



Vegetative propagation of *Justicia spicigera* using natural rooting agents and computational chemical description of the metabolite clitorine

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ABSTRACT: Plant phytochemical substances such as extracts usually contain in their mixture auxin-type substances, cytokinins, gibberellins, and other metabolites with application in the vegetative propagation of seedlings; in this sense, the objective was to evaluate the vegetative propagation of *Justicia spicigera* (JE) expressed in their morphology, through the use cuttings immersed in rooting mixtures extracts of *Aloe vera* (AV) and *Clitoria ternatea* (CT), as well as computationally describe the metabolite clitorine. A completely randomized design was employed to evaluate AV and CT extracts as rooting agents in the vegetative propagation of cuttings with and without JE leaves, comparing them with a commercial rooting agent (Biotrack) and water, across 12 treatments. The results show a beacon of hope, with the 5% and 10% CT extract leading to more significant vegetative development of JE plants. The extracts indicate the presence of secondary metabolites; one of the main ones is clitorine, which, due to its flavonoid-like structure, has potential bioactive applications. For this reason, a computational screening of this gold-standard metabolite was conducted to describe its chemical structure and electronic properties, as well as to predict its potential intermolecular interactions.

Keywords: biostimulants; medicinal plants; plant hormones; *Aloe vera*; *Clitoria ternatea*.

Propagação vegetativa de *Justicia spicigera* usando agentes de enraizamento naturais e descrição química computacional do metabolito clitorina

RESUMO: Sustâncias fitoquímicas vegetais como os extratos suelen contêm em sua mistura substâncias de tipo auxiliar, citoquininas, giberelinas e outros metabólitos aplicados na propagação vegetativa de plântulas. Neste sentido, o objetivo foi a propagação vegetativa de estacas *Justicia spicigera* (JE) expressa em sua morfologia, através da utilização de misturas de extratos enraizantes à base de *Aloe vera* (AV) e *Clitoria ternatea* (CT), bem como descrever computacionalmente o metabólito clitorina. Foi realizado um projeto completamente ao ar livre para avaliar os extratos de AV e CT como enraizadores na propagação vegetativa de estacas com e sem folhas de JE, comparando-os com um enraizador comercial (Biotrack) e água, para um total de 12 tratamentos. Os resultados mostram que o extrato de CT a 5% e 10% gera um maior desenvolvimento vegetativo das plantas JE. O uso dos extratos condiciona a presença de metabólitos secundários, um dos principais é a clitorina, que por sua estrutura tipo flavonoide tem potenciais aplicações bioativas. Por essa razão, foi realizado um cálculo computacional deste metabólito padrão ouro para a descrição químico-computacional de estrutura e projeções eletrônicas para suas potenciais interações intermoleculares.

Palavras-chave: bioestimulantes; extratos vegetais; hormônios vegetais; plantas medicinais; *Aloe vera*; *Clitoria ternatea*.

1. INTRODUCTION

The *Justicia* genus is the most prominent representative of the Acanthaceae family, primarily found in the tropical and subtropical regions of South America (ANAYA et al., 2018; PÉREZ-VÁSQUEZ et al., 2022). *Justicia spicigera* (JE) is known for its hypoglycemic activity, and studies have shown its bioactive potential in reducing blood levels of blood

glucose in the treatment of individuals with type II diabetes, hence its pharmacological importance (NÚÑEZ-TUESTA et al., 2022; JIMENEZ et al., 2023; THONGPHICHAI et al., 2023; LONDOÑO; DUEÑAS, 2024).

Generally, the propagation of medicinal plants is done through cuttings because this method allows the uniformity and quality of the seedlings by cloning selected genotypes of

the mother plants (ZEM et al., 2015; SILVA et al., 2021). For this, it is necessary to apply substances to the cuttings that promote rooting. However, it was discovered that there are consequences for the inappropriate use of chemicals as growth promoters in agricultural production concerning the plant's life cycle and in the animals and humans that consume them (CAMPOS, 2018).

Therefore, natural rooting agents have emerged as an alternative, with some proving to be as efficient as commercial ones (GUAMÁN et al., 2019; PEÑA et al., 2025). Among the plant extracts that yielded good results as rooting agents are *Aloe vera* and coconut water in Bamboo, *Gliricidia sepium*, and *Melissa officinalis* in blackberry plants (Borges et al., 2016), and *Aloe vera* in passionflower species endemic to the Colombian Andes (CALEÑO; MORALES, 2019).

Chemistry computer applications, disseminated through free platforms such as the Swiss Institute of Biotechnology (Daina et al., 2019) (SwissADME), provide information through probabilistic projections of potential bioactivity. This is achieved through comparison of Structure-Activity Relationships (SAR) from two-dimensional structures, thereby obtaining structurally similar molecules. In contrast, in biological activity libraries, a chemical-computational similarity is projected to predict chemical, physicochemical, kinetic, and pharmacokinetic properties.

Given the promising results observed for vegetative reproduction through the use of rooting agents based on plant extracts, the objective was to evaluate the vegetative propagation of *Justicia spicigera* (JE) expressed in their morphology, through the use cuttings immersed in rooting mixtures extracts of *Aloe vera* (AV) and *Clitoria ternatea* (CT), as well as computationally describe the metabolite clitorine, to generate a standardized protocol for the effective reproduction of the JE species, it is proposed to take advantage of its massification for pharmacological use, especially in its hypoglycemic potential in the possible treatment of patients affected by diabetes.

