Fruit juices act as biocatalysts in the efficient synthesis of potentially bioactive imidazoles

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ABSTRACT
An efficient and eco-friendly itinerary has been carried out for the synthesis of imidazole derivatives (3a–3h) from the reactions between substituted aldehydes (1a–1h), benzil (2a), and ammonium acetate (2b) in Citrus limon L. juice, Vitis vinifera L. juice, and Cocos nucifera L. juice. The purity of compounds was confirmed by their melting point and thin-layer chromatography. All synthesized compounds (3a–3h) were characterized by 1H NMR, FTIR, and CHN analysis and tested for in vitro herbicidal activity against Raphanus sativus L. (Radish seeds). The compounds (3a–3h) were also evaluated for their antifungal activity against Rhizoctonia solani and Colletotrichum gloeosporioides by the poisoned food technique. Antibacterial activity was also determined against Erwinia carotovora and Xanthomonas citri by the inhibition zone method. Activity data showed that compounds 3f and 3c were most active against Raphanus sativus L. (root) and Raphanus sativus L. (shoot), respectively. Compound 3d is most active against Rhizoctonia solani and Colletotrichum gloeosporioides fungus at highest concentrations. Compound 3b has shown the maximum inhibition zone, i.e., 2.10–7.10 mm against Erwinia carotovora at 2000 µg/mL concentration. Maximum Xanthomonas citrii growth was inhibited by compounds 3c, showing the inhibition zone 1.00–5.00 mm at highest concentration.

Introduction
Heterocyclic compounds, especially those containing nitrogen atoms, have been the significant molecules in organic chemistry because of their extraordinary activities, particularly their anticancer activities (1–3). One-pot synthesis of medicinally important heterocycles, under environment-friendly conditions, has remained one of the crucial topics in organic and medicinal chemistry (4). Imidazole is a key heterocyclic moiety widespread in many bioactive compounds and in synthetic drugs (5). Imidazoles fused with other heterocycles, such as benzothiazole and benzimidazole, are found in the core of various drugs and natural products. Imidazoles have a broad range of biological activities, such as anticancer, antifungal, antiviral, antibacterial, antitubercular, anti-parasitic, anti-histaminic, anti-inflammatory, anti-neuropathic, anti-obesity, and antihypertensive (6–15). In 1858, imidazole was synthesized by...
Heinrich Debus from glyoxal and formaldehyde, but the yield in this method was quite low (16). Later in 1977, Van Leusen synthesized imidazole using a three-component reaction with aldimines and tosylmethyl isocyanide, but the yield was low (17). In 1996, Zhang et al. reported the synthesis of imidazoles by Ugi four-component reactions of aryglyoxals, primary amines, carboxylic acids, and isocyanides (18). In 2003, a novel one-pot synthesis of tetra-substituted imidazoles under solvent-free conditions and microwave irradiation was reported by Balaie et al. (19). Wolkenberg et al. also reported a facile and efficient method for the synthesis of imidazoles from 1,2-diketones and aldehydes in NH4OAc (20). However, most of these methods have some drawbacks, such as using hazardous solvents, strongly acidic or basic conditions, expensive procedures, laborious work-up processes, and longer reaction times. Thus we turned our efforts to develop a green method for the synthesis of substituted imidazoles. Recently, fruit juices are being used as biocatalysts in the synthesis of heterocyclic compounds because of their eco-friendly character, non-hazardous, readily available, and cost-effective nature. Therefore, in this paper, we reported a one-pot three-component reaction between substituted aldehydes, benzil, and ammonium acetate in Citrus limon L. juice, Vitis vinifera L. juice, and Cocos nucifera L. juice, respectively (Scheme 1). The progress of the reaction was monitored by a thin-layer chromatography using Hexane: Ethyl acetate (80:20, v/v) as an eluent.

