UNIVERSIDADE VILA VELHA-ES

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

ATIVIDADE INDUTORA DE QUINONA REDUTASE DE PRODUTOS DE ORIGEM VEGETAL

SILVIA CRUZ GOES COUTINHO

VILA VELHA-ES

AGOSTO, 2023

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Tese apresentada à Universidade Vila Velha como pré- requisito do Programa de Pós-graduação em Ciências Farmacêuticas, para obtenção do grau de Doutora em Ciências Farmacêuticas.

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SUMÁRIO

LISTA DE ABREVIATURAS E SIGLAS

ABTS - 2,2-azinobis-(3-etilbenzotiazol-6-sulfonato)

ANOVA - Análise de Variância

BHT - 2,6-tert-butil-1-hidroxi-tolueno

COSY - Espectroscopia de Correlação

DMSO - Dimetilsulfóxido

DP - Desvio Padrão

DPPH - 2,2-difenil-1-picril-hidrazil

EE - Extrato Etanólico

EH - Extrato Hexânico

EDTA - Ácido etilenodiamino tetra-acético

EPM - Erro Padrão da Média

FAD - Dinucleotídeo de Flavina e Adenina

FRAP - Poder antioxidante de redução férrica

HMBC - Heteronuclear Multiple Bond Correlation

Hepa1c1c7- Células de hepatoma de rato

IC50 - concentração do extrato requerida para reduzir a quantidade de radicais livres por 50%

IFN - Interferon

IL - Interleucina

LPS - Lipopolissacarídeo

MDA - Malondealdeído

MIC - Concentração Mínima Inibitória

MTT - (brometo de 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazólio)

NADPH - Fosfato de dinucleótido de nicotinamida e adenina

NFkB - Fator De Transcrição Nuclear Kappa B

NPS - Nitroprussiato de Sódio

NRU - *Neutral Red Uptake*

iNOS - Óxido nítrico sintase induzível

PDB - *Protein Data Bank*

QR -Quinona redutase

RMN - Ressonância Magnética Nuclear

TNFα - Fator de Necrose Tumoral

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RESUMO

Coutinho, Silvia Cruz Goes, Dr., Universidade Vila Velha – ES, Agosto de 2023. **Atividade indutora de Quinona Redutase de Produtos de origem vegetal.** Orientadora: Profª. Drª Denise Coutinho Endringer.

Os produtos naturais são fontes de novas drogas utilizados pela humanidade há séculos e possuem papel fundamental na descoberta de novos medicamentos para doenças infecciosas, câncer e outras doenças neurodegenerativas até os dias de hoje. Muitos desses produtos naturais são capazes de alterar o metabolismo de carcinógenos por intermédio da indução de diversas substâncias envolvidas na destoxificação do organismo. Uma das estratégias para a proteção de células dos eventos iniciais da formação de tumores, utilizando produtos naturais inclui a diminuição de enzimas metabólicas responsáveis pela geração de espécies reativas de oxigênio que atuam principalmente na ativação de carcinógenos, as chamadas enzimas de fase 1, ao passo que enzimas de fase 2, que participam do processo de destoxificação do organismo frente a carcinógenos, são estimuladas, promovendo a desativação de radicais e eletrófilos envolvidos nos processos celulares. A família *Sapotaceae* tem se destacado por diversas espécies apresentarem atividades antioxidantes, quimiopreventivas e citotóxicas. Os metabólitos secundários apresentam como principal função evitar ou reduzir os danos causados pela presença de parasitas, vírus, predadores, insetos e outros artrópodes, variações físico-químicas do meio ambiente, e para atrair polinizadores. Os óleos essenciais são metabólitos secundários sintetizados por plantas que possuem como principais características a sua volatilidade, o forte odor, aspecto oleoso, pouca solubilidade em água além de apresentarem pouca estabilidade em ambientes com luz, ar e calor. A utilização de óleos essenciais na medicina popular para o tratamento, prevenção e cura de algumas doenças ocorre há diversos anos. Este trabalho teve como objetivo investigar a atividade antioxidante, anti-inflamatória e indutora da quinona redutase de produtos naturais. No primeiro capítulo foi avaliado a atividade antioxidante e quimiopreventiva de câncer de extratos de *Labramia bojeri* e posterior biofracionamento com consequente elucidação da estrutura do isolado, obtendo-se o acetato de *a*-amirina. Os resultados demonstraram que extratos de folhas de *L. bojeri* possuem atividades antioxidantes e apresentaram atividade citotóxica em células de macrófagos testadas neste trabalho. O extrato hexânico de folha demonstrou atividade quimiopreventiva de câncer quando testado em células Hepa1c1c7, além disso a atividade anti-inflamatória também foi confirmada por meio de ensaio da atividade inibitória de NF-κB. Pode-se sugerir que estes efeitos sejam devido ao acetato de *a*-amirina isolado no extrato hexânico das folhas da planta. Este estudo sugere que as propriedades quimiopreventivas desta substância sejam devido à indução da enzima quinona redutase representada *in silico*. O segundo capítulo teve

