Mathematical modelling of the extraction of essential oils from three Cameroonian plants (Syzygium aromaticum, Citrus sinensis L. and Thymus vulgaris), and optimisation of their inhibitory effects on Phanerochaete chrysosporium and Escherichia coli

Njouond Kamdem Donald

donaldkamdem6@gmail.com

University of Douala

Kenmogne Sidonie Beatrice

University of Douala

Nguikwie Kwanga Sylvie

University of Douala

Wansi Jean Duplex

University of Douala

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Mathematical modelling of the extraction of essential oils from three Cameroonian plants (*Syzygium aromaticum, Citrus sinensis L.* and *Thymus vulgaris*), and optimisation of their inhibitory effects on *Phanerochaete chrysosporium* and *Escherichia coli*.

[*]Njouond Kamdem Donald[a,b], Kenmogne Sidonie Beatrice[a,] Nguikwie Kwanga Sylvie[c] , Wansi Jean Duplex[a] ,

[a]Department of Chemistry, Faculty of Sciences, University of Douala, P.O. Box 24157 Douala - Cameroon

[b]Department of Process Engineering, Saint Jerome Catholic University Institute. Douala - Cameroon

Email: donaldkamdem6@gmail.com

[c]Department of Biochemistry, Faculty of Sciences, University of Douala, P.O. Box 24157. Douala - Cameroon

Email: nguikwie_sylvie@yahoo.fr

***Corresponding author**: Njouond Kamdem Donald

Email: donaldkamdem6@gmail.com

ABSTRACT

The present work employs a dual approach, namely, mathematical modelling of the hydrodistillation extraction of essential oils from Syzygium aromaticum, Citrus sinensis and Thymus vulgaris, followed by optimisation of their inhibitory activity on *Phanerochaete chrysosporium* and *Escherichia coli*. A comparative analysis was conducted on three extraction kinetics: Marafat and Beliard, SOVOVA, and pseudo second order. The SOVOVA model demonstrated the greatest efficacy, with coefficients of determination of 97.4% for S. aromaticum, 99.8% for C. sinensis and 98.9% for T. vulgaris, thus substantiating its capacity to accurately describe distillation kinetics. Subsequent to the procurement of the aforementioned oil yields, an optimisation of the biocidal formula was conducted via a constrained mixing scheme ($C \leq 28\%$ and $T \leq 28\%$) under JMP. The predictive models for the Y1 (MIC against P. chrysosporium) and Y2 (MIC against *E. coli*) responses presented R^2 values of 99.86% and 98.46%, respectively. The optimal minimum inhibitory concentrations were 772.9 µL/L for Y1 and 494.99 µL/L for Y2, which were obtained with 49% S. aromaticum, 23% C. sinensis and 28% T. vulgaris. The robustness of the model was validated by comparing the predictive values with the experimental results, which revealed low relative deviations (5.42% for Y1 and 3.9% for Y2), confirming the reliability of the mixture profile in guiding the formulation of a synergistic biocide.

Keywords: Hydrodistillation, Kinetic modelling, SOVOVA model, Essential oils of Syzygium aromaticum, Citrus sinensis and Thymus vulgaris, Multireaction optimisation, *Phanerochaete chrysosporium, Escherichia coli*, antimicrobial synergy

INTRODUCTION

In recent years, there has been a growing tendency for mankind to utilise a multitude of products derived from nature. Among these products, essential oils, which possess a multitude of beneficial properties, have been employed in a plethora of fields and for a multitude of purposes, including the manufacture of antimicrobial compounds, medicines and food additives. Essential oils are fragrant liquid oils, the composition of which is derived from complex mixtures extracted from a plethora of plant organs, including flowers and seeds [1]. To provide the most accurate description of the process of matter transfer during the extraction of essential oils from *Syzygium aromaticum, Thymus vulgaris* and *Citrus sinensis L.*, several models from the literature were tested. EXCEL software was used to solve the transfer equations numerically after adapting them to our experimental results. Additionally, the oils were subjected to several organoleptic (appearance, color and odor), physical (refractive index and density) and chemical (gas chromatography coupled with mass spectrometry: GC–MS) characterisation methods. Finally, the antimicrobial capacities of the three essential oils were assessed against *Eschiericha coli* (bactericidal effect) and *Phanerochaete chrysosporium* (fungicidal effect) to formulate a biocide for use in the protection of biosourced epoxy resins in cans. These two microorganisms were chosen because *Phanerochaete chrysosporium* is one of the most studied white rot fungi and is considered a model organism for the biodegradation of lignin and various pollutants. Its ability to produce a powerful ligninolytic enzyme system (lignin peroxidase, manganese peroxidase, laccase) has made it a subject of intense research in many fields. *Escherichia coli*, on the other hand, can be genetically modified to express complementary enzymes that facilitate the biodegradation of lignin by products and plastic polymers[2]. The synergistic action of these compounds is based on the ability of P. chrysosporium to fragment industrial lignocellulosic derivatives into smaller molecules, which *E. coli* can then metabolise into biodegradable compounds[2], [3].

Syzygium aromaticum is obtained by harvesting flower buds before they open and then drying them in the sun. The corolla, which is composed of folded petals at the top of *Syzygium aromaticum*, is referred to as the "*Syzygium aromaticum* head". *Syzygium aromaticum* is imported from Indonesia, which is one of the world's largest producers of this spice[4]. *Syzygium* aromaticum contains sugars, gums, tannins, chromones (eugenin and eugenitin), a ketone derivative (eugenone) and an essential oil (16%). The essential oil of *Syzygium aromaticum* is composed primarily of eugenol (80–90%) and eugenol acetate (3%). It is prescribed as a diuretic, stimulant, stomachic, tonic-cardiac, warming and remedy for diseases of the spleen; as a decoction in milk or sugar water; or as an oral powder. For toothache and mouth infections, *Syzygium aromaticum* is chewed or applied to the cheek as a poultice. *Syzygium aromaticum* oleate (oily macerate) is used in rubs as a revulsive and analgesic [5]. It is combined with certain creams and used in Western Sahara to impart shine, suppleness, and vigor to hair. Additionally, it is employed to impart flavour to fruit juices, coffee, and milk.

Thymus vulgaris is a member of the Lamiaceae family and is one of the most widely used plant families worldwide as a source of spices and extracts with interesting medicinal qualities. The essential oil of *Thymus vulgaris* CT thymol has anti-infectious properties and stimulates and regenerates the liver, whereas the essential oil of *Thymus vulgaris* CT thymol is antibacterial but hepatotoxic at high doses. Another advantage of essential oils is that they do not require the addition of chemical preservatives in cosmetic preparations that contain oils, including creams, gels, and ointments [6][7]. The hydrodistillation method has become increasingly popular for the extraction of essential oils from plant materials because of the simplicity of its installation and ease of conduction.

Citrus sinensis L peel is generated in large quantities as an agro-industrial byproduct that deserves to be recycled. This byproduct can even cause environmental pollution problems because of the high chemical and biological oxygen demand associated with it, due to its quantity of essential oil, pectin and sugar if it has not been processed [8]. The incorporation of these compounds into the formulation of certain food products could offer potential avenues for their valorisation. One of the most significant components of *Citrus sinensis L* peel is essential oil [9]. This growing interest is due to the various properties of essential oils, particularly their anticancer, antiviral, antibacterial, insecticidal and antioxidant properties.

Research on *Phanerochaete chrysosporium* has highlighted its key role in the degradation of lignocellulosic products because of its ligninolytic enzymes, particularly lignin peroxidase (LiP) and manganese peroxidase (MnP). These enzymes catalyse the fragmentation of lignin into phenolic compounds, facilitating their biodegradation. Studies have also explored the heterologous expression of these enzymes to improve their efficiency in industrial environments[10].