To standardize the reaction condition, we started our study with the three-component reactions of 3,4-Dimethoxybenzaldehyde (20 mmol), benzyl (20 mmol), and ammonium acetate (40 mmol) in Citrus limon L. juice (Table 1, Entry 1–4). It was observed that when the amount of Citrus limon L. juice was 4.0 mL in the reaction mixture, the yield of the product was 87% and the time of completion of the reaction was more, i.e., 2.5 h (Table 1, Entry 1). But when the amount of Citrus limon L. juice was increased from 4.0 mL to 8.0 mL, then the yield of the product was also good, i.e., 86% and the time of completion of reaction was reduced (Table 1, Entry 3). This result encourages us to explore the same model reaction in a solvent-free medium at room temperature in Vitis vinifera L. juice and Cocos nucifera L. juice, respectively. The excellent yields of the product, i.e., 87% and 89% were obtained, respectively, when the concentration of Vitis vinifera L. juice and Cocos nucifera L. juice was 8.0 mL in the reaction mixture and time of completion of the reaction was also less (Table 1, Entry 3). The data of this study are presented in Table 2. After the completion of the reaction, solid products were collected by simple filtration and then recrystallization in methanol to afford pure imidazole derivatives (3a–3h). All the synthesized imidazole derivatives are shown in Figure 1. The structure of synthesized compounds was confirmed by 1H NMR, FTIR, CHN analysis, and a comparison of their melting points with those of reported compounds. The compound viz. 2-(4-methoxyphenyl)-4,5-diphenyl-1H-imidazole (3b) displayed IR absorptions at 3317.3, 3063.3., 1592.9, 1210.5, and 1447.6 cm$^{-1}$ indicating NH, C=CH, C=C aromatic, OCH$_3$, and C=N, respectively and melting point 229–230°C. From the spectral study it was found that

![Scheme 1](image)

**Scheme 1.** Synthesis of substituted imidazole derivatives (3a–3h).
Table 1. Model reaction of 3,4-Dimethoxybenzaldehyde (20 mmol), benzyl (20 mmol), and ammonium acetate (20 mmol) using Citrus limon L. juice, Vitis vinifera L. juice, and Cocos nucifera L. juice as a catalyst.

| Entry | Catalyst concentration (mL) | Method A | Method B | Method C |
|-------|----------------------------|----------|----------|----------|
|       | Time (h) | Yield (%) | Time (h) | Yield (%) | Time (h) | Yield (%) |
| 1     | 4.0      | 2.5      | 87       | 2.5      | 62       | 9        | 43       |
| 2     | 6.0      | 1.8      | 79       | 2.0      | 79       | 8        | 52       |
| 3     | 8.0      | 1.5      | 86       | 1        | 87       | 5        | 89       |
| 4     | 10.0     | 1.0      | 60       | 40 min   | 50       | 6        | 80       |

Table 2. Physical data of substituted imidazole derivatives (3a–3h).

| S. No | Product | Ar          | Method A | Method B | Method C |
|-------|---------|-------------|----------|----------|----------|
|       |         |             | Time (h) | Yield (%) | Time (h) | Yield (%) | Time (h) | Yield (%) | m.p. (°C) |
| 1     | 3a      | 2-OHPh      | 4        | 92       | 3        | 86       | 1        | 88       | 119–120   |
| 2     | 3b      | 4-OCH3Ph    | 2        | 81       | 5        | 83       | 1        | 85       | 229–230   |
| 3     | 3c      | 4-CIPh      | 5        | 79       | 4        | 92       | 7        | 89       | 229–230; (Lit. 228–230) (21) |
| 4     | 3d      | 4-BrPh      | 3.5      | 80       | 1.5      | 79       | 2        | 85       | 194–196   |
| 5     | 3e      | 3-OHPh      | 9        | 80       | 8        | 81       | 5        | 80       | 254–255   |
| 6     | 3f      | 4-CH3Ph     | 3        | 83       | 4.5      | 80       | 4        | 80       | 233–235   |
| 7     | 3g      | 3-NO2Ph     | 7        | 88       | 2        | 78       | 3        | 78       | 197–198 (Lit. 198–200) (22) |
| 8     | 3h      | 4-OH-3-OCH3Ph | 7       | 88       | 3.5      | 81       | 5        | 89       | 200–202   |

Figure 1. Substituted imidazole derivatives (3a–3h).