como objetivo o estudo da atividade quimiopreventiva de câncer *in silico* e *in vitro* de 33 principais substâncias presentes em óleos essenciais. Os resultados demonstraram que, das 33 substâncias testadas, 15 apresentaram atividade quimiopreventiva de câncer resultante da indução da quinona redutase *in vitro,* corroboradas pela análise de *docking* molecular *in silico* da ligação entre o complexo da enzima Quinona Redutase, FAD e as substâncias isoladas. As substâncias isoladas que apresentaram atividade *in vitro* de indução da quinona redutase e mais baixas energia de *docking* foram o Valenceno, Eugenol e (+)Carvona. Este trabalho demonstra pela primeira vez que a atividade quimiopreventiva destas substâncias ocorre devido à indução da quinona redutase. A análise *in silico* da predição da farmacocinética e toxicidade das 33 substâncias foi realizada e demonstrou que todos atendem à regra de Lipinski e apresentariam boa biodisponibilidade oral entretanto o p-Cimeno e γ-Terpineno são fatais se forem ingeridos.

Palavras-chave: Labramia bojeri, Óleos Essenciais, Atividade Quimiopreventiva de câncer, Quinona redutase

ABSTRACT

Coutinho, Silvia Cruz Goes, Dr., Universidade Vila Velha – ES, august 2023, **Quinone Reductase Inducing Activity of producys of plant origin.** Advisor: Profª. Drª Denise Coutinho Endringer.

Natural products are sources of new drugs used by mankind for centuries and play a key role in the discovery of new drugs for infectious diseases, cancer and other neurodegenerative diseases to this day. Many of these natural products are capable of altering the metabolism of carcinogens through the induction of various substances involved in the detoxification of the organism. One of the strategies for protecting cells from the initial events of tumor formation, using natural products, includes the reduction of metabolic enzymes responsible for the generation of reactive oxygen species that act mainly in the activation of carcinogens, the so-called phase 1 enzymes, while that phase 2 enzymes, which participate in the body's detoxification process against carcinogens, are stimulated, promoting the deactivation of radicals and electrophiles involved in cellular processes. The Sapotaceae family has stood out because several species have antioxidant, chemopreventive and cytotoxic activities. Secondary metabolites have the main function of preventing or reducing damage caused by the presence of parasites, viruses, predators, insects and other arthropods, physicochemical changes in the environment, and to attract pollinators. Essential oils are secondary metabolites synthesized by plants that have as main characteristics their volatility, strong odor, oily appearance, low solubility in water, in addition to presenting little stability in environments with light, air and heat. The use of essential oils in folk medicine for the treatment, prevention and cure of some diseases has been going on for several years. This work aimed to investigate the antioxidant, anti-inflammatory and quinone reductase inducing activity of natural products. In the first chapter, the antioxidant and cancer chemopreventive activity of extracts of Labramia bojeri was evaluated and subsequent biofractionation with consequent elucidation of the structure of the isolate, obtaining a-amyrin acetate. The results demonstrated that L. bojeri leaf extracts have antioxidant activities and showed cytotoxic activity in macrophage cells tested in this work. The hexane leaf extract demonstrated cancer chemopreventive activity when tested in Hepa1c1c7 cells, in addition the anti-inflammatory activity was also confirmed by assay of NF-κB inhibitory activity. It can be suggested that these effects are due to a-amyrin acetate isolated in the hexane extract of the leaves of the plant. This study suggests that the chemopreventive properties of this substance are due to the induction of the enzyme quinone reductase represented in silico. The second chapter aimed to study the chemopreventive activity of cancer in silico and in vitro of 33 main substances present in essential oils. The results showed that, out of the 33 substances tested, 15 showed cancer chemopreventive activity resulting from in vitro quinone reductase induction, corroborated by the in silico

