solvent if an organic reaction allows for the separation of products through distillation or extraction; in this situation, both the catalyst and solvent can be recycled and used again.

Without the use of solvents, organic reactions can also be conducted in a supercritical medium of CO_2 (one method is microwave irradiation); both methods are regarded as environmentally friendly.

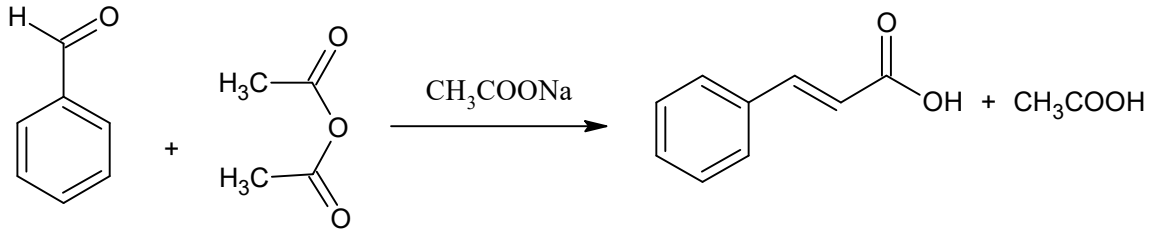
In many reactions, toluene can take the place of more harmful benzene; biodegradable solvents are another viable green option [12]. In the fields of medicinal chemistry, pharmaceutical engineering, and industry, green technologies are also used, particularly for drug administration and the treatment of tropical as well as other diseases [13–14]. This one and biosynthesis are distinguished in some explanations of green synthesis; the biosynthesis of substances and materials involves use of such plant extracts, fungus, algae, or microorganisms. The green synthesis is generally focused on reducing the impact of harmful compounds.

Green synthesis by Knoevenagel condensation principle and procedure:

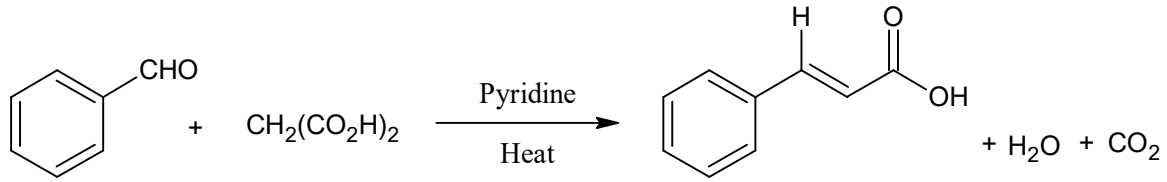
Knoevenagel condensations occur when active methylene molecules combine with aldehyde and ketones [15]. Weak bases like amines as well as carboxylate anions are used to catalyze them [16]. Malonic acid acts as the active methylene component in Knoevenagel condensations, which typically entail spontaneous decarboxylation. They are also referred to as Doebner condensations or Doebner variations of the Knoevenagel condensation because of this [17–18].



The Knoevenagel reactions are just a refined Aldol Condensation that produces C-C bonds through the nucleophilic addition of an active hydrogen molecule to an aldehyde or ketone in the influence of a basic catalyst. The C-H bond in the active hydrogen molecule can be deprotonated either by basic catalyst [19].



Benzaldehyde Acetic acid anhydride cinnamic acid Acetic acid



Benzaldehyde Malonic acid trans-cinnamic acid

MATERIAL AND METHODS

Chemicals: All chemicals used were analytical grade.

Green synthesis approach for synthesis of cinnamic acid:

0.10 moles of newly distilled benzaldehyde, 0.11 mole of malonic acid, 25 mL of 95 percent ethanol, and 2.5 mL pyridine are put in a round-bottomed flask with a reflux condenser. The mixture is cooked for six to eight hours with a mild reflux (during two lab periods). The enormous crystal mass is broken up with a spatula once the mixture has cooled, and the resulting reaction mixture is then chilled in an ice bath. The solid is collected using a Buchner funnel, and then 2 X 3 mL of cold 95 percent ethanol are used to wash it away. After filtering, the crystals of the crude cinnamic acid are recrystallized from ethanol and dried by air. The product weighs 11–12.6 g and is solid white (75-85 percent). Note the melting point of the produce [20].