2. MATERIALS AND METHODS

2.1. Study area and selection of plant material

The experimental area was located at the Technical University of Cotopaxi “La Maná Extension”, in the La Playita Experimental Center, (Figure 1 Coordinates ~0° 56' S, 79° 13' W), at an altitude of 200 m.a.s.l. The site is characterized by a warm-humid tropical climate, with an average annual temperature of 23°C and precipitation ranging from 1000 to 2000 mm (INAMHI, 2025).



Figura 1. Localização do Centro Experimental “La Playita”, Extensão La Maná (Cantão La Maná, Província de Cotopaxi, Equador).

Figure 1. Location of the “La Playita” Experimental Center, La Maná Extension (Cantão La Maná, Cotopaxi Province, Equador).

Mature cuttings were selected, free of phytosanitary problems, with 3 and 4 nodes. The choice of cuttings with this number of nodes was based on physiological considerations: each node represents a meristematic region capable of generating roots and/or shoots, and previous studies have indicated that 3-4 nodes ensure a sufficient balance between root initiation potential and the maintenance of photosynthetically active leaves. Additionally, this range allows the cutting to store sufficient carbohydrate reserves to sustain early growth while minimizing the risk of desiccation.

2.2. Preparation of solutions and extracts

To formulate and obtain the 5% extract, a volume of 500 mL of distilled water was initially sterilized by boiling. Subsequently, 25 g of previously dried and crushed CT biomass was added, left to rest for 30 minutes, and filtered. Consequently, the amount of crushed biomass is included in the 10% extract, taking 50 g of CT leaves in 500 mL of the solvent used (distilled water). For the aloe extract, 25 g and 50 g of parenchyma (gel) without aloe exocarp (5% and 10%, respectively) were weighed in the same manner, brought to a final volume of 500 mL, and mixed homogeneously for 20 minutes using a magnetic stirrer at 3000 rpm.

Biotrack-O2 (Commercial Rooting Agent), which is composed of gibberellic acids, concentration [100 ppm], auxins [100 ppm], cytokinins [100 ppm], amino acids approximately 4%, algae extract 12%, basinosteroids [120 ppm], and finally potassium at 2.5%. Following the manufacturer's instructions, 1.5 mL of Biotrack-O2 was added, making up 1000 mL of previously sterilized distilled water (boiled); the JE cuttings were then immersed in containers containing the rooting substances for 24 hours.

2.3. Experimental design

A Completely Randomized Design (DCA) was used, with a factorial arrangement of (2 x 2 x 2 + 4), where factor A represents the types of cuttings with two levels (with leaves and without leaves), factor B represents the extracts with two levels (CT and AV), factor C represents the variation of concentrations (two levels 5 and 10%), finally considered four witnesses, thus having a total of 12 treatments. Considering five repetitions, the factors evaluated were: JE cuttings at two levels with leaves (E1) and without leaves (E2), plant extracts at two levels, CT at 5% (C1) and 10% (C2). And AV in concentrations of the extracts at 5% (A1) and 10% (A2) and the Biotrack controls identified as (AB), distilled water called (AD) this was used because it is good option for plant propagation, since it eliminates all impurities, that is it free of minerals, chlorine and other chemicals that can damage or hinder the growth or the cutting or the new plant.

The mathematical model for factor analysis appears below:

$$Y_{ijk} = \mu + \tau_i + \beta_j + \gamma_k + (\tau\beta)_{ij} + (\tau\gamma)_{ik} + (\beta\gamma)_{jk} + (\tau\beta\gamma)_{ijk} + u_{ijk} \quad (01)$$

where: Y = independent variable; μ = average of treatments; τ = factor A (types of cuttings); β = factor B (extracts); γ = factor C (concentrations); I = factor A levels (with leaves and without leaves); j = factor B levels (CT and AV); k = factor C levels (5% and 10%); $(\tau\beta)_{ij} + (\tau\gamma)_{ik} + (\beta\gamma)_{jk} + (\tau\beta\gamma)_{ijk}$ = effects produced by interactions.

2.4. Variables evaluated

The number of cuttings ready for transplanting to the field was reported. This was carried out 60 days after planting in polyethylene polymer (PET) bags, where the cuttings were recorded in the plots and their percentages were reported. The comparison of the effects of each treatment was carried out by taking five plants at random to evaluate the variables at 15, 30, 45, and 60 days: plant height, root length, number of shoots, number of leaves, fresh weight of the plant, weight of the root system and volume of the root system.

Five *Justicia spicigera* plants were randomly selected from the available plant material in each treatment, ensuring uniformity in size, age, and phytosanitary status. This number was determined to adequately represent biological variability, facilitate experimental management, and allow for robust statistical analysis of the results.

The evaluations were conducted at predetermined intervals, taking into account the rooting cycle and vegetative development reported for the species, as well as research history on the propagation of related ornamental species.

During the trial, management practices included controlled irrigation, application of rooting treatments according to the experimental design, preventive phytosanitary monitoring, and maintenance of optimal environmental conditions of light and temperature. These actions ensured uniform plant development and the reliability of the measurements taken.