With respect to *Escherichia coli*, several studies have focused on its potential as a host organism for the expression of proteins involved in the degradation of lignocellulosic polymers. Research has identified and characterised specific enzymes derived from *P. chrysosporium*, such as dioxygenases, which can be expressed in *E. coli* to catalyse the degradation of aromatic compounds derived from lignin[11]. The synergistic action of *P. chrysosporium* and *E. coli* is based on their complementary enzymatic mechanisms. While *P. chrysosporium* breaks down lignin into smaller molecules, *E. coli* can metabolise these byproducts into biodegradable compounds. This synergy is particularly promising for the bioremediation of lignocellulosic plastic waste and industrial effluents containing lignin derivatives.

This solution is both extant and pragmatic; thus, the principal aim of this research is to formulate a biocide complex capable of protecting a biosourced epoxy resin derived from the polymerisation of lignocellulosic derivatives. It is imperative that the product can be protected against any microbiological attack that may prove effective.

A plethora of studies have been conducted on the subject of oil extraction via hydrodistillation methods, with the aim of addressing the separation efficiency, composition and usefulness of essential oils [12]. A number of studies have focused on the effects of microorganisms such as *P. chrysosporium* and *E. coli* on the degradation of organic matter and lignocellulosic residues, but very few have explored the kinetics of hydrodistillation and the synergistic effects of essential oils from *Syzygium aromaticum*, *Citrus sinensis* and *Thymus vulgaris* as biocides for the protection of epoxy resins from biomass in boxes, particularly against the synergistic effects of *P. chrysosporium* and *E. coli.* The undertaking of kinetic studies could contribute not only to the accumulation of fundamental knowledge but also to the improvement of the optimisation, operation and industrial processes of distillation. Kinetic models play a pivotal role in hydrodistillation processes, both in terms of technological advancement and the economic prospects of the sector.

The formulation of a biocide targeting *Phanerochaete chrysosporium* and *Escherichia coli* could offer several major benefits. First, it could protect lignocellulosic materials and their polymer derivatives against biodegradation, thereby extending their lifespan in industrial applications. Second, it could help preserve wooden infrastructure, particularly that exposed to humid environments where these microorganisms proliferate. The employment of an efficacious biocide has the potential to mitigate the risk of microbiological contamination in industrial processes that utilise biobased materials.

1. MATERIALS AND METHODS

The following experimental procedure was employed:

1. 1. Biological material

The plants *Citrus sinensis L.*, *Syzygium aromaticum* and *Thymus vulgaris* were harvested from Bertoua in the East Cameroon region. The different plants were used 4 days after harvest for *Citrus sinensis L.*, 19 days after harvest for *Syzygium aromaticum* and 9 days after harvest for Thymus vulgaris.

The processing of *Syzygium aromaticum* involved sorting and brief rinsing before hydrodistillation in a 2.5 L flask with a 1:3 matter-to-water ratio. Following distillation, a mixture of hydrolysate and essential oil was obtained. The essential oil was dehydrated with anhydrous sodium sulfate to remove residual water, filtered with Wattman filter paper and stored in amber glass jars until analysis and use.

The dead branches of *Thymus vulgaris* were removed and distilled without prior rinsing, using a 1:3 ratio of material to solvent. Following distillation, a mixture of hydrolysate and essential oil was obtained. The essential oil was dehydrated with anhydrous sodium sulfate to eliminate residual water, filtered with Wattman filter paper and stored in amber glass jars until analysis and use.

The citrus fruits were washed in tap water, peeled and cut into small pieces approximately 1 cm2 in circumference on the fifth day after harvest. The pieces (1 kg) were then introduced into a 2.5 L flask for hydrodistillation with a matter-to-water ratio of 1:3. Following the distillation process, a mixture of hydrolysate and essential oil was obtained. The essential oil was dehydrated with anhydrous sodium sulfate to remove residual water, filtered with Wattman filter paper and stored in amber glass jars until analysis and use.

The organoleptic characteristics (appearance, color and odor) of the three samples were recorded. Ten milliliters of each sample was poured into a 20-milliliter glass. To observe the sample, it was ensured that there was sufficient light, that direct light was avoided and that there were no sources of color nearby and no strange odors. The test samples were prepared in accordance with TCVN 11424:2016.

Physical properties: The physical properties of the three essential oils, particularly their density and specific gravity, were determined. The density was determined via the following calculation method:

$$\varphi = \frac{sample\ mass}{volume\ of\ the\ weighed\ sample} \quad (1)$$

To determine the density, it was first necessary to weigh a clean, dry bottle. This was then followed by the addition of distilled water to the bottle, which was also weighed. Finally, the same volume of essential oil was added to the same bottle, which was also weighed.

$$density = \frac{mass\ of\ a\ precise\ quantity\ of\ oil}{mass\ of\ an\ equal\ volume\ of\ water} \quad (2)$$

The calculation of essential oil yields was based on the various yields obtained in this work, which were calculated on the basis of fresh plant matter.

$$Yield(\%) = \frac{mass\ of\ oil\ extracted}{mass\ of\ friache\ starting\ material} \times 100 \quad (3)$$

1. 2. Kinetic modelling of the hydrodistillation of essential oils from our three plants

Extraction modelling is applied to assess the variable conditions affecting extraction. It is also recognised as an essential step in the design of an efficient extraction method [13].

The first-order kinetics are given by the following equation:

$$-\frac{dm}{dt} = km \text{ when } -\frac{dm}{dt} = kdt \text{ when } ln\frac{m_0}{m} = kt \text{ when } ln\frac{1}{1-u(t)} = kt \quad (4)$$

where m is the concentration of essential oil extracted at time t, m0 is the initial concentration of essential oil before the start of extraction, and u(t) is the fraction of essential oil extracted [14].

To find the model that best fits our different extractions, several models were tested, and the parameters related to these models as well as the coefficients of determination were determined.

1. 2.1. Mafarat and Béliard model

This model is based on the premise that matter transfer is constrained by convection in the liquid film. The solute mass balance is expressed by equation (5):

$$\dot{m} = \frac{dC_s(t)}{dt} = -KA\big(C_s(t) - C_l(t)\big) = -K'(C_S(t) - C_l(t)) \qquad (5)$$

The mass flow rate of the solute transferred (ṁ) is expressed in grams of solute per kilogram of solid matter per second. The overall coefficient of matter transfer per unit area (K) is given in metres squared per minute. The exchange area of the solid phase (A) is expressed in square metres. The overall coefficient of matter transfer (K') is given in minutes per unit. Finally, Cs(t) and Cl(t) are the concentrations of solute in the solid and liquid phases, respectively, expressed in grams of solute per kilogram of solid and grams of solute per kilogram of liquid. The concentration of solute in the solid phase (in g of solute. kg-1 of solid) and Cl(t), the concentration of solute in the liquid phase (in g of solute. kg-1 of liquid), are also considered. The authors assumed that diffusion rather than solubilisation is the limiting process [15]. The solute partition coefficient between the two phases is equal to unity, and the loss of mass in the solid phase due to solute diffusion is compensated by an equivalent gain in mass of solvent migrating into the solid phase [16]. The solute mass balance can also be written as equation (6).$sMC_s(0) = sM\overline{C_s(t)} + (1-s)MC_l(t)$ (6)

where M (g) is the total mass of the filtered solution, s (dimensionless) is the mass of the solid phase relative to the total mass of the solution, and Cs (0) (g.kg-1) is the initial solute content in the solid phase. By combining equations (5) and (6), the Mafarat-Béliard model can be defined by equation (7), which is called the Mafarat-Béliard I model.

$$C_l(t) = sC_S(0)\left[1 - e^{-\left(\frac{K'}{1-S}\right)t}\right] \qquad (7)$$

This equation can be expressed in another equivalent form (equation (6)), known as the Mafarat and Béliard II model. The product of the ratio s and Cs (0) is equal to the solute concentration of the solid phase after an infinite time C_l (∞). λ is the ratio between the total mass transfer coefficient K' and the 1-s coefficient.

$$C_l(t) = C_l(\infty)\left[1 - e^{-\lambda t}\right] \qquad (8)$$

The introduction of a power b in equation 7 makes the latter pseudodiffusive, known as the Mafarat and Béliard pseudodiffusive model (equation 8), and improves the result of this model.

$$C_l(t) = C_l(\infty)\left[1 - e^{-\lambda t}\right]^b \qquad (9)$$

To test their validity, it is necessary to compare the predictions of these models with experimental measurements [17][18].

