SUPPLEMENTARY MATERIAL

Revisiting the nucleophilicity concept in a comprehensive biomass valorization experiment: from papaya seeds to thiourea motifs

Raquel V. dos Santos¹, Gil M. Vianaᵇ, Anderson Felipe S. Moreiraᶜ, Vitor S. Nóbregaᶜ, Vitor A. S. da Silvaᶜ, Luiz Fernando B. Maltaᶜ,*⁻, Lucia C. S. Aguiarᶜ, Jaqueline D. Senraᵃ

ᵃInstituto de Química, Universidade do Estado do Rio de Janeiro, 20550-900 Rio de Janeiro – RJ, Brasil
ᵇFaculdade de Fármacia, Universidade Federal do Rio de Janeiro, 21941-614 Rio de Janeiro – RJ, Brasil
ᶜInstituto de Química, Universidade Federal do Rio de Janeiro, 21941-903 Rio de Janeiro – RJ, Brasil

*e-mail: lfbmalta@iq.ufrj.br
MATERIALS AND METHODS

Preparation of the material for class

The seeds of *Carica papaya* used in these studies were obtained from ripe papaya fruits of the golden variety, supplied by G. T. M. Filho or bought in the markets of Rio de Janeiro. The seeds were kept at low temperature (-18°C) before their use. We recommend its storage until 4-5 months. A manual peeling process led to the removal of the seed sarcotestas. The seeds were then crushed with a mortar and pestle before the extraction.

Identification of the extract components and BITC quantification by HPLC

The first qualitative test to confirm the BITC presence was the thin layer chromatography ($R_f = 0.89$). The quantification of BITC present in the organic extracts from hydrodistillation and Soxhlet methods was carried out in a Shimadzu liquid chromatography equipped with a pump model LC-10AD and a DAD detector SPD-M10A VP. The column used was a 250 x 4.6 mm Hypersil® (5mm). The wavelength was fixed in 252 nm. The mobile phase was a hexane: ethyl acetate (98:2, v/v) mixture with a flow rate of 1 mL.min$^{-1}$. The external padronization method was used for the BITC quantification (retention time was 10.1 min). After the chromatographic experiments, the collected fractions were analyzed by ESI-MS (positive mode) to confirm the peak identity (M+H). Concerning the hydrodistillation, after the complete co-distillation of BITC (observed by the absence of turbidity), the organic phase was separated from the aqueous phase, dried with anhydrous Na$_2$SO$_4$ and evaporated under reduced pressure for the extract quantification. The extract obtained from Soxhlet was also dried with anhydrous Na$_2$SO$_4$ and evaporated under reduced pressure for the quantification. In the Figure 1S, it is shown the calibration curve of BITC used for the quantification of the extracts.
**Figure 1S.** Calibration curve of BITC

**Table 1S.** Validation of the calibration curve for the BITC quantification

| Entry | Concentration of the BITC solution (mg/mL) | BITC peak area | Recovery tax |
|-------|-------------------------------------------|----------------|--------------|
| 1     | 0.1964                                    | 2280856        | 97.2%        |
| 2     | 0.0975                                    | 1272968        | 97.0%        |
| 3     | 0.0768                                    | 890866         | 96.2%        |
| 4     | 0.0299                                    | 346663         | 93.6%        |

**Table 2S.** Quantification of BITC in the papaya seeds extract

| Entry | Method            | BITC (%)*     |
|-------|-------------------|---------------|
| 1     | Hydrodistillation | 91.2          |
| 2     | Soxhlet/ n-hexane | 10.3(1)       |
| 3     | Soxhlet/ n-pentane| 15.9(1)       |
| 4     | Soxhlet/ n-pentane| 13.5(2)       |

*Mean values from duplicate. (1) After 5h. (2) After 7h. Experiment conducted in the out class period by a group of students.
Figure 2S. Comparison between hydrodistillation and Soxhlet methods for the BITC extraction (obtained from the report prepared by the students)

Figure 3S. Chromatogram of the extract obtained from Soxhlet. The peaks were identified by ESI-MS
**Figure 4S.** Chromatogram of the extract obtained from Hydrodistillation. The peaks were identified by ESI-MS

**Figure 5S.** $^1$H NMR of BITC (CDCl$_3$, 400 MHz, δ ppm): 7.5-7.32 (m, 5H); 4.7 (s, 2 H)

**Waste disposal**

This approach was based on an strategy that intent to minimize the risks and improve the green chemistry credentials. However, since we propose the use of pentane/
hexane during the first step (extraction of BITC), we recommend their recovery by distillation. This procedure can avoid costs of discard and contributes to the pedagogical objectives to be attained with the remediation alternatives of hazardous wastes.

**Nucleophilicity tests**

After the recovery of the organic extract from hydrodistillation, students carried out an additional liquid-liquid extraction of the aqueous phase with n-hexane. Then, they partially evaporated the total volume (70 mL) under reduced pressure and divided the resultant BITC solution (10 mL) in eight parts of 1 mL. To each solution deposited on a ceramic board about 0.1 mL amine was added by the students. For the instantly prepared thioureas, the precipitates were isolated through filtration, washed in hexane and 5 mL of HCl (10% w/v), dried and characterized by TLC and $^1$H NMR.