2.5. Data analysis

An Analysis of Variance (ANOVA) was performed to estimate differences between the treatments evaluated and the interactions. For variables that presented significant effects, Tukey's multiple comparison tests were performed using the Statistic for Windows (2024) software, with a significance level of $P < 0.05$. To assess the normal distribution of the data, the Kolmogorov-Smirnov test and the Bartlett test for homogeneity of variances are employed.

2.6. Chemical-computational description of secondary metabolites

A state-of-the-art review was conducted regarding primary metabolites in the CT species, identifying potential compositions of substances that can contribute to vegetative propagation. Differentiating compounds of phenolic origin, flavonoids, and essential oils. Only the highest representative will be considered the gold standard for the chemical-computational description of the structure and electronic projections for its potential intermolecular interactions (MUKHERJEE et al., 2008).

3. RESULTS

3.1. Rooting percentage

After 60 days of sowing, the rooted cuttings were counted, differentiating those that showed evidence of shoots and roots, resulting in 100% rooted cuttings, considering 30 cuttings per treatment, and yielding 360 JE plants. This demonstrated the effectiveness of using plant extracts in the rooting process of JE (BORGES et al., 2016; ORTIZ et al., 2017; MAMANI, 2024). In the chemical composition of CT and AV, considerable amounts of flavonoids and essential amino acids, which can be the promoters of apical meristems in JE seedlings, are present through the processes of cell multiplication and growth (MUTHUKUMARAN et al., 2018; MAULANA et al., 2019).

To evaluate the effect of the rooters on the vegetative growth of the JE species, only the impact on the variables plant height, number of leaves, number of shoots, plant weights, length, weight, and volume of roots in both the plants was considered plants reproduced by cuttings with leaves and without leaves, whose results are summarized in Tables 2 and 3, reporting the more significant vegetative development that was observed when cuttings with leaves reproduced the plants.

Table 2. Height, number of leaves and shoots of *Justicia spicigera*.
Tabela 2. Altura, número de flohas e brotos de *Justicia spicigera*.

Treatments		Plant height (cm)	Number of leaves	Number of shoots
stakes with leaves	Water	32,20bc	27,35a*	05,20a
	Biotrak O ₂	37,72bc	35,45a*	07,60b*
	AV 10%	37,52bc	52,70bc	11,05c*
	AV 5%	39,25bc	54,30bcd	11,25c*
	CT 10%	39,87bc	64,35ef	16,00e*
	CT 5%	37,35bc	60,85def	14,65de
Leafless stakes	Water	37,75bc	31,15a*	06,60ab
	Biotrak O ₂	46,27c*	44,75b*	08,80b*
	AV 10%	30,12a*	47,00bc	11,00c*
	AV 5%	35,80bc	55,85bcd	12,95cd
	CT 10%	37,15bc	69,85f*	15,65e*
	CT 5%	34,90b*	61,60def	14,35de

Note: *Significantly different means according to the statistical test Tukey's $P \leq 0,05$.

Nota: *Médias significativamente diferentes segundo o teste estatístico de Tukey a $P \leq 0,05$.

Table 3. Growth indicators of *Justicia spicigera*.
Tabela 3. Indicadores de crescimento da *Justicia spicigera*.

Treatments		Plant weight (g)	Root length (cm)	Root weight (g)	Root volume (cm ³)
stakes with leaves	Water	09,18a*	27,85a*	03,62ab	03,30a*
	Biotrak O ₂	12,18ab	43,30b*	06,99d*	07,80e*
	AV 10%	16,67cd	29,03a*	05,32c*	05,00bc
	AV 5%	16,95d*	30,35a*	05,58c*	06,50c*
	CT 10%	16,59cd*	27,50a*	04,68bc	05,95cd*
	CT 5%	13,79bc	27,33a*	03,39a*	05,55bcd
Leafless stakes	Water	11,24ab	28,61a*	03,53a*	03,15a*
	Biotrak O ₂	18,29d*	39,58b*	08,37e*	07,70e*
	AV 10%	09,31a*	28,88a*	03,60ab	04,50b*
	AV 5%	12,52b*	27,23a*	03,31a*	05,30bcd*
	CT 10%	13,53b*	26,90a*	04,01ab	05,95cd
	CT 5%	11,74ab*	26,77a*	04,11ab	05,30bcd*

Note: *Significantly different means according to the statistical test Tukey's $P \leq 0,05$.

Nota: *Médias significativamente diferentes segundo o teste estatístico de Tukey a $P \leq 0,05$.

3.2. Plant height and root length

The height of the plant in the cuttings with leaves, observed after 45 days, shows significant differences between the treatments. The 5% AV treatment showed the best results (45 cm), but at the end of the trial, at 60 days, there were still significant differences between treatments, although the use of 5% CT yielded better results (60 cm). Regarding the cuttings without leaves, significant differences were observed between the treatments throughout the evaluated period; the treatment where the Biotrack was applied showed the highest plant height (47.8 cm), while the lowest height was 32.8 cm. The growth of plants during their

propagation is due to the role played by growth promoters and regulators in induction and cell division (Figure 2), among which are cytokinins, many of which have been reported in plant extracts (ALCANTARA et al., 2019; PAREDES et al., 2021).

