

***In silico* investigation of *Annona coriacea* species candidates with effect on the central nervous system**

Investigação *in silico* de candidatos da espécie *Annona coriacea* com efeito no sistema nervoso central

Túlio Nunes Pinto¹

Weldes Francisco da Silva Júnior¹

Leonardo Luiz Borges²

Abstract

Annona coriacea, known by names such as "araticum", "araticum smooth" and "marolo", is a plant popularly used for its antiprotozoal, antirheumatic, anthelmintic, and anti-inflammatory properties, among others. In addition, studies with mice showed anxiolytic and antidepressant activity. This study sought to identify and elucidate the potential mechanisms involved in the activities above. The method of choice employed to predict biological activity, pharmacodynamics, and toxicity was *in silico* tools. In addition, docking and redocking were performed. Among 28 eligible compounds, two were the most promising, trigonelline and gallic acid. This study opens perspectives for *in vitro* and *in vivo* studies with these isolated structures.

Keywords: *In silico* modeling; Organic chemicals; Mental disorders.

Resumo

Annona coriacea, conhecida por nomes como "araticum", "araticum liso" e "marolo", é uma planta popularmente utilizada por suas propriedades antiprotozoária, antirreumática, anti-helmíntica e anti-inflamatórias, entre outras. Estudos com camundongos mostraram atividade ansiolítica e antidepressiva. Nesse estudo, procuramos identificar e elucidar os potenciais mecanismos envolvidos nas atividades supracitadas. O método de escolha empregado para prever atividade biológica, farmacodinâmica e toxicidade foi o uso de ferramentas *in silico*. Além disso, foi performed *docking* e *redocking*. Dentre 28 compostos elegíveis, dois foram os mais promissores, a trigonelina e o ácido gálico. Este estudo abre perspectivas para estudos *in vitro* e *in vivo* com essas estruturas isoladas.

Palavras-chave: Simulação *In silico*; Docagem Molecular; Novo Fármaco em Investigação.

¹ Graduando em Medicina pela Pontifícia Universidade Católica de Goiás (PUC Goiás).

² Doutor em Ciências Farmacêuticas, professor da Pontifícia Universidade Católica de Goiás (PUC Goiás) e da Universidade Estadual de Goiás (UEG).



INTRODUCTION

The *Annona coriacea* species, popularly known as “araticum”, “araticum-liso” and “marolo”, is a small tree from the Annonaceae family, commonly found in the North, Northeast, Midwest, and Southeast regions of Brazil. However, it can be found in other countries such as Paraguay, Bolivia, and the tropics of the African continent. This species is popularly used in producing homemade solutions with antiprotozoal, antirheumatic, anthelmintic, and anti-inflammatory purposes, as well as mitigating neuralgias and headaches. In addition, some studies indicate that some species of the genus have anxiolytic and antidepressant properties^{1,2}.

Studies carried out in the last 20 years were able to isolate substances that are possibly responsible for the effects. Among them are acetogenins, some alkaloids, diterpenes and phenolic components, and flavonoids. Among the last two categories mentioned, we have the compounds: gallic, caffeic, p-coumaric acid, catechin, epicatechin, rutin, quercetin, quercetin, and luteolin, which are of interest for their potential antidepressant and anxiolytic effects².

Psychiatric disorders (PD) are diseases characterized by emotional, cognitive, motivational, and socialization changes. They are highly hereditary, with genetic factors responsible for 20 to 90% of susceptibility to these diseases. Due to their high prevalence, early onset, and persistence, these Central Nervous System (CNS) diseases contribute significantly to the global disease burden³.

According to the Global Burden of Disease study carried out in 2010, PD accounted for 7.4% of the total disability-adjusted life years (DALY) and 22.9% of the total years lived in disability (YLD – Years Lived with Disability), making it the fifth leading cause of DALY and the first cause of YLD in the world⁴. Surveys carried out recently by the World Health Organization (WHO) indicate that approximately 700 million people in the world suffer from some mental disorder and problems related to drug and alcohol abuse, and this has been causing great suffering for the individual in their social, individual, and personal and family life⁵.