molecular docking analysis of the binding between the Quinone Reductase enzyme complex, FAD and the isolated substances. The isolated substances that showed in vitro quinone reductase induction activity and lower docking energy were Valencene, Eugenol and (+)Carvone. This work demonstrates for the first time that the chemopreventive activity of these substances occurs due to the induction of quinone reductase. The in silico analysis of the prediction of the pharmacokinetics and toxicity of the 33 substances was performed and demonstrated that all of them meet Lipinski's rule and would have good oral bioavailability, however p-Cymene and γ-Terpinene are fatal if ingested.

Keywords: Labramia bojeri, Essential Oils, Cancer chemopreventive activity, Quinone reductase.

INTRODUÇÃO GERAL

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O conhecimento tradicional e os relatos científicos demonstram que as plantas medicinais são ricas fontes de compostos biologicamente ativos, que podem ser utilizados para o tratamento de diversas doenças inclusive alguns tipos de câncer (MARRELI, 2021) Os principais benefícios de compostos químicos isolados ou suas misturas encontradas em frutas, vegetais, feijões e outras fontes vegetais incluem efeitos anti-inflamatórios, antioxidantes, antibacterianos, antifúngicos e outros efeitos benéficos à saúde (KOKLESOVA et al., 2020). O reino vegetal, no geral, é capaz de produzir mais de 200.000 ativos que são de interesse para os humanos, com diversas funções farmacêuticas para uma ampla gama de doenças, incluindo vários tipos de câncer (AFRIN; HUANG; LUO, 2015; SHIH; MORGAN, 2020).

Com os avanços da ciência nos últimos anos, é possível investigar com qualidade diversos tipos de plantas que são usadas há gerações por diversas populações tradicionais ao longo dos anos para fins terapêuticos. Recentemente, substâncias naturais de plantas estão no centro do interesse científico devido à sua atividade anticancerígena (KUBATKA et al., 2017; KOKLESOVA et al., 2020). Existem evidências que sugerem uma correlação entre maior consumo de alimentos ricos em fitoquímicos e menor risco de desenvolvimento de câncer (HOSSEINI e GHORBANI, 2015).

Produtos naturais de origem vegetal isolados são utilizados há muitos anos no desenvolvimento de medicamentos de combate às doenças, especificamente o câncer (PAN; CHAI; KINGHORN, 2012). Além disso, diversos trabalhos avançaram nos últimos anos na descoberta de novos agentes quimiopreventivos de câncer vindo de inúmeras espécies vegetais, abrindo portas para o entendimento para os mecanismos de prevenção do câncer (MAJOLO et al., 2019; SIDDIQUI et al., 2022).