The compound viz. 2-(4-bromophenyl)-4,5-diphenyl-1H-imidazole (3d) showed a $^1$H NMR spectrum in DMSO-$d_6$, displayed a multiplet at 7.54–7.93 δ, integrating for the proton of the aryl group, a singlet at 13.09 δ integrating for one proton of the NH group, and melting point 194–196°C. The compound viz. 3-(4,5-diphenyl-1H-imidazol-2-yl) (3e) phenol displayed IR absorptions at 3188.9, 3316.0, 3063.1, 1592.8, and 1449.0 cm$^{-1}$ indicating NH, OH, C=CH, C=C aromatic, and C=N, respectively, and melting point 254–255°C. The compound viz. 4,5-diphenyl-2-(p-tolyl)-1H-imidazole (3f) showed a $^1$H NMR spectrum in CDCl$_3$, displayed a multiplet at 7.12–7.98 δ integrating for the proton of the aryl group, a singlet at 9.89 δ integrating for one proton of the NH group.
and melting point 233–235°C. The compound (3f) also displayed IR absorptions at 3136.3, 3063.2, 1593.0, and 1448.6 cm⁻¹, indicating NH, C=CH, C=C aromatic, and C=N, respectively. The compound viz. 2-(3-nitrophenoxy)-4,5-diphenyl-1H-imidazole (3g) showed ¹H NMR spectrum in CDCl₃, displayed a singlet at 10.05 δ integrating for one proton of the NH group, a multiplet at 7.43–8.39 δ integrating for the proton of the aryl group, a singlet at 8.62 δ integrating for one proton of the =CH group, a doublet at 8.14 δ integrating for the proton of the aryl group, and melting point 197–198°C. The compound viz. 4-(4,5-diphenyl-1H-imidazol-2-yl)-2-methoxyphenol (3h) displayed IR absorptions at 3183.8, 3315.0, 3063.5, 1592.7, 1210.2, and 1430.4 cm⁻¹ indicating NH, OH, C=CH, C=C aromatic, OCH₃ and C=N, respectively, and melting point 200–202°C. To show the beauty of the current protocol, the previous protocols and their yields for the synthesis are summarized in Table 3. We observed that Citrus limon L. juice, Vitis vinifera L. juice, and Cocos nucifera L. juice catalyst give the best catalytic activity in terms of product yield and reaction time compared to other catalysts in the literature. Therefore, the present procedure for the synthesis of imidazole derivatives is considered a sustainable and eco-friendly protocol. The possible mechanism for the formation of substituted imidazoles is shown in Scheme 2. It involves the condensation of dicarbonyl compounds such as benzil with an aldehyde in ammonium acetate, which is a good source of ammonia. The aryl aldehyde and benzil are activated first by acid catalyst by nucleophilic attack on carbonyl groups. The catalyst converts ammonium acetate to ammonia, which forms an intermediate with an activated aldehyde. This intermediate reacted with activated benzil, and then cyclization takes place to form substituted imidazoles.

**Herbicidal activity**

All compounds (3a–3h) were tested for herbicidal activity against Raphanus sativus L. at 200, 150, 100, and 50 µg/mL concentrations, as shown in Table 4. Results were shown in the form of primary screening. All compounds were diluted to 1000 µg/mL concentration as a stock solution. Herbicidal activities of compounds were evaluated against Raphanus sativus L. by the inhibitory effect of compounds on the growth of weed roots and shoots. The percentage of inhibition growth was calculated from mean differences between treated and control. From the herbicidal activity results, we observed that compound 3f exhibited maximum percentage growth inhibition, i.e., 93.33 against Raphanus sativus L. (root), whereas compound 3c exhibited maximum percentage growth inhibition, i.e., 87.50 against Raphanus sativus L. (shoot) at 200 µg/mL concentrations. The compounds 3f and 3c showed broad-spectrum herbicidal activity because of methyl and chloro substitution at the phenyl ring. The box plot and graphical representation of the herbicidal activity of all compounds against Raphanus sativus L. are shown in Figures 2–5.