Sovová [17] developed a model that takes into account cells broken during the grinding stage and intact cells. This model is written for two types of particles: spherical and flat.

1. 2. 2 Sovová model

As demonstrated in Figure 2, the model under consideration processes two distinct types of particles.

a. Model assumptions

To model the matter transfer process, simplifying assumptions must be made. These included the following:

- Essential oils can be considered single compounds.
- The particles are identical in size, shape and essential oil content.

For each particle:

- The fraction of essential oil in the broken cells of the plant was noted as 'r'.
- The fraction of essential oil in intact plant cells is noted as '1-r'.

The system is considered to behave like a fluidised bed with three phases (solid, liquid and vapour).

In mass balance, the mass of water in the vapour phase, in the condenser and in the separator is neglected in relation to the mass of water in the flask.

$$x_v = k_v x_w = k_v k_w x_b \qquad (10)$$

The following variables are defined:

X_v: mass concentration of oil in the vapour phase, g (oil)/kg (vapour)

x_s: mass concentration of oil remaining in the hydrosol, g (oil)/kg (water)

x_w: mass concentration of oil in boiling, g (oil)/kg (vegetable matter)

The liquid–vapour partition coefficient, k_v

The liquid/broken cell partition coefficient, k_w

The initial concentration in the core of particles composed of broken cells is equal to the concentration of untreated particles (x_u):

$$x_i(t = 0) = x_u \qquad (11)$$

The symbol "x_u" represents the mass concentration of untreated essential oil, expressed in grams (oil) per kilogram (dry matter).

The concentration of essential oil in water (hydrolat) is assumed to be constant (xs). The yield over time is calculated as follows:

$$Y = \frac{Q}{N}\int_0^t (x_v - x_s)dt \quad (12)$$

The commencement of the extraction process is defined as the instant at which the initial droplets of condensation reach the separator.

The mass balance of the essential oil within the extractor assumes that equilibrium is established between the broken cells, the boiling water and the vapour phase almost instantaneously[16]. Consequently, the transfer of material for intact cells is simulated as a quasistationary process, with the evolution of the solute fraction over time written as follows:

$$-\frac{dx_i}{dt} = \frac{k}{\lambda}(x_i - x_b) \quad (13)$$

At $t = 0$, a proportion of the essential oil present in the readily accessible broken cells has already been dissolved in the boiling water. In this instance, the essential oil balance in the broken cells and in the boiling water is expressed as follows:

$$rN(x_u - x_b) = N_w x_w, pour\ t = 0, \quad (14)$$

In consideration of the initial hypotheses, upon traversing the global mass balance of the essential oils in the extractor for homogeneous particles and integrating the equation of this mass balance, we obtain:

$$C_\infty = \frac{Q_r}{N} = x_u(1 - \beta(1 - \gamma k_w)) \quad (15)$$

1. 2.3 Pseudo-second-order kinetic model

In accordance with the second-order kinetic law, the rate of dissolution of the material to be extracted from the plant into the solution is described by equation (4).

$$\frac{dC_t}{dt} = k(Cs - Ct)^2 \quad (16)$$

The second-order extraction kinetic constant (k), the extraction capacity (Cs) and the solute concentration in solution (Ct) are the variables in this equation. The equation can be solved by making several assumptions[19][20]. The extraction process

is assumed to be equivalent to the diffusion of solute from plant cells into the solution[21]. Consequently, the solute concentration at saturation of the solution under the same conditions was considered to be constant (6)[22]. In the initial and boundary conditions, from time t = 0 to t, where the solute concentration varies from Ct = 0 to Ct, the integration of the second-order kinetic law leads to $C_t = \frac{kC_s^2}{1+C_s kt}$ (17)

1.3. Antibacterial assays

1.3.1. Agar–disc diffusion method

The essential oils were first screened for their antibacterial activity against one bacterial strain (*Escherichia coli*) via the agar–disc diffusion method according to CLSI guidelines (CLSI, 2012), with some modifications. Briefly, a fresh bacterial suspension prepared in sterile saline and adjusted to 0.5 McFarland was used to inoculate Mueller–Hinton agar plates (Biokar diagnostics, Beauvais, France). Then, sterile paper discs (6 mm in diameter) were applied to the surface of each plate and impregnated with 10 µL of essential oil. The plates were incubated at 4°C for 2 h and then incubated at 37°C for 18–24 h. The diameters of the inhibition zones were measured in millimeters. All the experiments were carried out in triplicate.

1.3.2. Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the formulated biocide was determined via the liquid microdilution method, which allows the antimicrobial activity against bacteria and fungi to be assessed. This approach is in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI).

a. Preparation of inoculum and dilutions

The inoculum was adjusted to a standardised density of 0.5 McFarland (~10^8 CFU/mL for bacteria and ~10^7 CFU/mL for fungi). Serial dilutions of the extract were

performed in an appropriate culture medium (Mueller–Hinton broth for bacteria and RPMI 1640 for fungi)[23]. The microplates were then subjected to an incubation period at 37°C for bacteria and 28–30°C for fungi, with a duration of 24–48 hours. Confirmation was performed via resazurin staining.

1.4.Mixture design and statistical analysis

To assess the ternary antimicrobial effects of the essential oils studied, a centroidal mixing design was employed, incorporating upper constraints for the essential oils of *Thymus vulgaris* and *Citrus sinensis L*., with a resulting value of X less than or equal to 0.28. The factors represent the fraction of each essential oil in the mixture, which varies from 0 to 1 only for *Syzygium aromaticum* essential oil and from 0 to 0.28 for *Thymus vulgaris* and *Citrus sinensis L*. in the design space. Constraints were established with respect to the yield of essential oils from both *Thymus vulgaris* and *Citrus sinensis L*., as well as the recognised antimicrobial properties of the essential oils derived from Thymus vulgaris L.fi

Figure 3 shows the points of the designed experiments. The vertices of the triangle (1, 2, 3) correspond to the three essential oils alone. The midpoints of the three sides of the triangle (4, 5, 6) consist of binary combinations and the central point (centroid) and the three augmented points (8, 9, 10) ascribed to the ternary combinations. As shown in Table 1, the complete experimental design for each microorganism consisted of 9 experiments, including one replication of the central point. The experiments were performed in random order (Table 1).

The dependent variables (responses) were the MICs against *E. coli* and *Phanerochaete chrysosporium*, which were assessed via the microdilution method as described in Section 1.3.2.

Afterwards, the data were fitted to a special cubic polynomial model applying least squares regression to estimate the unknown coefficients in Eq. (18):

$$Y = b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3$$

where Y is the response, bi is the magnitude of the effect of each component X_i, b_{ij} is the magnitude of the interactive effect of two components and b_{ijk} is the magnitude of the interactive effect of the three components on the response. X_i denotes the proportion of component (i) in the mixture. This analysis was carried out via SAS JMP Pro software, version 17.

2. RESULTS AND DISCUSSION

2.1 Sensory and physicochemical characteristics of the essential oils of Syzygium *aromaticum, Citrus sinensis L.* and *Thymus vulgaris*.