Ultimamente, aumentou-se o interesse na determinação da atividade antioxidante de produtos naturais (SAMET et al., 2019), no qual estudos apontam que esses componentes também são responsáveis pela atividade como os óleos essenciais que podem ter diversas ações benéficas para o combate de doenças, variando de acordo com as plantas utilizadas e as formas de extração de seus compostos (AMORATI; FOTI; VALGIMIGLI, 2013; WEI; SHIBAMOTO, 2007). A capacidade antioxidante das plantas pode contribuir para prevenção de vários distúrbios de saúde, sejam agudos ou crônicos, como inflamatórios, alérgicos,

trombóticos, diabéticos, cardiovasculares, câncer e outros (BAUTISTA-HERNÁNDEZ et al., 2021; GALEOTTI et al., 2018; JAGANJAC; TISMA; ZARKOVIC, 2021; MENG et al., 2020).

Produtos naturais são potenciais candidatos na produção de compostos com atividades quimiopreventivas de câncer. Diversas espécies vegetais vêm sendo estudadas na busca por compostos que podem futuramente se tornarem medicamentos eficazes. Estudos recentes mostraram que alguns compostos naturais tem capacidade quimiopreventiva de câncer potentes (SILVA et al., 2018; EL-HAWANY et al., 2018; AHMAD et al., 2022). Os metabólitos secundários encontrados em diversas espécies de plantas, como por exemplo, flavonóides e triterpenóides são mediadores bem conhecidos da enzima Quinona Redutase com papéis apreciáveis na quimioprevenção de câncer (CHENG et al., 2010; FAHEY e STHEPHENSON, 2002).

A quimioprevenção de câncer ocorre pela eliminação dos efeitos dos carcinógenos pela inibição ou regulação negativa de enzimas, como aromatase e óxido nítrico sintase induzível (iNOS), que são capazes de gerar espécies cancerígenas (AGGARWAL e SHISHODIA, 2006). Por outro lado, a quimioprevenção do câncer também pode ser alcançada pela ativação ou regulação positiva de enzimas anticarcinogênicas, que incluem enzimas citoprotetoras de processamento de eletrófilos, como glutationa S-transferases, bem como superóxido dismutase e NAD(P)H: quinona redutase (QR1) (ROSS et al., 2000; PROCHASKA e TALALAY, 1988).

Muitos produtos naturais e sintéticos foram empregados para prevenir a carcinogênese ou metástase de câncer (SPORN, 1976). A pesquisa de quimioprevenção aumentou visivelmente com informações avançadas sobre a carcinogênese e a detecção de alvos moleculares potentes para impedir o processo de carcinogênese (GEORGE et al., 2021).

Estudos recentes têm demonstrado que plantas conhecidas podem ser ainda mais exploradas no âmbito da quimiopreveção de câncer por meio de novas técnicas acerca de suas aplicações já conhecidas (AHMED et al., 2022). Análises de antioxidantes, atividades quimiopreventivas, testes moleculares e técnicas de docking molecular podem trazer novos conhecimentos sobre espécies anteriormente já estudadas.

As metodologias de *docking* molecular são de grande importância no desenvolvimento de novos fármacos. Esse método visa prever o modo de ligação experimental e a afinidade de uma pequena molécula dentro do sítio de ligação do receptor alvo de interesse (GUEDES; DE MAGALHÃES; DARDENNE, 2014).

Neste contexto, este trabalho objetivou a investigação da atividade antioxidante, anti-inflamatória e indutora da enzima quinona redutase em produtos naturais. Considerando os resultados obtidos por meio das metodologias propostas, o trabalho foi dividido em dois capítulos. O primeiro capítulo apresenta os resultados dos estudos químicos, atividades antioxidantes e anti-inflamatória e efeito quimiopreventivo de *Labramia bojeri in vitro e in silico.* O segundo capítulo descreve a atividade quimiopreventiva de câncer em diversos isolados de óleos essenciais por meio de análises *in silico* e *in vitro*.

Os dados encontrados nesse trabalho confirmam a hipótese de que os extratos de produtos naturais e substâncias isoladas possuem propriedades antioxidantes, anti-inflamatória e quimiopreventiva de câncer.