**Antimicrobial evaluation**

**Antifungal activity**

All synthesized compounds (3a–3h) were screened for their fungicidal activity against 2 fungal strains viz. Rhizoctonia solani and Colletotrichum gloeosporioides by the poisoned food technique method. DMSO was used as a negative control against fungal strains. The result of the antibacterial activity of tested compounds is shown in Table 5. Most of the synthesized compounds have a moderate to good activity against R. solani and C. gloeosporioides. Compounds 3e and 3g showed no antifungal activity at all concentrations against R. solani. Compound 3f has shown no growth inhibition up to 500 µg/mL concentrations against R. solani. Compound 3f exhibited 23.69 and 38.25% growth inhibition against R. solani fungus at 1000 µg/mL and 2000 µg/mL concentrations, respectively. Compounds 3a, 3c, and 3f showed no antifungal activity at all concentrations against C. gloeosporioides. Compound 3g has shown no growth inhibition up to 2000 µg/mL concentrations against C. gloeosporioides.
inhibition at lower concentrations. Compound 3g exhibited 11.69 and 30.82% growth inhibition against *C. gloeosporioides* fungus at 1000 µg/mL and 2000 µg/mL concentrations, respectively. Antifungal activity results concluded that compound 3d was the most promising against *R. solani* and *C. gloeosporioides*. This result

Scheme 2. Possible mechanism for the synthesis of substituted imidazoles (3a–3h).

Table 4. Herbicidal activity of substituted imidazoles (3a–3h).

| Compounds | Growth Inhibition (%) |
|-----------|------------------------|
|           | Root (µg/mL) | Shoot (µg/mL) | Root (µg/mL) | Shoot (µg/mL) | Root (µg/mL) | Shoot (µg/mL) | Root (µg/mL) | Shoot (µg/mL) |
| 3a        | 25.00 ± 0.81  | 50.00 ± 0.86  | 66.60 ± 1.41 | 83.33 ± 0.99  | 47.50 ± 0.98  | 56.25 ± 1.07  | 71.25 ± 1.01 | 81.25 ± 1.05  |
| 3b        | 64.60 ± 1.06  | 75.38 ± 1.95  | 85.36 ± 1.51 | 92.66 ± 1.16  | 36.95 ± 1.03  | 51.36 ± 1.05  | 64.80 ± 1.12  | 84.02 ± 0.30  |
| 3c        | 16.60 ± 0.63  | 33.33 ± 1.00  | 58.33 ± 0.93 | 83.33 ± 1.52  | 37.50 ± 1.53  | 52.50 ± 0.93  | 68.75 ± 1.71  | 87.50 ± 0.91  |
| 3d        | 50.00 ± 0.92  | 66.60 ± 0.38  | 80.00 ± 0.83 | 90.00 ± 0.83  | 38.46 ± 0.51  | 53.80 ± 1.01  | 66.15 ± 0.64  | 83.07 ± 0.85  |
| 3e        | 28.13 ± 1.02  | 49.17 ± 0.34  | 65.63 ± 0.94 | 84.12 ± 0.95  | 40.25 ± 0.91  | 51.36 ± 1.57  | 67.63 ± 1.10  | 80.36 ± 0.99  |
| 3f        | 66.60 ± 0.86  | 76.60 ± 0.99  | 86.60 ± 0.93 | 93.33 ± 0.98  | 47.69 ± 1.01  | 61.50 ± 1.00  | 72.30 ± 1.00  | 84.61 ± 1.12  |
| 3g        | 33.33 ± 0.93  | 50.00 ± 1.44  | 75.00 ± 0.78 | 91.66 ± 1.57  | 56.25 ± 1.52  | 68.75 ± 1.66  | 81.20 ± 1.00  | 86.25 ± 1.01  |
| 3h        | 48.66 ± 1.01  | 61.36 ± 1.00  | 78.12 ± 1.00 | 89.66 ± 1.16  | 46.32 ± 0.96  | 58.96 ± 1.89  | 71.30 ± 1.01  | 83.66 ± 1.13  |

Note: All values are mean ± S.D.
Figure 2. Box plot of substituted imidazoles (3a–3h) against *Raphanus sativus* L. (root).