Therefore, it becomes of medical interest to create new drugs so that the range of therapeutic options that can be used to treat these disorders can be expanded. The use of *in silico* tools is established to research new substances with possible pharmacological activity. The design of *in silico* drug prototypes involves the study of the structure-activity relationship (SAR) to studies that consider the pharmacokinetics (absorption, distribution, metabolism, and excretion) of the compounds (ADME). The advantage of *in silico* studies is the speed of execution, low cost, and the ability to reduce the use of laboratory animals⁶⁻⁸.

Within this context, this work aimed to investigate new candidates with TP activities obtained from *Annona coriacea* using *in silico* tools.

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METHODS

A search was performed in PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), ScienceDirect (<https://www.sciencedirect.com/>), and Scielo (<https://www.scielo.br/?lng=pt>), to find substances with a possible effect on the central nervous system, already characterized, from the species *Annona coriacea*. For this, articles published between 2000 and 2021 were searched using the descriptors “*Annona coriacea*” and “chemical compounds”.

After identifying the compounds already elucidated in this species, their structural data were collected using the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). Soon after organizing the structures, the PASS Prediction server (<http://www.way2drug.com/passonline/>) was used to predict the probable biological activities of the cataloged structures⁹, with the aim of finding those most likely to have actions on the central nervous system. Through the PASS prediction server, we consider the values of Pa and Pi as > 0.7 and < 0.05 , respectively. These values represent the probability of a given substance being active (Pa) or inactive (Pi) for a suggested biological property, which was used as criteria for the inclusion of structures of interest. Through the SwissADME (<http://swissadme.ch/>)¹⁰ and ProtoxII (https://tox-new.charite.de/protox_II/)¹¹ servers, it was possible to perform both pharmacokinetic and toxicological prediction analysis, respectively. In addition, to verify the most likely targets of the binding compounds obtained, the Swiss Target Prediction website was used¹².

The next step was molecular docking. Molecular docking is a structure-based drug design method that simulates molecular interaction and predicts the binding mode and affinity between receptors and ligands obtained in previous study phases¹³. To create the docking models, the program used was GOLD Suite 5.7.0. Next, 2D interaction maps and pharmacophoric mapping figures were generated by Discovery Studio 3.5 Visualizer software. Finally, the 3D images were built with the PyMOL Molecular Graphics System software, version 2.0.

Finally, to validate the models produced with the molecules found in this study, the redocking was performed using a structure in which the target protein and its ligand were co-crystallized. For all parameters used during the process, default values were employed, and the structures were subjected to 10 runs of genetic algorithms using the CHEMPLP function.

RESULTS AND DISCUSSION

After searching for articles on the Pubmed, ScienceDirect, and Scielo platforms, 28 substances were found present in the *Annona coriacea* species, which occur in the Cerrado. After pharmacokinetic prediction analysis (absorption from the gastrointestinal tract (GIT), “druglike”



classification according to Lipinski, interaction with cytochromes, and permeability of the blood-brain barrier), 11 compounds were selected (Table 1).

Table 1. Compounds present in *Annona coriacea* species selected after pharmacokinetic evaluation.

Compound	Absorption from the gastrointestinal tract	“Druglike” classification	Interaction with cytochromes	Permeability of the blood-brain barrier
Caffeic acid	High	Yes; 0 violations	None	No
Crolecinic acid	High	Yes; 0 violations	CYP2C9	Yes
Ellagic acid	High	Yes; 0 violations	CYP1A2	No
Gallic acid	High	Yes; 0 violations	CYP3A4	No
Annonalide	High	Yes; 0 violations	None	No
Catechin	High	Yes; 0 violations	None	No
Epicatechin	High	Yes; 0 violations	None	No
Kaempferol	High	Yes; 0 violations	CYP1A2, CYP2D6 e CYP3A4	No
Quercetin	High	Yes; 0 violations	CYP1A2, CYP2D6 e CYP3A4	No
Trigonelline	High	Yes; 0 violations	None	No

Source: Table prepared by the authors.

In addition, as a selection filter, their toxicity levels, probable toxic effects, and, finally, targets of interest for the study were determined. Considering the possible toxic effects and the LD50 test, the number of compounds was reduced to 7 (Table 2).

Table 2. Compounds present in *Annona coriacea* species selected after an assessment of toxic potential.