$^1$H NMR characterization of thioureas

The $^1$H NMR analyses were recorded at 400 MHz on a Varian Gemini 200 or Bruker 400 spectrometers and chemical shifts were expressed in parts per million relative to internal standard TMS. The acquisition of the $^1$H NMR spectra of each thiourea was carried out in CDCl$_3$ with 5 mg of precipitate.

*Thiourea T1 (N-benzyl, N’-isopropyl thiourea)*

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\text{NMR } ^1\text{H [400 MHz, CDCl}_3, \text{ Figure S3]}: 7.38-7.27 (m, 5\text{H}), 6.06 (s, 1\text{H}), 5.69 (s, 1\text{H}), 4.63 (s, 2\text{H}), 4.16 (s, 1\text{H}), 1.17 (d, J = 6.5 \text{ Hz, 6H}). \text{ R}_f = 0.25
\]
Thiourea T2 (N-benzyl, N′-butyl thiourea)

NMR $^1$H [400 MHz, CDCl$_3$, Figure S4]: 7.38-7.27 (m, 5H), 6.17 (s, 1H), 5.91 (s, 1H), 4.65 (s, 2H), 3.35 (s, 2H), 1.52 (m, 2H), 1.30 (sex, $J = 7.4$ Hz, 2H), 0.89 (t, $J = 7.4$ Hz, 3H). $R_f$ = 0.57

Thiourea T3 (N-benzyl, N′-cyclohexyl thiourea)

NMR $^1$H [400 MHz, CDCl$_3$, Figure S5]: 7.38-7.28 (m, 5H), 6.11 (s, 1H), 5.75 (s, 1H), 4.63 (s, 2H), 3.83 (s, 1H), 1.95 (m, 2H), 1.61 (m, 4H), 1.35 (m, 2H), 1.15 (m, 2H). $R_f$ = 0.47

Thiourea T4 (N,N′-dibenzyl thiourea)

NMR $^1$H [400 MHz, CDCl$_3$, Figure S6]: 7.34-7.22 (m, 10H), 6.13 (s, 2H), 4.61 (s, 4H). $R_f$ = 0.36

Thiourea T5 (N-benzyl, N′-(2-hydroxyethyl) thiourea)

NMR $^1$H [400 MHz, CDCl$_3$, Figure S7]: 7.35-7.26 (m, 5H), 6.94 (s, 1H), 6.66 (s, 1H), 4.64 (s, 2H), 3.70 (t, $J = 4.8$ Hz, 2H), 3.56 (s, 2H), 2.69 (s, 1H). $R_f$ = 0.04
*Thiourea T6 (N-benzyl, N'-morpholinyl thiourea)*

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\begin{array}{c}
\text{NMR } ^1H \text{ [400 MHz, CDCl}_3, \text{ Figure S8]: } 7.38-7.28 \text{ (m, 5H)}, 5.65 \text{ (s, 1H)}, 4.87 \text{ (d, } J = 4.8 \text{ Hz, 2H)}, 3.80 \text{ (t, 4H)}, 3.74 \text{ (t, 4H)}. \text{ Rf = 0.09} \\
\end{array}
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*Figure 6S. $^1H$ NMR spectrum of thiourea T1*
Figure 7S. $^1$H NMR spectrum of thiourea T2

Figure 8S. $^1$H NMR spectrum of thiourea T3
Figure 9S. $^1$H NMR spectrum of thiourea T4

Figure 10S. $^1$H NMR spectrum of thiourea T5
In-class questions and student feedback

For the comparison of the student skills, prelab and postlab activities were introduced. According to the responses we could conclude some main difficulties from the students: i) efficiency of hydrodistillation x Soxhlet, ii) differences between basicity and nucleophilicity, iii) NMR theory. In general, we recommend teacher stress out an introduction to the distillation theory and the more simple fundamentals of organic compounds characterization by physical methods. In the second part, a possible correlation between the pk values and the product yield is a possible alternative to stimulate the students to differentiate the nucleophilicity and basicity concepts.

One of the positive aspects was that students realized that it was necessary to think about the questions through the analysis of results. This student feedback indicated a greater desire to learn beyond the knowledge taught in the lab. Indeed, we felt the pre- and postlab activities encouraged students to think more independently and reinforced the pedagogical importance of problem-solving approach.
Prelab exercises

1- Compare the different types of natural products isolation concerning their principles and applications.
2- Chromatographic techniques are invaluable tools for the qualitative and quantitative analysis. Cite the main advantages/disadvantages of the liquid chromatography.
3- What are the general information obtained from the $^1$H NMR spectrum?
4- What makes a good nucleophile/electrophile?

Postlab exercises

Questions about experiment 1

1 – Why BITC can co-distill with water? Observation: B.P.$^{\text{BITC}} = 242-243^\circ$C
2 – How can we explain the differences in the selectivity of the BITC extraction in both methods used in the class?
3 – Based on the theoretical background, suggest other interesting substrates from natural sources.

Questions about experiment 2

1 – Discuss the main differences between basicity and nucleophilicity. How they are usually measured?
2 - What factors can contribute to increase nucleophilicity?
3 – Suggest a general mechanism for the reaction between an amine and BITC exploiting the TS and the solvent influence.