Figure 3. Herbicidal activity of substituted imidazoles (3a–3h) against *Raphanus sativus* L. (root).
Figure 4. Box plot of substituted imidazoles (3a–3h) against *Raphanus sativus* L. (shoot).

Figure 5. Herbicidal activity of substituted imidazoles (3a–3h) against *Raphanus sativus* L. (shoot).
may be due to the substitution of the bromo group on the phenyl ring. The box plot and graphical representation of the antifungal activity of all compounds against *Rhizoctonia solani* and *Colletotrichum gloeosporioides* are shown in Figures 6–9.

**Antibacterial activity**

The propitious antifungal activity of synthesized compounds (3a–3h) has inspired authors to test further for antibacterial activity. All synthesized compounds (3a–3h) were tested for their *in vitro* antibacterial activity against two bacterial strains, *Erwinia carotovora* and *Xanthomonas citri*, by the inhibition zone method using DMSO as the negative control. The results of the antibacterial activity of synthesized compounds are shown in Table 6. Compound 3f has shown no inhibition zone at lower concentrations. Compound 3f exhibited a 0.60 mm inhibition zone against *Erwinia carotovora* at 2000 µg/mL concentration. Compound 3g has shown no inhibition zone at 250 µg/mL concentration. Compound 3g has shown 1.00, 2.00, and 5.00 mm inhibition zone against *Erwinia carotovora* at 500, 1000, and 2000 µg/mL concentrations, respectively. Compound 3a has shown no inhibition zone at all concentrations against *Xanthomonas citri*. Compound 3e has shown no inhibition zone at lower concentrations. Compound 3e has shown 0.40 mm inhibition zone against *Xanthomonas citri* at 2000 µg/mL concentration. Compound 3f has

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**Table 5. Antifungal activity of substituted imidazoles (3a–3h).**

| Compounds | *Rhizoctonia solani* (conc.) µg/mL | *Colletotrichum gloeosporioides* (conc.) µg/mL |
|-----------|----------------------------------|------------------------------------------|
|           | 250    | 500    | 1000   | 2000   | 250    | 500    | 1000   | 2000   |
| 3a        | 22.85 ± 1.44 | 57.14 ± 1.55 | 65.71 ± 0.73 | 77.14 ± 0.97 | a      | a      | a      | a      |
| 3b        | 20.78 ± 0.78 | 51.67 ± 1.03 | 63.29 ± 0.20 | 76.91 ± 1.39 | 22.80 ± 1.23 | 42.49 ± 1.12 | 64.87 ± 1.43 | 81.13 ± 0.99 |
| 3c        | 21.21 ± 0.21 | 39.39 ± 0.77 | 63.63 ± 1.35 | 78.78 ± 0.77 | a      | a      | a      | a      |
| 3d        | 23.69 ± 1.16 | 42.22 ± 1.21 | 68.17 ± 0.14 | 82.69 ± 0.65 | 21.36 ± 1.00 | 46.66 ± 0.41 | 67.59 ± 0.47 | 83.54 ± 1.09 |
| 3e        | a      | a      | 23.69 ± 0.67 | 38.25 ± 0.51 | 10.69 ± 0.97 | 23.54 ± 0.48 | 37.28 ± 2.14 | 66.39 ± 0.51 |
| 3f        | a      | a      | a      | a      | a      | a      | a      | a      |
| 3g        | a      | a      | 23.69 ± 0.67 | 38.25 ± 0.51 | a      | a      | a      | a      |
| 3h        | 25.54 ± 1.43 | 45.38 ± 2.77 | 67.58 ± 0.70 | 80.15 ± 2.23 | 18.90 ± 0.50 | 38.69 ± 0.51 | 59.34 ± 0.99 | 77.82 ± 1.46 |

Note: All values are mean ± S.D.

a: No Growth inhibition.