Compound	DL50	Probable toxic effects
Caffeic acid	2.980 mg/kg - toxicity class 5	Hepatotoxicity, carcinogenicity, immunotoxicity, and androgen receptor (AR) inhibition
Ellagic acid	2,991 mg/kg - toxicity class 4	Carcinogenicity
Gallic acid	2.000 mg/kg - toxicity class 4	Hepatotoxicity and carcinogenicity
Catechin	10.000 mg/kg - toxicity class 6	Carcinogenicity, mutagenicity, and inhibition of Mitochondrial Membrane Potential (MMP)
Epicatechin	10.000 mg/kg - toxicity class 6	Carcinogenicity, mutagenicity, and inhibition of Mitochondrial Membrane Potential (MMP)
Kaempferol	3.919 mg/kg - toxicity class 5	Hepatotoxicity, Mutagenicity, Aryl Hydrocarbon Receptor (AhR) inhibitor, Aromatase, Estrogen Receptor Alpha (ER), Estrogen Receptor Ligand Binding Domain (ER-LBD), and Mitochondrial Membrane Potential (MMP)
Trigonelline	3.720 mg/kg - toxicity class 5	Hepatotoxicity and carcinogenicity

Source: Table prepared by the authors.



As the last exclusion criterion, the possible targets of each substance were investigated through the SwissTargetPrediction platform, excluding those that did not have targets related to a potential effect on the CNS. Thus, the two selected compounds were: trigonelline and gallic acid (Table 3).

Table 3. Substances found in the *Annona coriacea* species selected for the study after analyzing their potential targets and related psychiatric diseases.

Compound	Interest targets	Diseases likely related
Gallic acid	Catechol O-methyltransferase / COMT	Schizophrenia, schizotypal and schizophrenia, Panic Disorder, and psychosis, paranoid
	AC1	Transient global amnesia
Trigonelline	SIRT2	Mood disorders
	CHRM1	Schizophrenia and Attention Deficit Hyperactivity Disorder

Source: Table prepared by the authors.

Trigonelline, or N-methyl nicotinic acid, is an alkaloid derived from pyridine nucleotides widely distributed in the plant kingdom. According to *in vitro* studies, trigonelline has several biological activities, including antibacterial function against *S. mutans* (a bacteria related to the development of caries in humans), regeneration of dendrites and axons of cortical neurons, leading to improved spatial memory in rats, and inhibition of the invasion of cancer cells in the liver¹⁴.

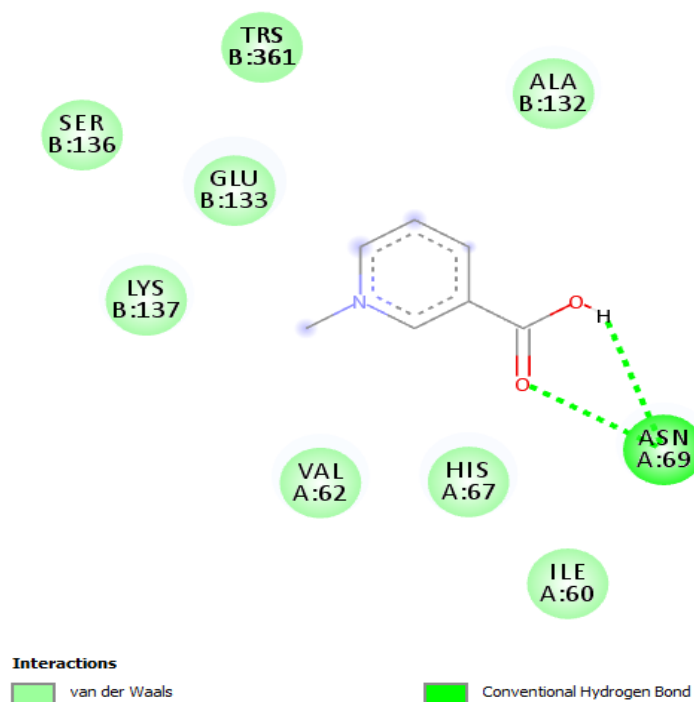
Cognitive dysfunction is considered by many to be the main symptom of the psychopathology of mood disorders and affects multiple areas, such as attention, processing speed, memory, and executive activities¹⁵. Studies show that the continuous production of pro-inflammatory mediators is related to the genesis of neuronal damage and structural and functional alteration of vital areas for affective and cognitive processing, including the prefrontal cortex and hippocampus¹⁶.