Conventional method of synthesis of cinnamic acid:

A dry 250 ml round bottom flask is filled with 10.5g (10ml) of benzaldehyde, 15g (14ml) of acetic anhydride, and 6g of finely powdered sodium acetate. The RB flask's contents are well combined, and the reaction mixture is heated in an oil bath at 160°C for 60 minutes before being raised to 170–180°C for three hours. The contents of the flask are emptied into a 500ml RB flask that contains 50ml of water and is set up for steam distillation operation while they are still hot (90-100°C). After being briefly washed with hot water, the flask 1's contents are transferred to the flask 2. By progressively adding a saturated sod, the resultant solution in the 500 ml RB flask is rendered alkaline. Shake carbonate with vigorous. The mixture is steam-distilled until all unreacted benzaldehyde is eliminated and the distillate is crystal-clear. The majority of undesirable byproducts are removed from the distillation flask's contents by cooling and suction filtration. By pouring conc. HCL slowly in tiny amounts at regular intervals and continuously shaking till the evaluation of CO2, the filtrate is carefully brought to an acidic pH. Cinnamic acid that results from chilling is separated into white crystals, filtered in a Buchner funnel, briefly washed with cold water, then thoroughly drained and dried at 100°C.

ANTIBACTERIAL ACTIVITY:

Preparation of agar medium:

Using the dehydrated medium, prepare MHA as directed by the manufacturer. Distilled or deionized water should be used to prepare media. The medium must boil and be heated while being stirred frequently. By autoclaving for 15 minutes at 121 °C, sterilize. After sterilization, measure each preparation's pH; it will be between 7.2 and 7.4 at ambient temperature. This is accomplished by either permitting a small amount of medium to gel around a pH meter electrode or by macerating a minimal quantity of medium in a small amount of distilled water. To 40–50°C, cool the agar medium. In a clean, flat petri dish made of glass or plastic, pour the agar to a uniform depth of 4 mm. To be allowed to set. Plates should be dried in an incubator at 30-37°C for no longer than 30 minutes, or until any excess moisture has evaporated, before use. The media needs to be wet but without any visible water droplets. Water droplets could cause swarming bacterial growth, which might produce precise findings. They can also quickly become polluted [21].

Inoculums:

Take three to five colonies with just a wire loop from a pure bacterial culture that is no older than 48 hours or older, excluding organisms with poor growth. Colonies should be transferred to 5ml of Trypticase-Soy or 0.9 percent saline. The broth should be incubated at 30°C or a temperature that promotes growth until it reaches or surpasses the turbidity of 0.5 Mac Farland standards (prepared by adding 0.5 ml of 0.048 M $BaCl_2$ to 99.5ml of 0.36 NH_2SO_4; commercially available). When using 0.5 MacFarland (which has been vigorously shaken before use), compare the turbidity of the test bacterial culture to that of the control sample on a white background with a black line in sharp contrast. Arrow identifies the tube with the appropriate turbidity. By adding sterile broth or saline, turbidity can be reduced.

Inoculation of plates:

Enter the standardized bacterial suspension with a clean cotton swab. By quietly pressing the swab on the tube wall on a level beyond the liquid, extra inoculums can be removed. By streaking the agar with the inoculum-containing swab, inoculate it. Repeat the rubbing process after rotating the plate by 60 degrees. Repetition twice. The inoculums will be distributed evenly as a result. To allow for the absorption of extra moisture, let the medium's surface dry for 3 to 5 minutes, but no more than 15 minutes.

Selection

There should be a limit on the antimicrobial agents that are tested. To make the test practicable and applicable, only one representative from each category of related drugs should be used, including those that are prescribed for veterinary use to treat and prevent disease and those who can be useful for epidemiological other research purposes. Use antibiotic discs that you bought from a dependable supplier. The disc has a 6 mm diameter roughly. Disks should be carefully stored at 2 to 8 °C in a desiccant-filled container that is tightly sealed. It is not advisable to utilize expired discs.