The essential oils extracted from *Thymus vulgaris*, *Syzygium aromaticum*, and *Citrus sinensis* presented notable differences in both sensory and physicochemical parameters (Table 1), which can be attributed to the distinct phytochemical profiles and structural features of the plant materials.

In terms of essential oil yield, *Syzygium aromaticum* presented the highest yield (4.054%), followed by Thymus vulgaris (0.359%) and [24]*Citrus sinensis* (0.222%). The high yield of *S. aromaticum* is consistent with its well-documented richness in volatile oils, particularly eugenol, which constitutes more than 80% of clove essential oils[25]. In contrast, *T. vulgaris* and *C. sinensis* are known to contain lower essential oil contents, which is in agreement with previous reports [24][26].

In terms of density, *S. aromaticum* oil was the densest (1.0135 g/mL), reflecting the presence of heavier aromatic compounds such as eugenol. Essential oils with densities greater than those of water are typically rich in oxygenated compounds [27]. *T. vulgaris* and *C. sinensis* oils presented relatively low densities (0.8700 and 0.8197 g/mL, respectively), which is characteristic of oils with relatively high contents of monoterpenes such as thymol and limonene.

The moisture content in the plant materials prior to extraction ranged from 6.78% (*S. aromaticum*) to 11.14% (*T. vulgaris*). The lower moisture content of *S. aromaticum* may contribute to more efficient oil recovery, as excess water in plant tissues can inhibit oil diffusion during distillation [28]. Conversely, higher moisture in *T. vulgaris* and *C. sinensis* may dilute the oil concentration or lead to partial hydrolysis of sensitive compounds during extraction.

Sensory analysis further supported the chemical distinctions between the oils. *T. vulgaris* oil is pale yellow, bitter, and herbaceous, a profile attributed to thymol and carvacrol, two bioactive monoterpenes with antimicrobial and antioxidant properties [24]. *S. aromaticum* oil is colourless with a spicy odour and taste, which is consistent with its high eugenol content, which also imparts analgesic and antiseptic properties [25]. *C. sinensis* oil is pale yellow, sweet in taste, and citrusy in odor, reflecting the predominance of limonene, a compound widely used in perfumery and food flavouring for its pleasant fragrance and mild taste [26].

Overall, the combination of yield, physicochemical parameters, and sensory profiles provides insight into the potential applications of these oils. *S. aromaticum* appears to be more suitable for medicinal and antimicrobial use because of its high yield and potent sensory profile. *T. vulgaris* offers a balance between aromatic complexity and moderate yield, whereas *C. sinensis*, despite its lower yield, remains valuable in the food and cosmetic industries because of its sweet and refreshing properties.

Following the evaluation of sensory and physicochemical properties, the extraction kinetics of the essential oils from *Thymus vulgaris*, *Syzygium aromaticum*, and *Citrus sinensis* were investigated to understand the dynamics of oil release during hydrodistillation."

2.2. Experimental Oil Extraction

The extraction kinetics of essential oils from *Thymus vulgaris*, *Syzygium aromaticum*, and *Citrus sinensis* under hydrodistillation revealed marked differences in both yield and extraction profiles (Figure 4).

T. vulgaris presented the highest essential oil yield, reaching approximately 5% within 600 min, after which the yield plateaued. This rapid and early increase suggests a high essential oil content and a favourable matrix for distillation, which is consistent with previous findings on Lamiaceae species, which are known for their high glandular trichome density facilitating oil release [29]. In contrast, *S. aromaticum* and *C. sinensis* presented significantly lower yields, with both remaining below 1% even after prolonged extraction. The extraction profiles for these species gradually increased without clear plateaus, indicating either a lower concentration of essential oils or a more resistant matrix. In particular, for *C. sinensis*, the yield was less than 0.5%, which aligns with prior reports that citrus peels yield modest amounts of oil under hydrodistillation due to their thicker epidermal layers [12]. The extraction behavior also suggests different diffusion and desorption mechanisms among the plant matrices. For T. vulgaris, oil release appears to follow a rapid extraction phase dominated by washing or surface oil diffusion, followed by a slower phase likely governed by internal diffusion. This biphasic pattern is less evident in *S. aromaticum* and *C. sinensis*, where the rate of extraction remains nearly linear, indicating possible resistance to mass transfer or low oil content. These observations underscore the importance of plant species and matrix properties in determining essential oil yield and suggest that T. vulgaris is the most promising candidate among the three for industrial-scale essential oil recovery via hydrodistillation. Following the yield evaluation, three mathematical models were employed to simulate and compare the kinetics of essential oil extraction for the three plant species.

2.3. Comparative evaluation of the kinetics models

Table 2 presents the kinetic parameters of the three extraction models. Sovova, Marafat and Béliard, and pseudo-second-order methods were applied to essential oils extracted from *Syzygium aromaticum, Thymus vulgaris,* and *Citrus sinensis L.* The objective was to identify the most appropriate model for describing the extraction kinetics on the basis of the fitted parameters and the determination coefficient (R^2).

- *Syzygium aromaticum*

The Sovova model provides a very good fit, with a high determination coefficient ($R^2 = 0.974$), indicating that it accurately describes the extraction kinetics. In contrast, the Marafat and Béliard model yields a very poor fit ($R^2 = 0.089$), suggesting that it is inadequate for representing this system. The pseudo-second-order model shows a moderate fit ($R^2 = 0.23$), which is better than those of the Marafat and Béliard models but significantly less satisfactory than those of Sovova.

- *Thymus vulgaris*

The Sovova model also demonstrated excellent agreement with the experimental data for T. vulgaris, with $R^2 = 0.989$. The Marafat and Béliard model again performs poorly ($R^2 = 0.138$), and the pseudo second-order model yields an almost negligible fit ($R^2 = 0.002$), making it unsuitable.

- *Citrus sinensis L.*

In the case of C. sinensis, the Sovova model provides the best fit overall ($R^2 = 0.998$). The pseudo-second-order model also shows a good fit ($R^2 = 0.89$), although it is slightly inferior to Sovova. The Marafat and Béliard model again displays poor performance ($R^2 = 0.231$).

The Sovova model, which was originally developed for supercritical CO_2 extraction, incorporates mass transfer between the solid and fluid phases and has been shown to be adaptable to thermal extraction processes such as hydrodistillation [30]. The consistently poor performance of the Marafat and Béliard models may be attributed to their limited capacity to represent the complexity of mass transfer within the plant matrix. The pseudo-second-order model, typically used for adsorption kinetics [31], provides acceptable fits only in the case of C. sinensis.

The Sovova model (figures 2, 3 and 4) demonstrated the highest determination coefficients across all three essential oils, indicating its superior capacity to describe extraction kinetics. It is therefore recommended for future modelling and optimisation of essential oil extraction from these plant materials.

2 .4. Interpretation of GC–MS Chromatograms

Figure 5 presents the GC–MS chromatograms of essential oils from Syzygium aromaticum, Thymus vulgaris, and Citrus sinensis L. from top to bottom. Each chromatographic profile reveals a complex mixture of volatile organic compounds with varying retention times and mass–charge ratios (m/z), indicative of a broad range of chemical structures and volatilities.

2.4.1 *Syzygium aromaticum*

The chromatogram of *S. aromaticum* (top) shows several prominent peaks, with one major peak at approximately 6.54 minutes, corresponding to eugenol (C10H12O2), which is consistent with previous reports identifying this phenolic compound as the primary constituent of clove essential oil [26]. The additional peak included methyl salicylate (RT = 4.77 min), a known anti-inflammatory ester [32][33]. Sesquiterpenes such as α-cubebene (RT = 4.84 min), α-humulene, and β-caryophyllene have been reported [34][35][36]. Recent GC–MS studies have confirmed that eugenol can account for up to 84.63% of the total oil composition, followed by β-caryophyllene (23.58%) and eugenol acetate (11.37%) [37]. These findings support the recognised antioxidant, antimicrobial, and anti-inflammatory activities of clove oil [25].