According to analyzes carried out using the SwissTargetPrediction platform, one of the targets that may be influenced by Trigonelline is Carbonic Anhydrase 1 (CA1) (Figure 1A). Carbonic anhydrases (CAs) are a large family of zinc metalloenzymes that catalyze the reversible hydration of carbon dioxide.

Redocking of the co-crystallized ligand with CA1 was successfully performed, resulting in RMSD values lower than 0.5 for the 10 generated poses (Figure 1B).



A:



B:

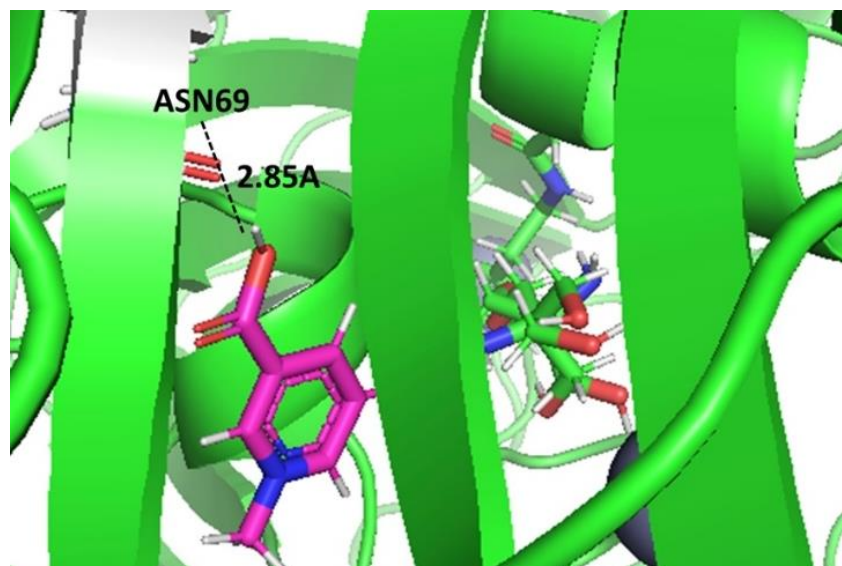
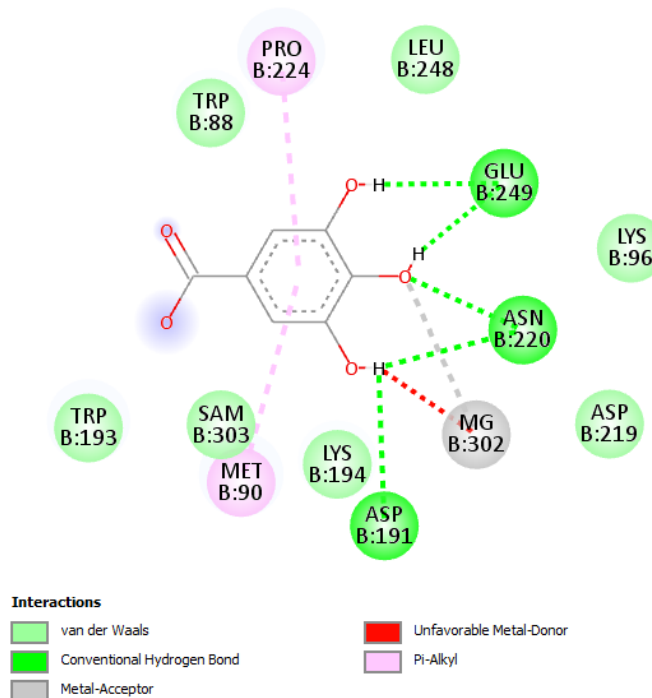


Figure 1.A - 2D graphic representation of the trigonelline pose 1 molecule found in *Annona coriacea* species interacting with the target carbonic anhydrase I (9PDB ID: 2NMX). **B** - Image with a 3D representation of trigonelline with carbonic anhydrase I and the intermolecular distance of the hydrogen bond with the ASN69 residue.