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**Figure 6.** Box plot of substituted imidazoles (3a–3h) against *Rhizoctonia solani*.
Figure 7. Antifungal activity of substituted imidazoles (3a–3h) against *Rhizoctonia solani*.

Figure 8. Box plot of substituted imidazoles (3a–3h) against *Colletotrichum gloeosporioides*.
shown no inhibition zone at lower concentrations. Compound 3f has shown 1.70, 2.30 mm inhibition zone against *Xanthomonas citri* at 1000 and 2000 µg/mL concentrations, respectively. Maximum *Erwinia carotovora* growth was inhibited by compound 3b showing inhibition zone 2.20–7.10 mm. Maximum *Xanthomonas citri* growth was inhibited by compound 3c showing inhibition zone 1.00–5.00 mm. This inhibition may be due to methoxy and chloro groups on phenyl groups. The box plot and graphical representation of the antibacterial activity of all compounds against *Erwinia carotovora* and *Xanthomonas citri* are shown in Figures 10–13.

**Experimental**

All reagents used were of analytical grade. All the chemicals were purchased from CDH (Central Drug House), SRL (Sisco Research Laboratory) and Sigma-Aldrich. Melting points were determined with a Ganson electric melting point apparatus and were uncorrected. The progress of the reaction was monitored by thin-layer chromatography. The ¹H NMR spectra were recorded on a ‘Brucker Ac 400 F’ (400 MHz) nuclear magnetic resonance spectrometer. All chemical shifts are given as δ values (ppm) with reference to tetramethylsilane as an internal standard. The peak patterns are indicated as follows: s =
Figure 10. Box plot of substituted imidazoles (3a–3h) against *Erwinia carotovora*.

Figure 11. Antibacterial activity of substituted imidazoles (3a–3h) against *Erwinia carotovora*. 
Figure 12. Box plot of substituted imidazoles (3a–3h) against *Xanthomonas citri*.

Figure 13. Antibacterial activity of substituted imidazoles (3a–3h) against *Xanthomonas citri*. 
singlet, d = doublet, t = triplet, m = multiplet, and brs = broad singlet. Infrared spectra (4000–350 cm\(^{-1}\)) of the synthesized compounds were recorded in KBr pellets on a Perkin Elmer FT-IR-R2X spectrophotometer and their frequencies were recorded in cm\(^{-1}\). Elemental analysis was performed using a ThermoFinnigan CHN elemental analyzer. Coupling constant (\(J\)) values are expressed in Hz.

Bio-evaluation

Herbicidal activity

Solutions of 50 µg/mL, 100 µg/mL, 150 µg/mL, and 200 µg/mL of the test compounds in DMSO were prepared. Agar powder (5 g) was put into boiling distilled water (1L) until it dissolved and then cooled down to 40–50°C. The solution (2 mL), containing test compounds and melting agar (18 mL), was mixed, and this mixture was added to a Petri dish with a 4.5 cm diameter. The agar plate without test compound was used as an untreated control. Then 15 seeds of *Raphanus Sativus* L. (Radish) were put on the surface of the agar plate. The Petri dishes were covered with glass lids, and the cultivation conditions were kept at 25 ± 1°C and 12 h in the light and 12 h in the dark alternating for seven days. Seven days later, the root lengths and shoot lengths of *Raphanus sativus* L. were measured. The growth inhibitory rate related to untreated control was determined by the given formula (31).

\[
\text{%Inhibition} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100
\]

Antifungal activity

All synthesized compounds (3a–3h) were tested for their antifungal activity against *Rhizoctonia solani* and *Colletotrichum gloeosporioides*. Fungal species were grown in the laboratory on Potato dextrose agar (PDA) media. The antifungal activity was determined by the poisoned food technique method (32). The required amount of synthesized compounds dissolved in 1 mL of DMSO was incorporated aseptically into 99 mL aliquots of sterilized potato dextrose agar cooled at 45°C after brief shaking. Each lot of medium was poured into Petri dishes and allowed to solidify. DMSO was used as a negative control. Each dish was inoculated centrally with a 5 mm mycelial disk cut from the periphery of 2–3-day-old fungal colonies. Inoculated Petri plates were incubated in the dark 25 ± 2°C for 48–72 h, and colony diameters were measured periodically till the control dishes were nearly completely covered with fungus growth. All observations were made in triplicate. The degree of inhibition of growth was calculated from the mean differences between treatments and the control as a percentage of latter using the following formula:

\[
\text{%Inhibition} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100
\]