2.4.2 *Thymus vulgaris*

The middle chromatogram corresponds to *T. vulgaris* and is characterised by numerous peaks, notably between 6 and 20 min, indicating the presence of monoterpenes and oxygenated derivatives. The key constituents identified include thymol (RT = 19.199 min) and carvacrol (RT = 18.701 min); potent phenolic monoterpenes with known antimicrobial and antioxidant effects [24] [38]; and p-cymene, γ-terpinene, terpinen-4-ol, and carvacrol methyl ether, which contribute to the characteristic spicy aroma of thyme oil[38], [39][40][41]. According to recent findings, thymol constitutes up to 47.59% of the oil, γ-terpinene 30.90%, and p-cymene 8.41%, which confirms the strong bioactivity of the thyme essential oil.

2.4.3 *Citrus sinensis*

The chromatogram at the bottom represents *C. sinensis* oil, where the most intense peaks are observed below 10 minutes, confirming the dominance of monoterpene hydrocarbons, which are generally more volatile. The major components include α-thujene, β-pinene, and limonene (although not listed in Table 3, often

predominant in citrus oils) [39][42][43] and sesquiterpenes such as humulene, isoledene, and β-caryophyllene, which are observed at longer retention times (>18 min) [35][36][44]. Recent analyses of C. sinensis leaf oils reported high proportions of sabinene (8.25–28.81%), 2-carene (11.25–16.72%), and cis-β-ocimene (10.22–13.93%) [45][46]. These compounds are known for their antioxidant and aromatic properties.

2.5. Comparative Analysis of Essential Oil Profiles

This comparison highlights the distinctive chemical fingerprints and bioactivities of each essential oil. The dominance of eugenol in *S. aromaticum*, thymol in *T. vulgaris*, and monoterpenes in *C. sinensis* underlines their pharmacological and aromatic applications. These findings support their traditional uses and are consistent with contemporary research [16–44].

2.6. Analysis of Essential Oil Composition

Table 3 presents the chemical compositions of the essential oils extracted from *Syzygium aromaticum, Citrus sinensis L*., and *Thymus vulgaris*. A total of 36 volatile compounds, mainly monoterpenes, sesquiterpenes, oxygenated terpenes, phenols, and esters, were identified. This chemical diversity reflects the complex nature of essential oils and their wide range of biological activities [16–44]. The compounds exhibited retention times ranging from 4.770 to 22.068 minutes, indicating a broad spectrum of volatility and polarity. In general, more volatile and polar compounds (e.g., esters and alcohols) eluted earlier, whereas heavier sesquiterpenes appeared later.

The chemical profiles of the essential oils revealed the presence of several bioactive compounds with therapeutic and industrial potential. The predominance of phenolic compounds (e.g., eugenol and thymol), monoterpenes (e.g., p-cymene and β-pinene), and sesquiterpenes (e.g., β-caryophyllene) support their use as natural antimicrobial, anti-inflammatory, and antioxidant agents. These findings are in agreement with those of previous studies [16–44] and provide valuable insight into the phytochemical richness of these Cameroonian medicinal plants.

2.7. Optimal biocide composition for inhibiting the growth of *E. coli* and *Phanerochaete chrysosporium*

The present study adopted a mixture design methodology with upper constraints to optimise the inhibition of *E. coli* and *Phanerochaete chrysosporium* by minimising

the MIC (minimum inhibitory concentration) of a mixture of essential oils from *Syzygium aromaticum*, *Thymus vulgaris* and *Citrus sinensis L.*

Preliminary experiments were conducted to ascertain the effective sensitivity of the two types of microorganisms tested to the three essential oils studied. The results of the present study demonstrated that the growth of *Phanerochaete chrysosporium* was inhibited at minimum concentrations of 3581.45, 1584 and 2589 µL/L for the essential oils of *Syzygium aromaticum*, *Citrus sinensis* and *Thymus vulgaris, respectively*. Similarly, the growth of *E. coli* was inhibited at minimum concentrations of 1958.75, 929.26 and 2512.45 µL/L for the same oils.

2.7.1 Model of the mixing plan methodology and its suitability

The minimum inhibitory concentrations of the mixtures were obtained and analysed for nine different mixtures, the results of which are presented in Table 4. The values of these minimum inhibitory concentrations ranged from 694.44 to 2777.77 µL/L for *Phanerochaete chrysosporium* and from 694.44 to 1277 µL/L for *E. coli*. Second-order polynomial models were constructed for the two concentrations (Y_1 for *Phanerochaete chrysosporium* and Y_2 for *E. coli*) from the experimental data. These are expressed by equations 19 and 20:

$$Y_1 = 21286.28 - 18508.51X_1 + 5258.73X_2 - 422223.98X_1X_2 - 34942.35X_1X_3 - 127994X_2X_3 + 162890.85X_1X_2X_3 \quad (19)$$

$$Y_2 = -507.95 + 2902.39X_1 + 17879.74\,X_2 - 26747.58X_1X_2 - 1597.32X_1X_3 - 28034.55X_2X_3 + 16147.08X_1X_2X_3 \quad (20)$$

The minimum inhibitory concentration (MIC) of the mixture on *Phanerochaete chrysosporium* was denoted by Y1.

Y2 is defined as the minimum inhibitory concentration of the mixture on *E. coli*.

X1 is expressed as the percentage of essential oil derived from *Syzygium aromaticum*.

X2 is the percentage of essential oil derived from *Citrus sinensis L.*

X3 represents the percentage of essential oil derived from *Thymus vulgaris*.

2.7.2. Statistical analysis

The relevance of the mixture design models was verified via analysis of variance (ANOVA) and the coefficient of determination (R^2) of the MIC. ANOVA revealed significant results for the models at a 95% confidence interval. The determination of significant contributions was based on the probability values (P values) of the F test, as illustrated in Table 5.

The P value for the two responses was lower by 0.004 and 0.045 for Y1 and Y2, respectively (Y_1 and Y_2 represent the minimum inhibitory concentrations of the mixtures on *Phanerochaete chrysosporium* and *E. coli*, respectively), revealing that the

models were highly significant. The P value for the lack of fit for the response was not significant ($P < 0.05$), thus confirming the relevance of the models. The R^2 values for Y_1 and Y_2 were 99.86 and 98.46, respectively. As the values were greater than 0.80, the observed and predicted values of all the variables were considered to be strongly correlated [47]. Consequently, the proposed models were deemed adequate for representing the relationships between the independent variables and the response variables.

2.7.3. Optimal proportions for inhibition at minimum concentrations

The numerical optimisation of the mixture was conducted via JMP PRO 16 software, with the process informed by the findings of experimental investigations. The software in question is utilised for the purpose of conducting searches, the objective of which is to identify all possible combinations of factor levels whose requirements are imposed on responses and factors in a simultaneous manner.

To determine the most efficacious combinations, three independent variables (the proportions of essential oils from *Syzygium aromaticum* (X_1), *Citrus sinensis L.* (X_2) and *Thymus vulgaris* (X_3)) were defined within the ranges delineated in Table 4, and the MICs (Y_1 and Y_2) were set to the minimum values. The optimal proportions for the simultaneous inhibition of both microorganisms were as follows: 0. The study utilised a blend of essential oils, comprising 49% *Syzygium aromaticum*, 0.23% Citrus sinensis, and 0.28% *Thymus vulgaris*, to achieve complete inhibition of *Phanerochaete chrysosporium* at a minimum concentration of 772.9 µL/L and of *E. coli* at a minimum concentration of 494.99 µL/L. The study achieved a desirability of 0.95%, demonstrating the efficacy of the essential oil blend in addressing the target organisms. To achieve complete inhibition of microbial activity in the two microorganisms under investigation, the value selected for this purpose would be the higher of the two selected, i.e., 772.9 µL/L.