FUNDAMENTAÇÃO TEÓRICA

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Compostos bioativos

Compreende-se como metabolismo vegetal primário tudo que é produzido pelas plantas a partir de reações químicas e enzimáticas, que são comuns ao bom funcionamento celular basal com compostos altamente conservados e metabolismo secundário (compostos fenólicos, terpenos e compostos contendo nitrogênio), quando essas atividades estão intimamente ligadas à proteção e à sobrevivência às diversas modificações do ambiente (ERB; KLIEBENSTEIN, 2020; HARTMANN, 2007).

As plantas são capazes de produzir mais de 200.000 produtos naturais dos quais, muitos são de interesse para os humanos, com funções farmacêuticas para uma ampla gama de doenças (AFRIN; HUANG; LUO, 2015; SHIH; MORGAN, 2020). Alguns desses metabólitos, como artemisinina e o taxol, são importantes para o tratamento da malária e câncer de mama, respectivamente (MA et al., 2009; WILSON; ROBERTS, 2014).

Toda a síntese de metabólitos secundários e seu acúmulo estão diretamente ligados a fatores espaciais e temporais, com fortes influências dos fatores bióticos e abióticos. Esses processos ocorrem por meio de uma regulação transcricional espaço-temporal das vias metabólicas, controlada por uma rede complexa envolvendo diversas proteínas reguladoras conhecidas como fatores de transcrição (AFRIN; HUANG; LUO, 2015). A regulação transcricional é a mudança dos níveis da expressão gênica, sua regulação depende de todos os efeitos combinados das diversas propriedades estruturais dessas substâncias e de suas interações com os fatores de transcrição dos genes, que levam à produção desses metabóltios secundários com diversas funções para as plantas (AFRIN; HUANG; LUO, 2015; PAUWELS; INZÉ; GOOSSENS, 2009).

Família Sapotaceae

A família *Sapotaceae*, ordem *Ericales*, possui uma ampla gama de espécies, com cerca de 60 gêneros e 1300 espécies, distribuídas em três subfamílias: *Sarcospermatoideae, Sapotoideae e Chrysophylloideae*, sendo encontradas principalmente em florestas tropicais úmidas (DE LIMA et al., 2018; SWENSON; ANDERBERG, 2005). Esta família é conhecida pela grande diversidade morfológica

e sua importância medicinal para diversos povos tradicionais no mundo (BAKY; ELSAID; FARAG, 2022; DE LIMA et al., 2018).

Consiste em árvores ou arbustos com ampla distribuição mundial, embora a maior diversidade de espécies seja encontrada nas regiões tropicais e subtropicais da Ásia e América do Sul. Diversas espécies produzem frutos comestíveis, com ou sem uso econômico (BAKY et al., 2016; BAKY; ELSAID; FARAG, 2022; DE LIMA et al., 2018).

Diversas espécies desta família são utilizadas para tratar várias doenças em todo mundo. As doenças tratadas vão desde tratamento de bronquites, helmintos, amigdalite, bem como tratamento de reumatismo e sangramentos (BADUKALE et al., 2021; YADAV et al., 2012) (Tabela 1).

Tabela 1. Usos tradicionais relatados para espécies pertencentes à família *Sapotaceae.*

Espécie	Parte usada	Uso tradicional	Referência
Madhuca indica JFGmel.	Flores e folhas	Agente de resfriamento, tônico, amigdalite aguda, crônica, bronquite e anti-helmintos. Adstringente, afrodisíaco e emoliente.	(BADUKALE et al., 2021)
Mimusops sabor L.	Casca e caules Flores (loção)	Limpeza dos dentes Cura de feridas e úlceras.	(BALIGA et al., 2011)
Chrysophyllum albidum G.Don	Folhas	Emoliente, dor de estômago e tratamento de diarréia.	(EMUDAINOHWO et al., 2015)