Gallic acid (Figure 2A) is a phenolic compound known as 3,4,5-trihydroxybenzoic acid¹⁷. So far, this substance has demonstrated many effects, including anti-inflammatory, antinociceptive, antineoplastic, antioxidant, and antibacterial effects.

A:



B:

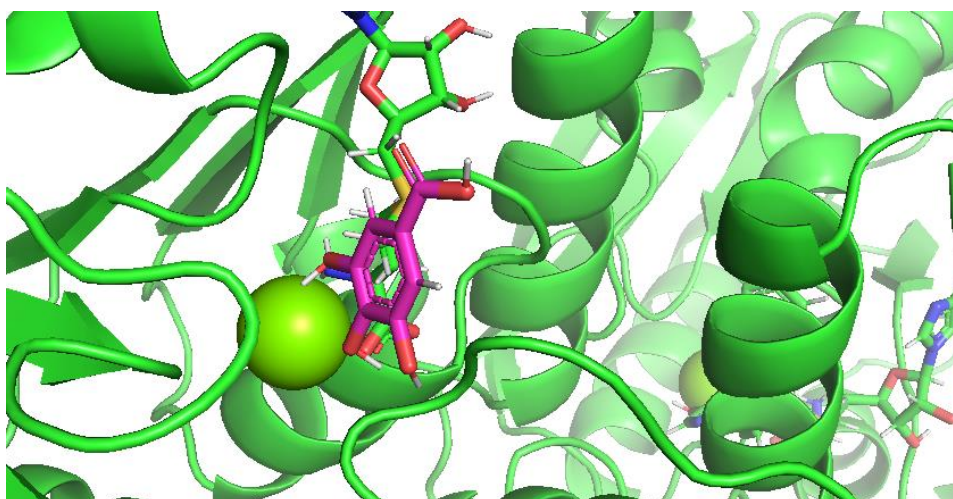


Figure 2A - 2D graphic representation of the gallic acid molecule found in the *Annona coriacea* species, interacting with the target COMT (catechol O-methyl transferase) (PDB ID: 4XUE). **B** - Image with 3D representation of gallic acid with COMT.

According to analyzes carried out using the SwissTargetPrediction platform, one of the targets that may be influenced by gallic acid is COMT, responsible for encoding the catechol-O-methyltransferase enzyme that catalyzes the degradation of catecholamines, including dopamine, a neurotransmitter relevant to cognitive function (Figure 2B)¹⁹. The effect is inhibitory, acting directly on the action of COMT. A study arrived through experimentation with cytosolic preparations of rat liver and cultured hepatocytes²⁰.



Redocking of the ligand co-crystallized with COMT was successfully performed, resulting in RMSD values lower than 0.5 for the 10 generated poses. The intermolecular distances between gallic acid and the COMT receptor were between 2Å and 4Å, corresponding to accurate docking (Figure 3A). Furthermore, 5 hydrogen bonds contribute to the interaction between gallic acid and the ligand interaction site (Figure 3B).

The findings found through the employability of *in silico* tools in the study of the relationship between trigonelline and CA1 support the results of other studies. Among them, authors bring us, through *in vivo* studies, that trigonelline can attenuate the symptoms of cognitive dysfunction through the suppression of oxidative and neuroinflammatory stress caused by lipopolysaccharide (LPS), an endotoxin produced by gram-negative bacteria²¹.

The same occurred concerning gallic acid and COMT. Authors, through experimentation with cytosolic preparations of rat liver and cultured hepatocytes, showed that gallic acid strongly inhibits the catalyzing role of COMT²⁰.

CONCLUSION

Considering the high prevalence, early onset, and persistence of PD, they significantly contribute to the suffering of millions of individuals, directly or indirectly, worldwide. Given these data, interest in medicinal plants' preventive and curative effects has been increasing. The *in silico* approach used in this study demonstrated that trigonelline and gallic acid are promising compounds with protective activity from the neuropsychiatric perspective of the man. The results presented here may help elucidate the effects of *Annona coriacea* extracts and encourage research with other plants that carry similar substances. Finally, this study provides information that enables future research in vitro and in vivo with these isolated structures.

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Contato para correspondência:

Túlio Nunes Pinto

E-mail:

tulionunesp@gmail.com

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