Control = mycelial growth in a control dish
Treated = mycelial growth in a treated dish

Antibacterial activity

Bacterial species, *Erwinia carotovora* and *Xanthomonas citri*, were grown on a Luria–Bertani medium in the laboratory. Antibacterial activity was evaluated using the inhibition zone method (33). 250, 500, 1000, and 2000 µg/mL synthesized compounds were prepared from the stock solution by taking the appropriate amount and diluting with DMSO. DMSO was used as a negative control. The circular paper disks of 10 mm diameter were prepared from Whatman’s Filter paper No. 1. The disks were kept in a Petri plate and autoclaved at 15 lbs pressure 20 min. Two paper disks were used for each concentration of the synthesized compounds. The excess solution absorbed by paper disks was removed by holding them vertically with sterile forceps. Such soaked disks were transferred aseptically to Petri plates containing media and bacterial suspension spread over the surface. The Petri plates were kept in an incubator at 25 ± 2°C overnight and then examined for the inhibition zone at these different concentrations of compounds. The experiment was performed in triplicate, and activity was determined based on the inhibition zone (in mm).

Statistical analysis

The experiments were performed in triplicates for each treatment, and the mean values were recorded and expressed as mean ± S.D. The descriptive statistics in the form of box-and-whisker diagram were also presented in this paper. The spacing between the different box parts indicates the degree of dispersion and skewness in the data. This is a convenient way of visually displaying the data distribution through their quartiles.

Composition and preparation of green catalyst

A general procedure for the extraction of *Citrus limon* L. juice: The main component of lemon juice is moisture (85%), carbohydrate (11.2%), citric acid (5–7%), protein (1%), ascorbic acid, or vitamin C (0.5%), fat (0.9%),
minerals (0.3%), fibers (1.6%), and some other organic acids. Due to citric and ascorbic acids (Vitamin C) in lemon juice, it acts as an acid catalyst in organic synthesis. Fresh lemon was cut with a knife, and then pieces were pressed in a fruit juicer to get the juice extract. Then the juice was filtered through cotton and then through a Whatman filter paper no 1 to remove solid material, to get clear juice to be used as a catalyst (34).

The method for preparation of Vitis vinifera L. juice: The main constituents of grape juice are water (70–80%), sugar content (150–250 g/L), and organic acids viz. citric, malic, and tartaric acids found in grapes. Fresh grapes were purchased from the local market, then washed thoroughly under running tap water, and rinsed thrice with distilled water. Grapes were squeezed, and juices were strained initially through a muslin cloth, then passed through a Whatman filter paper No. 1 (35).

The method of preparation of Cocos nucifera L. juice: Cocos nucifera is a member of the family Arecaceae (palm family). Its juice is also called coconut water and is used to treat high blood pressure, hypertension, and diarrhea-related dehydration. The main ingredients per 100 g of coconut juice of Cocos nucifera are water (94.99 g), carbohydrates (3.71 g), protein (0.72 g), fat (0.2 g), ascorbic acid (2.4 mg), and pantothenic acid (0.043 mg). Coconut juice also contains many natural bioactive enzymes, such as acid phosphatase, catalase, dehydrogenase, diastase, peroxidase, and RNA-polymerase etc. Due to ascorbic acid and pantothenic acid, coconut juice was obtained by perforating the fruit with a knife. The coconut juice was filtered using a filter paper Whatman no 1 for the elimination of residues to get clear juice to be used as a catalyst (36).