Espécie	Parte usada	Uso tradicional	Referência
Argania spinosa (L.) Skeels	Folhas, sementes e raízes.	Antidiabético	(MECHQOQ et al., 2021)
	Sementes	Trata queimaduras, eczemas, pele seca, cuidados capilares anti-hipercolesterolémicos e anti-reumáticos	
	Flores	Cosméticos para o rosto	
Labramia bojeri	Flores, frutos, folhas	Inseticida	(MARTINEZ et al., 2012)

Uma particularidade da família Sapotaceae é sua riqueza em saponinas (BAKY et al., 2016; BAKY; ELSAID; FARAG, 2022; TAPONDJOU et al., 2011). As saponinas triterpenos são comumente encontrados em quase todas as Ericales conhecidas, e os tipos triterpenóides, incluindo oleanano, ursano e lupano (triterpenos pentacíclicos), são os mais encontrados (AKIHISA et al., 2018; MACEDO et al., 2004).

Os extratos e várias preparações com partes das plantas da família *Sapotaceae* podem ser utilizados como medicamentos. Uma vez que as saponinas triterpenos demonstraram possuir uma ampla gama de atividades biológicas e farmacológicas, principalmente atividade citotóxica e quimiopreventiva, esses compostos parecem ser responsáveis, em parte, pela bioatividade dos extratos (AKIHISA et al., 2018). Logo, é muito importante que mais estudos sobre esta família sejam realizados, visto a gama de diversidade de espécies desta família.

Quimioprevenção e atividade citotóxica

A carcinogênese é um processo de vários estágios que levam a uma série de eventos de mudanças genéticas e epigenéticas que levam aos estágios iniciais, promoção e progressão do câncer nos seres vivos (GEORGE; DELLAIRE; RUPASINGHE, 2017; SEYFRIED; SHELTON, 2010). Já a quimioprevenção é dita como o uso de compostos naturais não tóxicos ou produtos químicos sintéticos com

a intenção de prevenir qualquer dos estágios do câncer (GEORGE; DELLAIRE; RUPASINGHE, 2017).

Os produtos naturais são utilizados há mais de 50 anos nessa função. Plantas e microrganismos são as principais fontes de potenciais compostos com ação quimiopreventiva (SIDDIQUI et al., 2022). Além disso, diversos trabalhos vêm avançando nos últimos anos na descoberta de novos agentes quimiopreventivos vindo de inúmeras espécies vegetais, abrindo portas para o entendimento para os mecanismos de prevenção do câncer (MAJOLO et al., 2019; SIDDIQUI et al., 2022).

De fato, os produtos naturais têm mostrado grande potencial no desenvolvimento de fármacos quimioterápicos, com enorme gama de estruturas moleculares e aplicações farmacológicas (PAN; CHAI; KINGHORN, 2012). Desta forma, 52% dos medicamentos para o câncer aprovados de 1981 a 2014 são produtos naturais ou seus derivados (NEWMAN; CRAGG, 2016). Excelentes fontes para o desenvolvimento de fármacos são os metabólitos secundários. A descoberta de suas estruturas podem contribuir com a melhora da seletividade desses fármacos, consequentemente melhorando sua absorção, distribuição e outras propriedades na ação anticancerígena (MAJOLO et al., 2019).

A casca da raiz de *Butyrospermum parkii* pertencente à família *Sapotaceae* apresentou atividade citotóxica contra linhagens celulares de adenocarcinoma de mama humano (MDA-MB 231), melanoma maligno (A375), carcinoma de cólon (HCT116) e glioblastoma multiforme (T98G) (TAPONDJOU et al., 2011). Outra espécie da família *Sapotaceae* também demonstrou importante atividade anticancerígena. O extrato de acetato de etila de frutos de *Argania spinosa* mostrou atividade citotóxica contra células de câncer de mama humano (MCF7) (BABILI et al., 2010).

Ainda hoje a busca por novos fármacos com ação quimiopreventiva de câncer se faz necessária, pois vários protocolos podem se beneficiar de plantas medicinais e todo seu potencial quimiopreventivo. Diversos compostos bioativos vegetais ainda podem ser descobertos com a grande gama de espécies ainda a serem estudadas na geração de novos produtos quimiopreventivos.