Regarding root length, it was significantly greater ($P < 0.05$) in the treatment with the commercial rooter (AB), with lengths of 54.4 cm and 48.0 cm, respectively, in cuttings with and without leaves. Concerning the treatments where natural rooting agents based on AV and CT extracts were used at concentrations of 5 and 10%, these results are contrary to those reported in other trials where greater root length was reported when they used natural rooting agents based on *Aloe vera* gel and coconut water in *Ficus benjamina* species (ALVARADO; MUNZÓN, 2019).

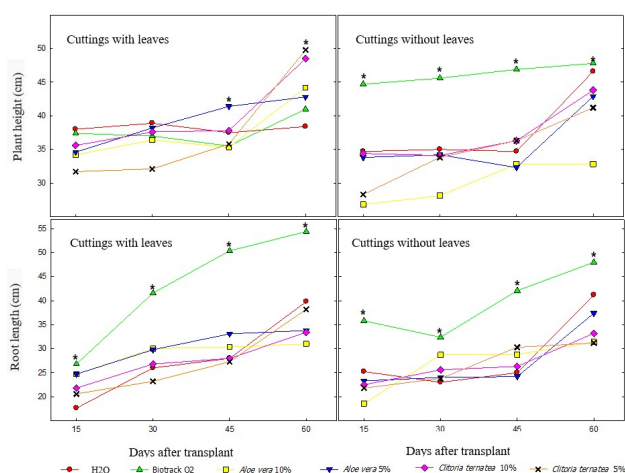


Figure 2. Effect of natural and synthetic rooting on plant height and length.

Figura 2. Efeito do enraizamento natural e sintético na altura e no comprimento da planta.

*Significant differences between treatments according to Tukey's test at 0.05. Each point represents the average of thirty values.

*Diferenças significativas entre tratamentos segundo o teste de Tukey a 0,05. Cada ponto representa a média de trinta valores.

3.3. Number of shoots and leaves

The highest number of leaves was observed in both cuttings with leaves and without leaves in the treatment with CT extract at a concentration of 10%, with 162 and 172 leaves, respectively. These numbers showed significant differences compared to the rest ($P < 0.05$), as seen in Figure 3. Plant extracts, especially those of *Aloe vera*, garlic, and moringa, serve as biostimulants that promote plant development, given their association at the rhizosphere level with growth-promoting bacteria, in addition to the presence of secondary metabolites and cyclotides that are contained in it, in the case of CT its use as a growth promoter is not reported, however, in its composition growth-promoting bacteria, secondary metabolites and cyclotides that can promote said growth were found vegetable (ZULFIQAR et al., 2020).

For the number of shoots, the best results were reported with the CT extract at concentrations of 10% and 5% for both cuttings produced with leaves (35 and 32) and without leaves (37 and 32), with statistically significant differences ($P < 0.05$) compared to the rest of the treatments. It is reported that in plant extracts, such as moringa, the presence of phytohormones, including Zeatin, a constituent of essential oils and proteins, may be associated with plant growth (ABUSUWAR; ABOHASSAN, 2017).

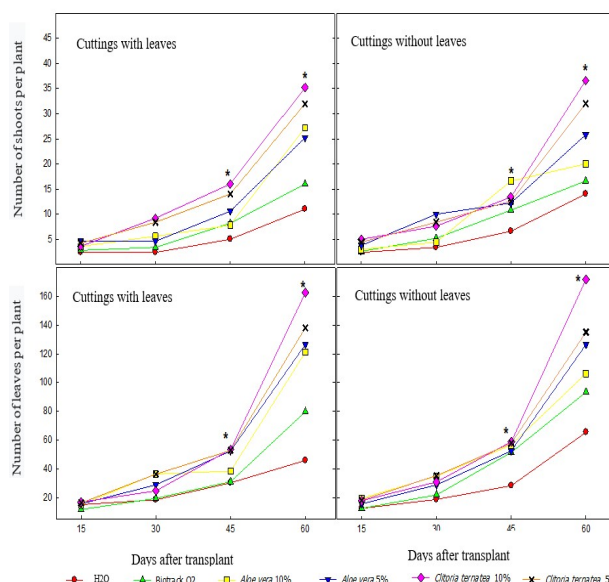


Figure 3. Effect of natural and synthetic rooting on the number of shoots and leaves.

Figura 3. Efeito do enraizamento natural e sintético no número de brotos e folhas.

*Significant differences between treatments according to Tukey's test at 0.05. Each point represents the average of thirty values.

*Diferenças significativas entre tratamentos segundo o teste de Tukey a 0,05. Cada ponto representa a média de trinta valores.

3.4. Plant weight

In the plant weight variable, differences were observed between the treatments during the evaluated period. The treatment with 10% CT extract as a rooting agent resulted in greater plant weight in both cuttings with and without leaves, at 42 and 32 g, respectively. Followed by those where CT was used at 5% and AV extract at 10 and 5% contractions. The lowest weight of plants in cuttings with leaves was observed when the synthetic rooting agent was used with 17.07 g, and when no rooting agent was applied, with 12.30 g. In contrast, in the cuttings reproduced without leaves, the lowest values were observed where no water was applied (18.75 g) or 10% *Aloe vera* gel (16.02 g) was used. The results are presented in Figure 4. The use of plant extracts, including microalgae, in combination with cytokinins, has been reported to increase the fresh weight of *Roselle hibiscus* plants.