By Cocos nucifera L. juice (Method C)

A mixture of substituted aldehydes (20 mmol) (1a–1h), 20 mmol benzil (2a), 40 mmol ammonium acetate (2b), and 8 mL of Cocos nucifera L. juice was stirred at room temperature. The completion of the reaction was monitored by thin-layer chromatography. The solid was separated out, then filtered, and washed with ice-cold water to get the products (3a–3h), which was further recrystallized with methanol. All compounds (3a–3h) were characterized by ¹H NMR, FTIR, and CHN analysis.

All the imidazole derivatives (3a–3h) were prepared according to Method A, B, and C.

Characterization data of selected compounds

2-(4-methoxyphenyl)-4, 5-diphenyl-1H-imidazole (3b): IR (νmax cm⁻¹) (neat): 3317.3 (NH); 3063.3 (C=CH); 1592.9 (C=C, aromatic); 1210.5 (OCH₃); 1447.6 (C=N)

2-(4-chlorophenyl)-4, 5-diphenyl-1H-imidazole (3c): Elemental Analysis found: C, 76.24; H, 4.57; Cl, 10.72; N, 8.47; Required: C, 74.32; H, 4.50

3-(4, 5-diphenyl-1H-imidazol-2-yl)-2-methoxyphenol (3e): IR (νmax cm⁻¹) (neat): 3183.8 (NH); 3315.0 (OH); 1593.0 (C=C, aromatic); 1449.0 (C=N)

3-(4, 5-diphenyl-1H-imidazol-2-yl)-phenol (3f): ¹H NMR (400 MHz, DMSO-d₆): δ 7.54–7.58 (m, J = 8 Hz, 2H, Ar-H); 7.59–7.63 (m, J = 8 Hz, 1H, Ar-H); 7.69–7.80 (m, J = 8 Hz, 2H, Ar-H); 7.87–7.93 (m, J = 8 Hz, 2H, Ar-H); 13.09 (s, 1H, NH)

4-(4, 5-diphenyl-1H-imidazol-2-yl)-2-methoxyphenol (3h): IR (νmax cm⁻¹) (neat): 3183.8 (NH); 3315.0 (OH);

The general method for the preparation of substituted imidazole derivatives (3a–3h)

By Citrus limon L. juice (Method A)

Substituted aldehydes (20 mmol) (1a–1h) were taken in a clean round-bottom flask. Then 20 mmol of benzil (2a), 40 mmol of ammonium acetate (2b), and 8 mL of Citrus limon L. juice were added, and the mixture was stirred at room temperature. The completion of the reaction was monitored by thin-layer chromatography. The solid was separated out, then filtered, and washed with ice-cold water to get the products (3a–3h), which was further recrystallized with methanol. All compounds (3a–3h) were characterized by ¹H NMR, FTIR, and CHN analysis.

By Vitis vinifera L. juice (Method B)

The mixture of substituted aldehydes (20 mmol) (1a–1h), 20 mmol benzil (2a), 40 mmol ammonium acetate (2b), and 8 mL of Vitis vinifera L. juice was stirred at room temperature. The completion of the reaction was monitored by thin-layer chromatography. The solid was separated out, then filtered, and washed with ice-cold water to get the products (3a–3h), which was further recrystallized with methanol. All compounds (3a–3h) were characterized by ¹H NMR, FTIR, and CHN analysis.
Conclusions

An efficient, simple, and solvent-free methodology has been developed for the synthesis of biologically active substituted imidazole derivatives (3a–3h). The present method has superior qualities, such as a simple and efficient catalytic system, a simple work-up, no use of toxic solvents, cheap and products, with excellent yields. All compounds (3a–3h) were also evaluated for their bio-efficacy in terms of herbicidal activity against *Raphanus sativus* L. (Radish) seeds, antifungal activity against *Rhizoctonia solani* & *Colletotrichum gloeosporioides*, and antibacterial activity against *Erwinia carotovora* and *Xanthomonas citri*. Based on biological activity data, we concluded that strong electronegative groups substitution at the phenyl ring exhibit a good activity profile compared to electron-donating groups.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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