Incubation

Plates should be incubated upside-down at 30 °C or another temperature that promotes growth. After 16–18 hours, look for the zone of inhibition. Longer incubation periods may be necessary for species with slow growth.

Reading:

Using a ruler with a 0.5mm graduation, measure the diameter of the inhibitory zones and note it down. The zone measurement should be rounded up to the closest millimeter. Chloramphenicol, 30 g (C-30), was utilized on the disc.

RESULT

Cinnamic acid's practical yield by green synthesis was determined to be about 75.03 percent, compared to 64.80 percent by the traditional approach. Cinnamic acid's melting point was discovered to be 132-134°C through green synthesis, compared to 130-132°C by traditional methods. It was discovered that the green approach's zone of cinnamic acid inhibition has a 16 mm diameter. The conventional technique revealed a zone of inhibition of cinnamic acid with a diameter of 15.6 mm.

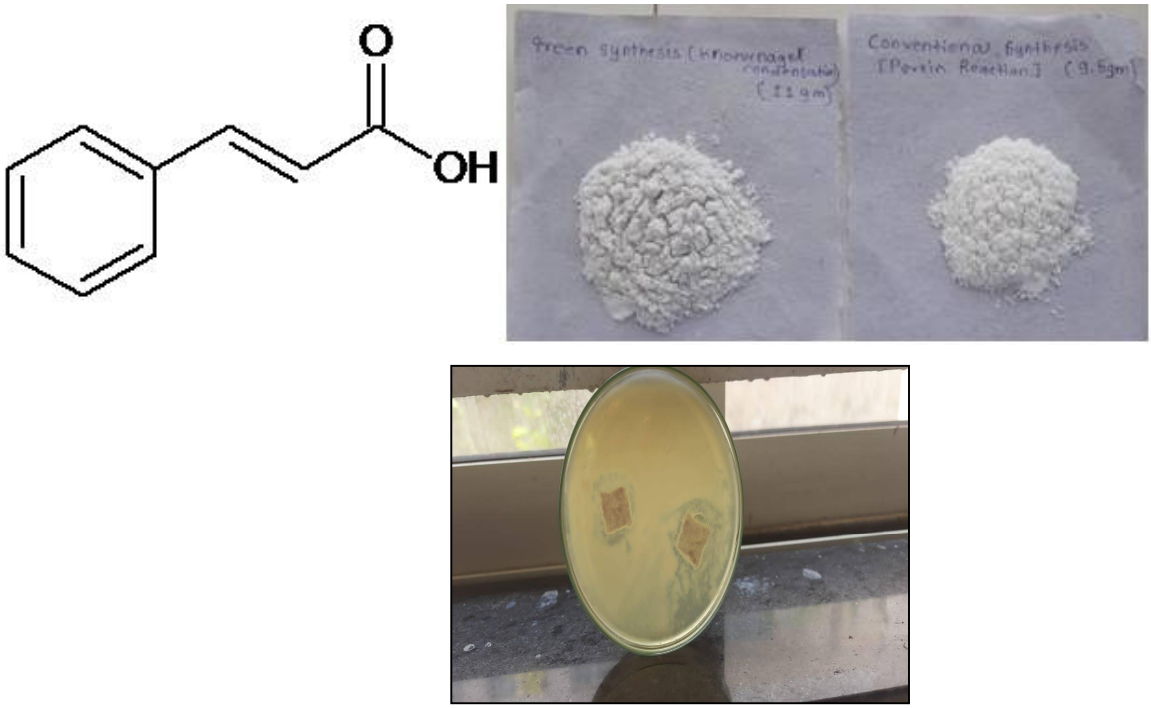


Figure 2. Disk diffusion method- for antibacterial activity of cinnamic acid

CONCLUSION

In the current study, we have reported on the Knoevenagel condensation-based synthesis of cinnamic acid. The primary goal of this study is to investigate the benefits of green synthesis. Under fully green guidelines, the current process is effective and economical. Their operations were quick, simple to control, and the resulting compounds were easily filtered by simply rinsing the mixture with environmentally friendly solvents. Synthesis of cinnamic acid by green synthesis gives more practical yield than the conventional method. Cinnamic acid is crucial to the development of antibacterial action.

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