The adequacy of the model was verified by comparing the experimental and predicted values (see Table 6). The relative errors between the predicted and experimental values were found to be less than 6%. The findings indicate that the mixing design methodology is a viable approach to optimise the effects of diverse oils on the microorganisms under investigation and that the regression models delineating the relationships between the various proportions and the characteristics of the mixtures obtained were precise and sufficient.

2.7.4. Optimal Composition and Impact on MIC

The mixture profiler (Figure 6) derived from the JMP PRO design identified a formulation with maximum bacterial inhibition at S = 49%, C = 23%, and T = 28%, respecting the constraints *Citrus sinensis L.* and *Thymus vulgaris* ≤ 28%. The combination of these constituents is advantageous because of the presence of clove eugenol (representing approximately 80% of S. oil), the principal antibacterial agent, sweet orange limonene, which facilitates membrane penetration, and thymol, which

complements the biocidal effect. The high R^2 values (99.86% for Y_1 and 98.46% for Y_2) confirm the model's fidelity to the experimental data, with simultaneous minimisation of MIC_1 and MIC_2 within the formulation space. The red and blue curves that intersect the profile are known as iso-response curves, denoting the trajectories on which all compositions exhibit equivalent responses of Y_1 and Y_2. The area under consideration is delineated by the values of the variables and their constraints (lozane area).

2.7.5. Molecular Mechanisms of Synergy

A number of studies have been conducted that provide evidence for a synergistic interaction between eugenol and thymol. As demonstrated by Sateriale *et al*., the combination of *Thymus vulgaris* and *Syzygium aromaticum* exhibited a substantial inhibitory effect on the growth of *Escherichia coli* and *Staphylococcus aureus*, with an FIC observed in both the liquid and vapour phases[48]. In this mechanism, eugenol disrupts the lipid bilayer, thereby creating a path for thymol to penetrate. Thymol, in turn, has been shown to disrupt intracellular enzymes and generate oxidative stress. Conversely, limonene modulates membrane fluidity and enhances the diffusion of phenols, thereby increasing the overall effectiveness of the mixture.

2.7.6. Comparison with literature data and practical constraints

Compared with unit extractions, the MIC of isolated T. vulgaris (0.021 mg/mL for *E. coli*) was exceeded by the optimised formula, thereby demonstrating the synergistic contribution of *Syzygium aromaticum* and *Citrus sinensis L*. The restriction of *Citrus sinensis L.* and *Thymus vulgaris* to 28% also aligns with industrial imperatives: volatile limonene operates in the vapour phase to extend the antibacterial activity, but its excessively high proportion could compromise the stability of the product. In conclusion, the increased expense of thymol and the safety recommendations (which indicate a potential for skin irritation beyond 30%) necessitate the calibration of its dosage. This justifies the upper limit retained for T in the profile.

2.7.7. Experimental validation and model robustness

To confirm the reliability of the JMP profiler, leave-one-out cross-validation and an independent goodness-of-fit test were performed according to the CLSI guidelines for broth microdilution [49]. These tests verified that the high R^2 values (99.86% for Y_1 and 98.46% for Y_2) were not the result of overfitting and that the MIC minimisation was reproducible outside the calibration sample, with potential margins of errors of 5.42 and 3.9% for Y1 and Y2, respectively (Table 6).

Conclusion

This approach, which combines kinetic modelling and a constrained mixing plan, has enabled the production of an optimised formulation of essential oils with very high antifungal and antibacterial activity. This has been achieved while controlling the composition for reasons of cost, safety and stability. The correlation between prediction and experimentation serves to reinforce the relevance of this system in the context of the development of natural biocides.

To extend this work, it will be necessary to test biocides under real conditions for the conservation of biosourced epoxy polymers. The durability of the antimicrobial effect in the can, the interaction with the polymer matrix, and the impact on the mechanical and aging properties are the next challenges to be met.

Authors' contribution statement

The lead author, NJOUOND KAMDEM Donald, was responsible for all experimental work, data analysis and the composition of the entire manuscript.

Co-author Kemmogne Sidonie Beatrice contributed to experimental planning, methodology definition and results validation.

Co-author Nguikwie Kwanga Sylvie contributed to results validation.

The supervision of all work was overseen by co-author Wansi Jean Duplex, who also provided regular scientific guidance and validated the final version of the manuscript.

All authors have perused and endorsed the final version of the document, and consent to its submission to the journal Discover Chemistry.

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Plant guidelines and Permissions to collect the plants/plant parts: It is noteworthy that none of the plant specimens utilised in this research are designated as protected species under Cameroon's prevailing forestry regulations.

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Figures

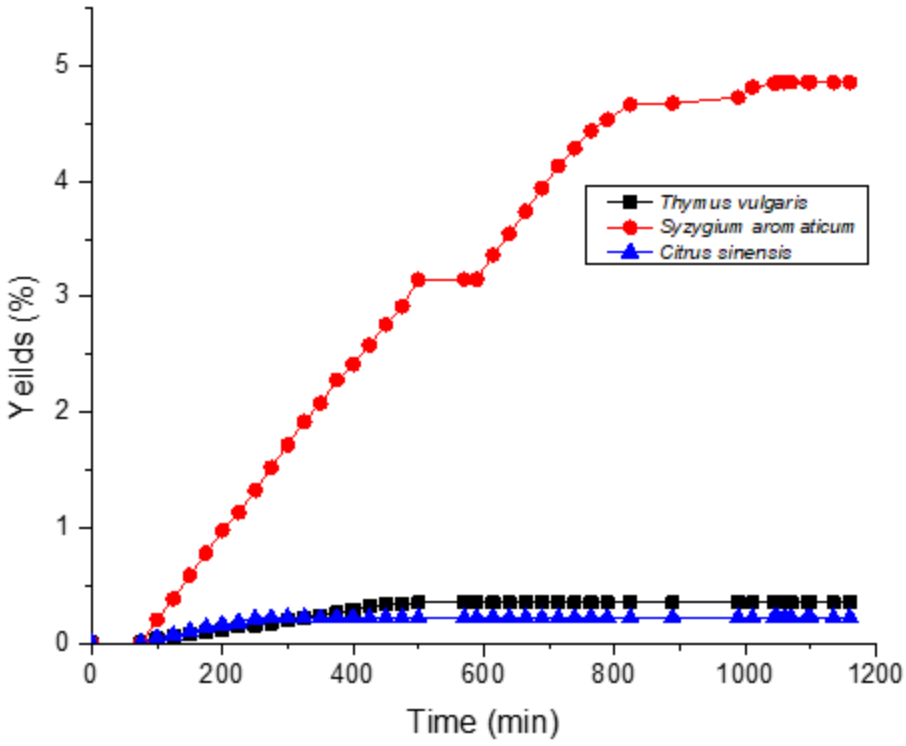

Figure 1

Yeilds evolutions of extraction of *Thymus vulgaris, Syzygium aromaticum* and *Citrus sinensis* essemtials oils over the time