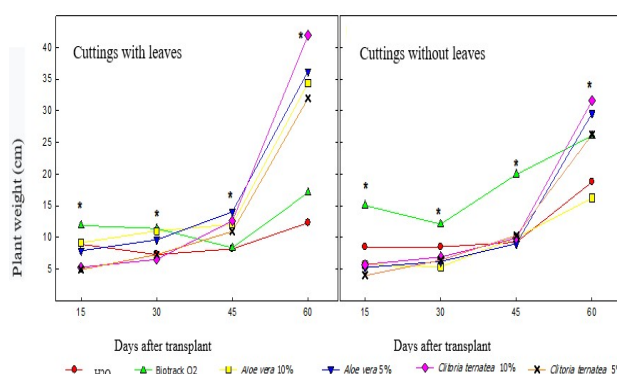


Figura 4. Efeito do enraizamento natural e sintético no peso das plantas, em estacas com e sem folhas.

Figure 4. Effect of natural and synthetic rooting on plant weight, in cuttings with and without leaves.

*Significant differences between treatments according to Tukey's test at 0.05. Each point represents the average of thirty values.

*Diferenças significativas entre tratamentos segundo o teste de Tukey a 0,05. Cada ponto representa a média de trinta valores.

3.5. Root weight and volume

For root length, the treatment using the synthetic rooter presented weight values of 10.97 and 10.37 g, respectively, for cuttings with and without leaves; this difference was statistically insignificant ($P < 0.05$). To the weight of roots when the rooting agent based on 10% CT extract was applied with 9.87 g, in the case of cuttings reproduced with leaves and 6.8 g without leaves. In comparison, the lowest value evidences the absence of rooter with a weight of 3.96 g. The use of plant extracts such as those obtained from *Cyperus rotundus* can promote root development due to the presence of indolebutyric acid in its composition, however, the presence of substances such as alkaloids, phenols and terpenes in high concentrations can have an effect allelopathic, affecting root development, this type of inhibitory substances are identified in the species CT (CHACAGUASAY et al., 2025).

For its part, the volume of the roots in both cuttings, reproduced with and without leaves, was greater when the synthetic rooting agent was used, with values of 8.6 and 10.2 cm³, respectively, showing statistical differences compared to the rest.

In the treatments where 10% CT was applied, the best performance was observed for cuttings without leaves, with a volume of 9 cm³. For both cuttings reproduced with and without leaves, the treatment that yielded the lowest root volume results was distilled water, with values of 4 cm³ and 4.4 cm³, respectively (Figure 5). The use of commercial rooting agents has been proven effective in promoting root development in various plants. However, the alternative use of extracts of microalgae and plant species is also possible due to the presence of growth-promoting hormones in their composition.

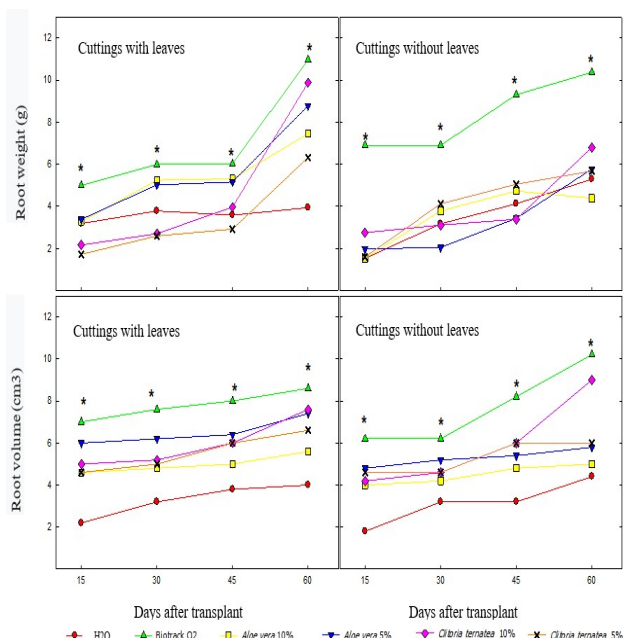


Figure 5. Effect of natural and synthetic rooting on the weight and volume of plant roots, in cuttings with and without leaves.

Figura 5. Efeito do enraizamento natural e sintético no peso e volume das raízes das plantas, em estacas com e sem folhas.

*Significant differences between treatments according to Tukey's test at 0.05. Each point represents the average of thirty values.

*Diferenças significativas entre tratamentos segundo o teste de Tukey a 0,05. Cada ponto representa a média de trinta valores.

The literature report reflects that the use of CT extracts, particularly at a concentration of 10%, is promising for promoting the growth of the JE species reproduced by cuttings with superior vegetative growth in many cases compared to the commercial rooting agent and the use of AV, which was tested effectively in other plant species. The effect of CT is attributed to the presence of secondary metabolites in its composition, which promote plant growth and provide a low-cost alternative for the propagation of ornamental and medicinal plants (BALDI et al., 2021).

3.6. Structural description of the clitorine metabolite

Among the flavonoid-type compounds, there is a metabolite specific to the species *Clitoria ternatea*, called clitorine, with molecular formula C₃₃H₄₀O₁₉, molecular mass 740.6640 g/mol, composition in elemental analysis C: 53.51%, H: 5.44%, O: 41.04%. As shown in Figure 5, the red core represents the functional structure of the flavonoids, a section that serves as the basis for projecting their potential uses (CORONEL et al., 2020).

