

Mixtures of *S.rebaudiana* Leaves and Stems Waste for *Steviol g. (Steviosides)* Synthesis Using Ethanol-Water Solvents

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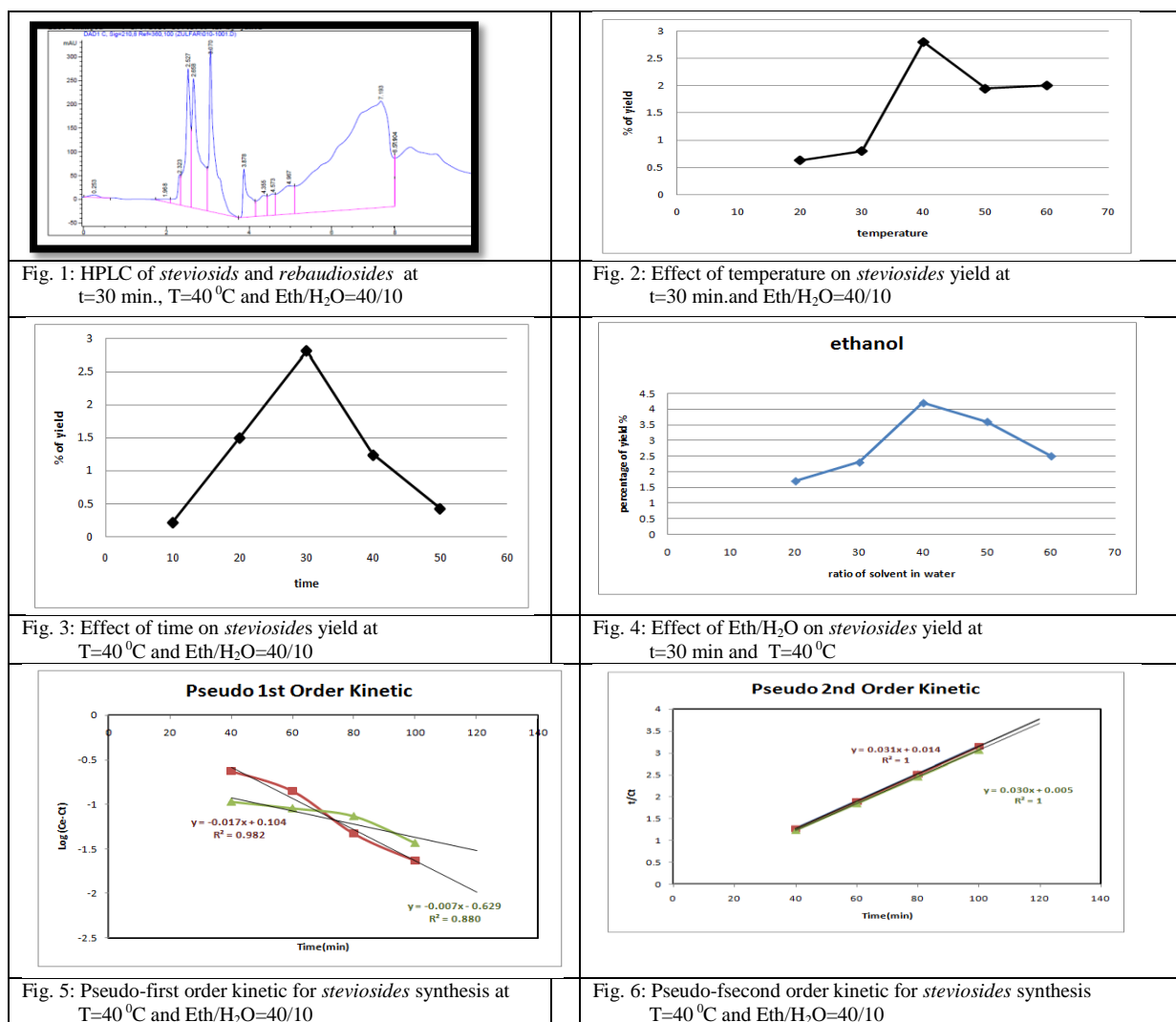
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Abstract

The *s.rebaudiana* leaves is used for *steviol g. (steviosides)* synthesis, but its stems were discarded without processing for added value products, thus this work aim to defeat this issues by using the mixtures of *s.rebaudiana* leaves and stems waste as an alternative feed stock for *steviosides*. The *steviosides* are originating from *S. rebaudiana* plants which has up 300 times sweetener than sugar from sugar cane (1), and better in term of taste quality due to less bitter than *reboudiosides* A (2,3,4). Among 150 *stevia* species, the only one with significant sweet tasting properties is *S. rebaudiana* (7,8). The *stevia* extracts may benefit people at risk of diabetes, as they may help to control blood glucose level (9,10). The *steviosides* synthesis was conducted by ultrasound-assisted extraction (UAE) using ethanol-water as combination of polarity solvents. The mixture of *S. rebaudiana* stems and leaves were dried in open shade then milled to 60 mesh powder using grinder. Then, a sample of 500 mg of the mixture of *S. rebaudiana* stems and leaves powder was sonicated with 50 mL of different percentage of ethanol in water (80:20, 60:40,40:60,20:80,100:0, v/v) in an ultrasonicator bath at various temperature from 20 °C – 50 °C for time of 10 minutes to 50 minutes. After extraction, the supernatant was filtered through microfiber filter disk using vacuum pump filter. The combined percolations were evaporated to dryness on a rotary evaporator at 55°C and dissolved in water. Then the sample were analyzed by High Performance Liquid Chromatography (HPLC) using Eurospher 100 – 5 NH₂ column at flow rate of 1mL/m³ and temperature of 35 °C. Next, the 2.5 mg of the standard solution were prepared by diluting with 80% ethanol in water into volumetric flask respectively in prior to sonicate them for 15 minutes. Beside that, standard solutions were prepared before running the HPLC. The prepared solutions were diluted by the stock solution of 0.25 mg/mL into a 10 ml volumetric flask for concentration ranging from 0.001 to 0. It was found, the mixtures of *s.rebaudiana* leaves and stems has been successfully synthesized for *steviosides*. The synthesis process has been affected by the variation of parameters such as time, temperature, and ratio of solvent to water. The maximum yield of 2.81 % was resulted by the time of 30 min, temperature of 40 °C and ratio of ethanol-water of 40 : 10 (v/v) or 80 : 20 (v/v). The kinetics of *steviosides* synthesis approved the second-order kinetic yielding good R² values from of 0.997 and k values of 0.031.



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