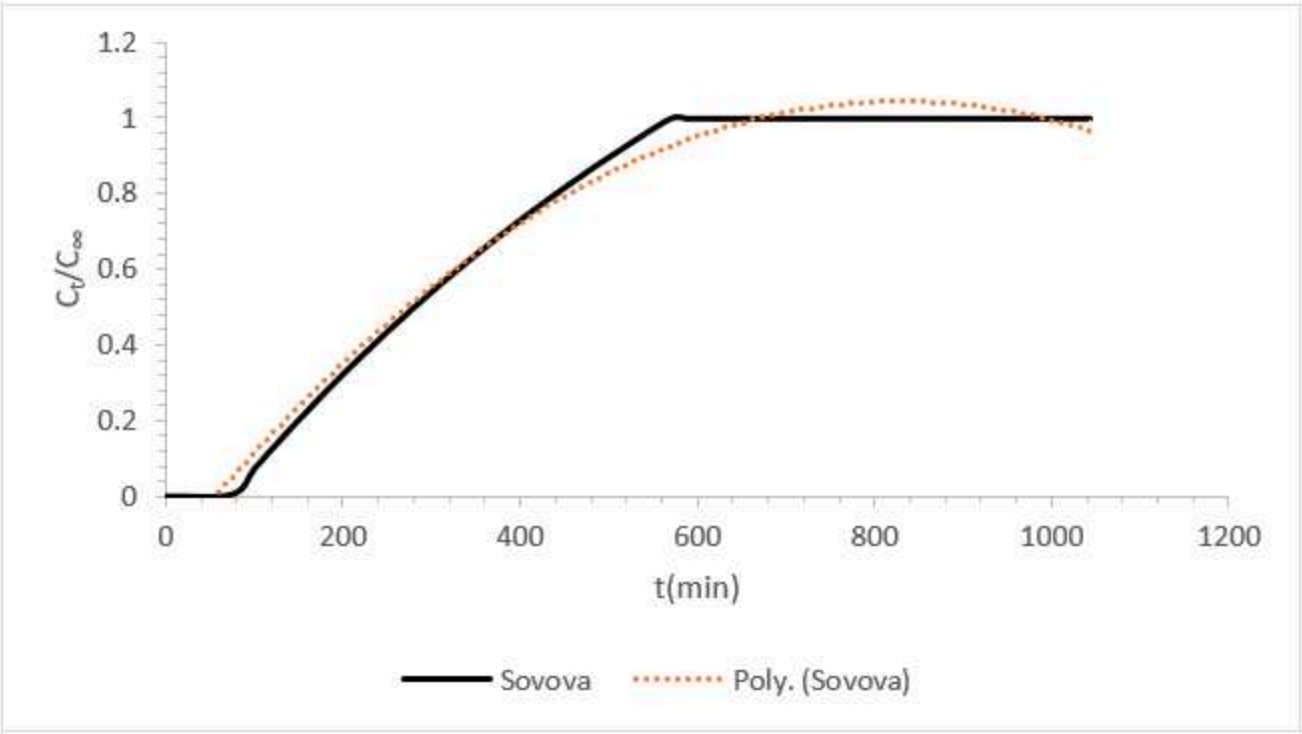

Figure 2

Sovova model applied to extraction of *Thymus vulgaris* essential oil

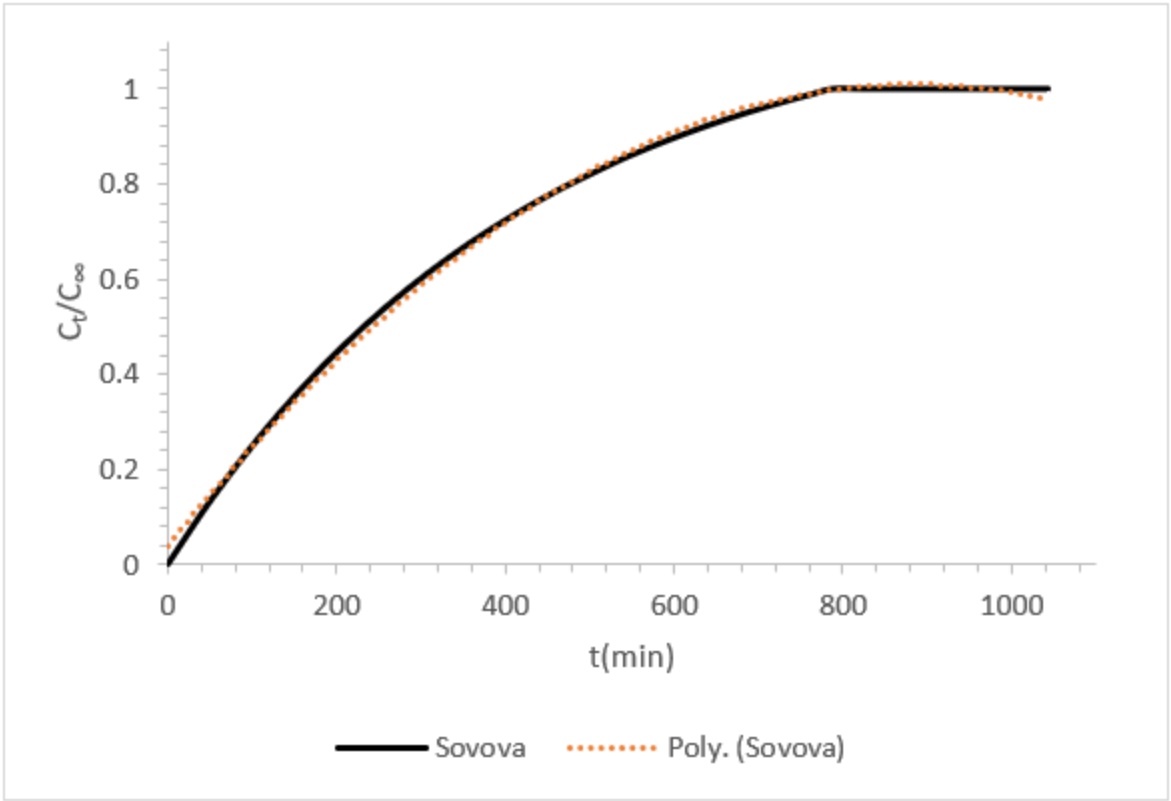

Figure 3

Sovova model applied to extraction of *Syzygium aromaticum* essential oil

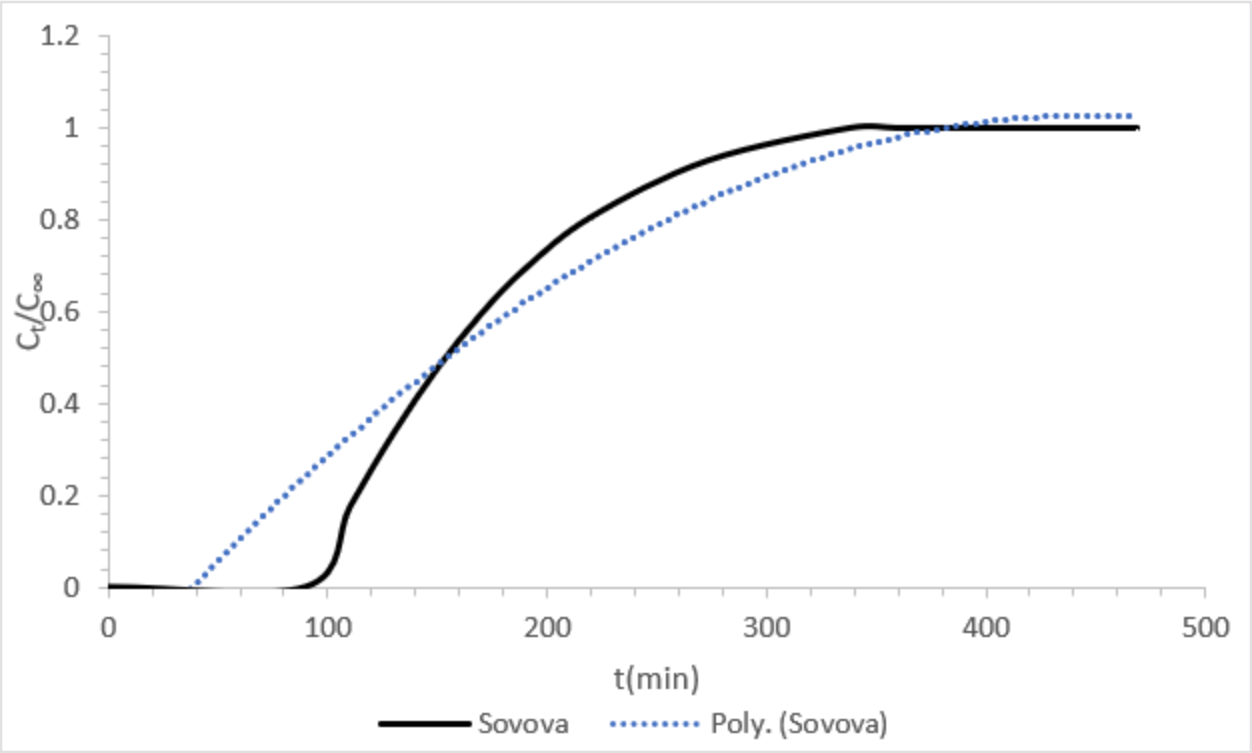

Figure 4

Sovova model applied to extraction of *Citrus sinensis L* essential oil