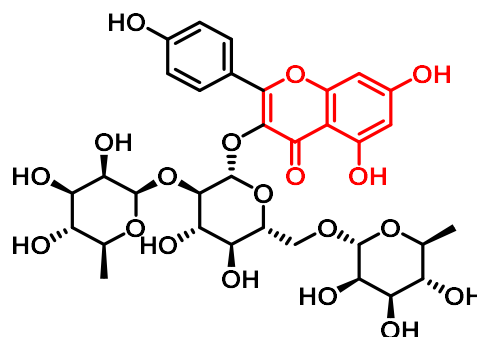


Figure 6. Chemical structure of the clitorine.

Figura 6. Estrutura química da clitorina.

The rest of the clitorine structure is composed of subunits of (2S,3R,4S,5S)-2-methyltetrahydro-2H-pyran-3,4,5-triol consecutively linked by ether-type bridges, the most voluminous section of this metabolite (Figure 7), which will provide the oxygen heteroatoms and their two pairs of free electrons, for the generation of hydrogen bond interactions. Likewise, by having oxydriol groups, they can donate hydrogen atoms to form interactions by coordinating a fluctuating bond with the two electrons shared by the oxygen atom.

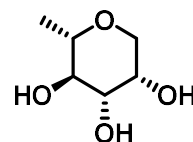


Figure 7. Complement subunits in the flavonoid structure.

Figura 7. Subunidades do complemento na estrutura flavonóide.

By projecting this structural subunit in its three-dimensional visualization (Figure 8), considering its attraction/repulsion interactions, bond distances, and dihedral angles, we obtain a deformed chair conformation, which, unlike the standard structure of cyclohexane, features an oxygen heteroatom within the cycle. This produces a distortion of the saddle projection. This is due to the atomic radius of the oxygen atom, its two unshared orbitals that each house a pair of unpaired electrons, its sp² hybridization, and

its distortion of the electronic cloud, which generates a dipole moment in the direction of the ether group.

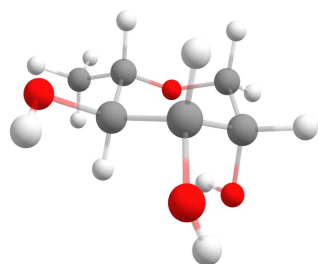


Figura 8. Conformação de subunidades tridimensionais de clitorina.
Figure 8. Three-dimensional clitorin subunit conformation.

For the global visualization of the clitorine (Figure 9), the 2D structure was spatially optimized by adding a force field of the MMFF92 type. This process was carried out using the Avogadro software version 1.2.0. Structurally, in a molecule with a considerable steric volume, the functional, structural core of flavonoids is planar. At the same time, the most voluminous subunits are located forward, and the individual subunit projects backward from the plane.

Using the Swiss Institute of Bioinformatics' (SwissADME) platform, it was possible to predict the biological activity of compounds with a clitorine base structure. Modeling was employed through computational comparison of their structure-activity relationship; this suggests that they may have a favorable response when used as protein receptors/couplings, hydrolases, and lyase-type enzymes. All results are reported as a product of a two-dimensional comparison of reported compounds (Figure 10).

Specific results for the molecular objectives indicate that the highest probability is obtained when interacting with proteins related to segments of the aldehyde and ketone type, and this interaction occurs primarily through marked dipoles. Thus, it is projected that they can interact with G-coupling proteins, for acetylco-linesterase, being a very reactive protein with various types of molecules, it has the same probability of bioactivity. Finally, clitorine can act with a lower probability of bioactivity with the lyase-type enzymes, mainly as inhibitors of enzymatic activity.

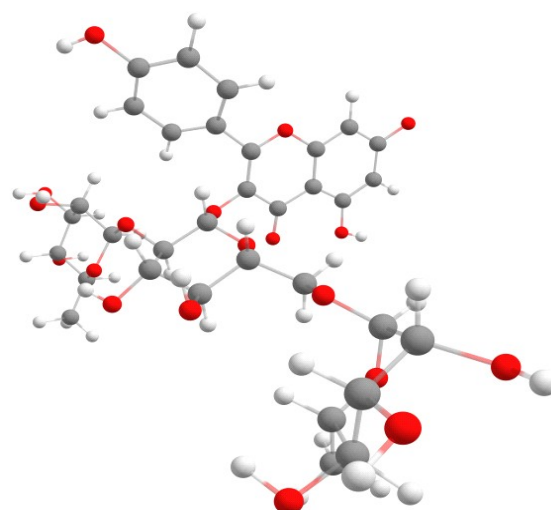


Figura 9. Conformação tridimensional global da clitorina.
Figure 9. Global three-dimensional conformation of the clitoris.