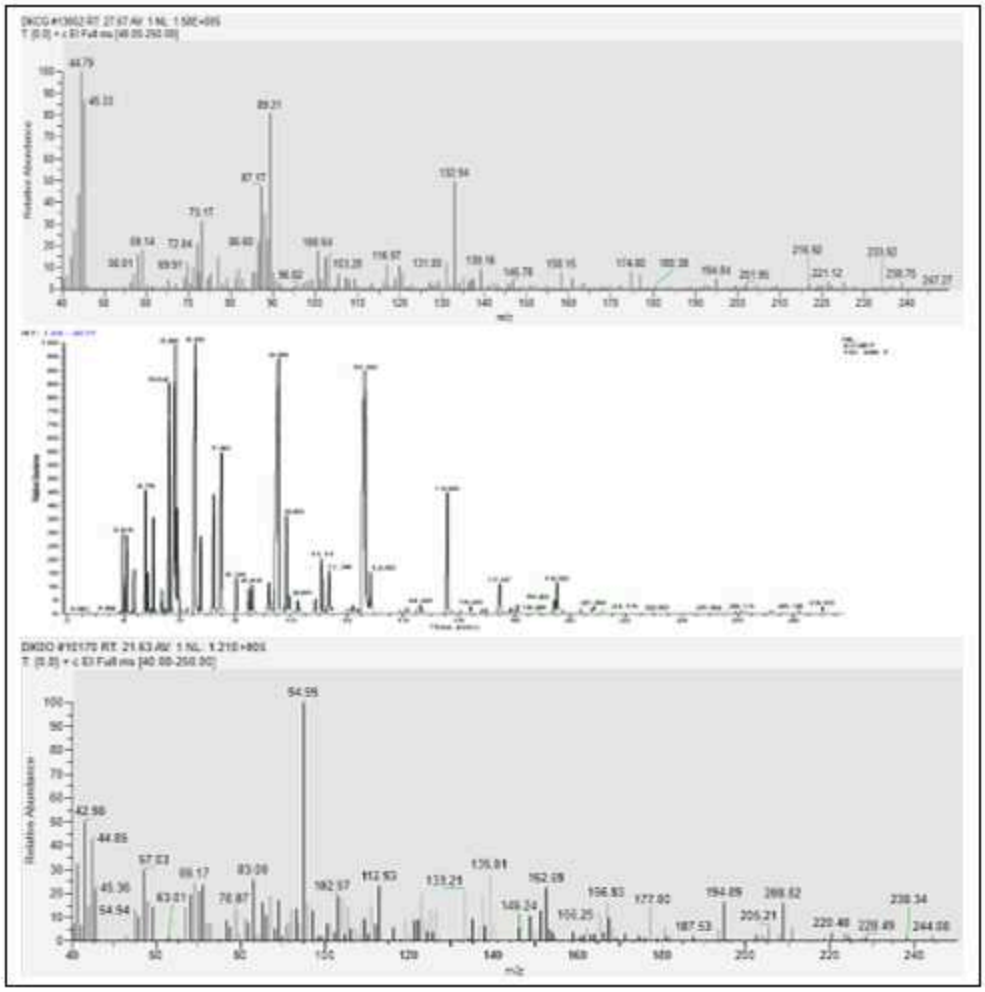

Figure 5

GC-MS Chromatogram respectively from top to bottom of Syzygium aromaticum, Thymus vulgarisand Citrus sinensis L.

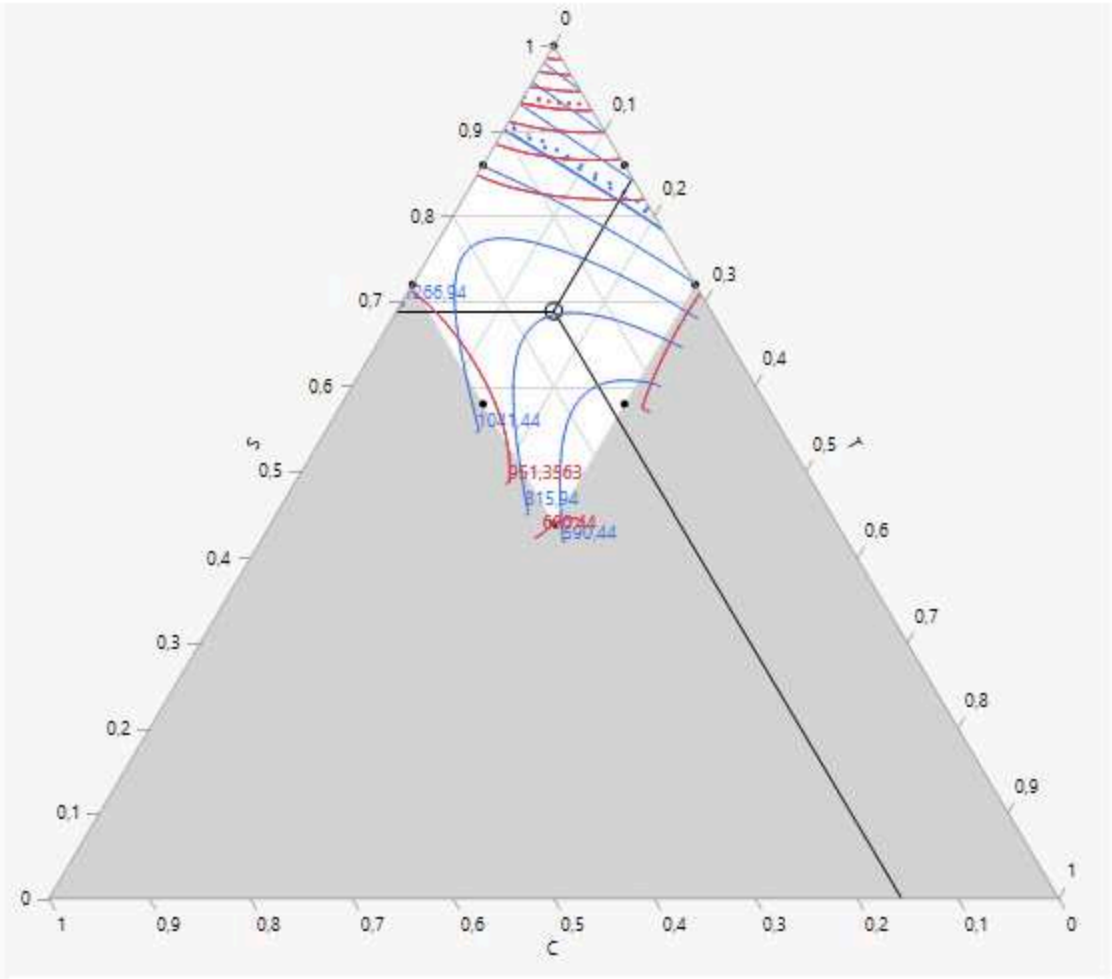

Figure 6

Biocide mixture Profiler

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TablesL.docx