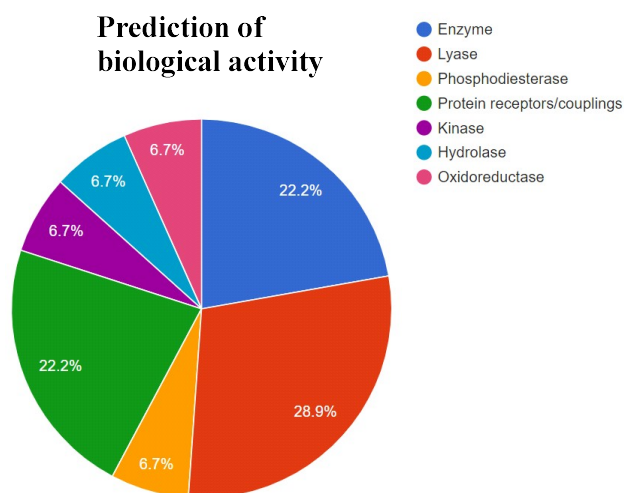


Figure 10. Prediction of clitoral biological activity.
Figura 10. Predição da actividade biológica da clitorina.

Table 4. Probabilistic bioactivity prediction report.

Tabela 4. Relatório probabilístico de predição de bioatividade.

ID	Probability	Classification	Molecular Aim
P15121 ²²	0.6709	Enzyme	Aldo-keto reductase family 1 member B1
Q9GZQ4 ²³	0.6601	G protein receptor/couplings	Neuromedin-U receptor 2
P08913 ²⁴	0.6601	G protein receptor/couplings	Alpha-2A adrenergic receptor
P18825 ²⁵	0.6601	G protein receptor/couplings	Alpha-2C adrenergic receptor
P22303 ²⁶	0.6601	Hydrolase	Acetylcholinesterase
P43166 ²⁷	0.2934	Lyase	Carbonic anhydrase 7f

The projection of the pharmacological potential of clitorine was carried out by comparing it with Lipinski's rules, which indicate whether a substance can be considered bioactive. The well-known Lipinski's rule of five is as follows: 1) Partition coefficient (log P) [-0.4 to +5.6]; 2) Molar refractivity (RF) [40 to 130]; 3) Molecular mass (MW) [160 to 500 g/mol]; Number of atoms [20 to 70]. In this way, when comparing the pharmacokinetic properties, the variables not met and the violations of Lipinski's rules reported by the SwissADME platform are listed in Table 5.

Table 5. Pharmacological potential of clitoria.

Tabela 5. Potencial farmacológico da clitoria.

Parameter	Compliance	Classification	Reported violation
Lipinski ²⁸	No	Masa molecular: Átomos polares: Donadores de puente de hidrógeno:	MW>500, NorO>10, NHOrOH>5

Since three of Lipinski's five rules are not met, it is stated that clitorine would not be a promising drug as it has a molecular weight out of range by 200 units, the number of polar atoms exceeds ten heteroatoms, and the number of atoms of hydrogen associated with a polar heteroatom exceeds five units, which indicates that it would generate intermolecular interactions with various substances, which can even be a false positive in computational and laboratory tests.

4. DISCUSSION

The vegetative multiplication of *Justicia spicigera* using extracts or natural substances is a relatively unexplored area, which allows for the use of agrochemicals (DELGADO, 2018). In this experiment, the development of agronomic variables was achieved when using plant extracts, which was even more significant than chemical rooting agents. By interpreting the results, it was determined that the treatment in which 10% Clitoria extract was applied generated significant results in terms of the number of leaves, number of shoots, plant height, and plant weight, providing a proposal for future research with this extract.

The results for plant height, number of shoots, and leaves, using rooting agents such as *Clitoria ternatea* and *Aloe vera*, reflected the highest values. The literature states that these rooting agents, composed of Natural and non-synthetic substances, have the potential to stimulate root growth and improve nutrient absorption, thus contributing to increased productivity and quality of the plant (Osuna-Fernandez, 2014; Miranda et al., 2023; García et al., 2024), in addition to conserving soil and water by reducing the need for synthetic fertilizers and agrochemicals (OSUNA-FERNANDEZ, 2014; ALI et al., 2020). Thus, various investigations in other crops have demonstrated that organic compounds used as rooting agents yield higher height values than synthetic ones (DOMÍNGUEZ et al., 2023; SOLANO et al., 2023; LIVIA et al., 2024).

The literature states that the use of rooters in plant production favors the development of important parameters at the time of transplant, including the height of the seedling and the number of shoots, among others. Furthermore, the development of seedlings in their initial stage is directly related to uniformity in germination, and this, in turn, can be attributed only to the characteristics of the substrate (ARÉBALO et al., 2019; SÁNCHEZ et al., 2020; VALLEJO et al., 2020). When applying natural rooting agents to tomato plants, the number of leaves was favored by 100% (Lazcano et al., 2021) something similar happened in coffee plant and short cycle crops (Ardiasa et al., 2020; Matamoros et al., 2020), a valuable indicator for the quality of the plant, since it favors photosynthesis (Espinosa et al., 2019), in addition to increasing the root system and, therefore, the nutrient absorption capacity; In this way, the plants will have greater anchorage and thus lodging can be avoided (PÉREZ et al., 2019).

For plant weight (Figure 3), it was noted that treatments with 10% CT and 5% AV showed the best results. A study where *Aloe vera* plus molasses was used as a rooting agent in *Hibiscus rosasinensis* reflected the greater weights of the plant (11.24 g), as well as the length of the root (5.58 cm), 30 days after sowing when applying this treatment, compared to the control (chemical rooting agent). These authors pointed out that different factors, such as lighting, substrate, and water, among others, can influence the behavior; however, the

presence of various metabolites, such as those possessed by *Clitoria ternatea* and *Aloe vera*, can affect the results of this research, due to the functions they have in the development of the plant.

On the other hand, a study in the cultivation of beans with the use of *Clitoria ternatea* as a rooting agent in concentrations of three and seven percent, reflected plant weights more significant than 17 g after 50 days of study, with differences significant compared to the rest of the treatments (SANTANA et al., 2022; GAVILANEZ et al., 2024). Thus, the literature reports that the leaves of *Clitoria ternatea* constitute an excellent forage (4 to 23% of fresh matter; 5 to 30% of dry matter; 20 to 27% of protein, rich in calcium, potassium, and vitamins). This is why it is efficient when used as a biostimulant. Other research highlights the use of organic rooting agents in plant species such as *Fragaria sp.* and highlights the importance of these compounds for plant development. However, they highlight the importance of defining appropriate percentages for their use (CHOQUE et al., 2021).

For root weight and volume, no significant differences were observed between the synthetic and natural rooting agents, an aspect highlighted by other authors when evaluating these products in the development of Raspberry, where the use of rooting agents such as Biofit (synthetic) allowed them to reach plant height greater than 40 mm after 15 days of study. These authors highlight the importance of these compounds and report that they are more efficient than organic compounds (Choque et al., 2021), an aspect that contradicts the results of the present investigation. The above described suggests the importance of establishing the appropriate concentrations to be able to use a natural product as a rooting agent, especially if it has alkaloids, phenols, and terpenes, as is the case of *Clitoria ternatea* (Jaafar et al., 2020), which can constitute elements that high concentrations can affect the development of the plant, an aspect to be clarified in future research.

Another investigation reported an increase in root mass, as well as root diameter, when applying organic rooting agents based on coconut water, willow extract, and *Aloe vera* in crops of *Fragaria sp.* These authors highlighted how the treatments where coconut water was fundamentally applied as a rooting agent were superior to the control (inorganic compounds). Thus, they reported that these compounds have plant growth-promoting substances, possibly the presence of hormones such as auxins, gibberellins, and cytokinins, which promote cell elongation of the cultures (CHOQUE et al., 2021; NALIMU et al., 2021). Aspects that can explain the results of this research when applying *Clitoria ternatea* and *Aloe vera*.

The literature highlights different studies where the use of organic rooting agents constitutes a way to achieve satisfactory results in the morphological characteristics of other crops. Thus, a study in Higo reflected the shortest rooting time with the treatment where lentil water was applied for 48 hours, followed by the treatment with coconut water for the same time. This research showed better behavior of the morphological variables of different crops when synthetic rooting phytohormones were replaced by *aloe vera* extract (TINCO, 2024). The above highlights that the use of organic and natural products acquires excellent economic, social, and environmental importance every day due to the security it offers to health and the environment, and can constitute a way for the use of these organic

compounds in agriculture, more precise studies to evaluate different concentrations, exposure time, and chemical composition of the rooting agent to be used are becoming increasingly necessary to achieve better agronomic responses (TINCO, 2024)

For its part, the computational chemical description of the metabolite clitorine was based on identifying the principal core of the flavonoid structures, which, with its voluminous aryl-type substituents and its multiple oxydryl groups, can form many intermolecular hydrogen bonding interactions. In sum, these dipole-type interactions allow us to know that the more segments that can react as nucleophiles, the more bioactive the molecule can be as a secondary metabolite. Furthermore, in the complementary analysis, it was evidenced that clitorine can present bioactivity mainly against enzymes, and that it can show activity as a drug; however, reflecting many restrictions makes it a false positive. Especially if the rooters must play essential roles in the development of the plant; They strengthen the root system and the anchoring of the crop to the substrate, and improve the acquisition, transportation and storage of soil resources.

In general, it should focus on the results obtained according to the statistical analysis, since it is assumed that this responds to the objective. Chemical formulas are not relevant, but they can be mentioned as a contribution to the forcefulness of the results and discussions.

5. CONCLUSIONS

The plant extracts of CT and AV at 5% and 10% showed effectiveness as rooting agents in the initial stage of adaptation, demonstrating the production of 100% of rooted cuttings, also taking into account the commercial rooting agent Biotrack-O2, consistent throughout the entire period. The plant extract that generated the best result was CT, both in the species reproduced with cuttings with and without leaves at concentrations of 5% and 10%, as better vegetative development of the JE plants was observed in those related to the variables number of leaves, plant height, number of shoots and plant weight.

The use of natural extracts improves vegetative growth and root development, expressed in the length, volume and weight of roots. It confirms the effects of the synthetic rooting agent, so the results suggest that the use of synthetic and natural rooting agents should be combined, in search of the synergistic effect on the vegetative variables and root growth of the JE species.

In the structural chemical analysis, the secondary metabolite clitorine was determined, one of the most notable since its properties are derived from the flavonoid nucleus, making it considered for bioactive applications; its potential is increased by the cyclic substituents located spatially forward and behind the three-dimensional plane derived from its core.

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Data availability: Study data can be obtained by email from the corresponding author.

Conflict of interest: The authors declare that they have no conflict of interest.



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