Atividade Antioxidante

Os antioxidantes são uma família de compostos de grande interesse para as várias áreas da pesquisa, principalmente as moléculas derivadas de plantas. Sua

capacidade de proteção contra as agressões oriundas das espécies reativas de oxigênio (EROs), justifica sua grande importância (AMORATI; VALGIMIGLI, 2018). Os antioxidantes possuem grandes variações, logo, são diferenciados em dois grandes grupos: os antioxidantes diretos, que são capazes de proteger os tecidos da oxidação e podem expressar seu potencial tanto *in vitro* quanto *in vivo* e os antioxidantes indiretos que não são capazes de oferecer a proteção propriamente dita e sim estimular a síntese de outros elementos na proteção do tecido, induzindo diversas enzimas antioxidantes (AMORATI; VALGIMIGLI, 2015).

Nos últimos anos, aumentou-se o interesse na determinação da atividade antioxidante de produtos naturais (SAMET et al., 2019), no qual os pesquisadores apontam que esses componentes responsáveis pela atividade antioxidante desses produtos, como óleos essenciais, podem variar de acordo com as plantas utilizadas e as formas de extração (AMORATI; FOTI; VALGIMIGLI, 2013; WEI; SHIBAMOTO, 2007). Essa capacidade antioxidante desses extratos contribui para a prevenção de vários distúrbios agudos e crônicos, como inflamatórios, alérgicos, trombóticos, diabéticos, cardiovasculares, câncer e outros (BAUTISTA-HERNÁNDEZ et al., 2021; GALEOTTI et al., 2018; JAGANJAC; TISMA; ZARKOVIC, 2021; MENG et al., 2020).

Plantas da família *Sapotaceae*, ao qual pertence a espécie *Labramia bojeri* (Figura 1) têm demonstrado serem grandes produtoras de compostos antioxidantes (BAKY et al., 2016). *Butyrospermum parki* apresentou atividade antioxidante contra Trolox ou hidroxitolueno butilado (BHT), contra DPPH, radicais livres e óxido nítrico (TAPONDJOU et al., 2011). Extrato alcoólico das folhas de *Mimusops elengi* demonstrou uma excelente atividade antioxidante pela atividade sequestrante de peroxinitrito, superóxido e ácido hidrocloroso (BISWAKANTH et al., 2012).

Diferentes métodos para determinar a capacidade antioxidante em extratos vegetais podem ser utilizados, dentre eles pode-se incluir o método de desativação de radicais livres DPPH, o poder antioxidante redutor do ferro (FRAP, do inglês *Ferric Reducing Antioxidant Power*) (TAPONDJOU et al., 2011). O método DPPH é bastante usado para avaliação da capacidade antioxidante (OLIVEIRA, 2015). Na presença de um antioxidante doador de H^+, ocorre redução do radical DPPH (KEDARE; SINGH, 2011), ocorrendo uma mudança de cor. Quanto maior a descoloração, maior a doação de H+ e atividade antioxidante da amostra (REDDY et al., 2012).

Figura 1. Imagem *Labramia bojeri* A.DC. Fonte: Autora

Óleos essenciais

Os óleos essenciais são misturas complexas de metabólitos secundários sintetizados por plantas aromáticas e possuem como principais características a sua volatilidade, o forte odor, aspecto oleoso e normalmente incolor, pouca solubilidade em água, além de apresentarem pouca estabilidade em ambientes com luz, ar e calor (ASBAHANI et al., 2015; BAKKALI et al., 2008). Os metabólitos secundários apresentam diversificadas funções, entre elas evitar ou reduzir os danos causados pela presença de parasitas, vírus, predadores, insetos, e outros artrópodes, variações físico-químicas do meio ambiente (luz, temperatura, pH, dentre outros), e para atrair

polinizadores (DE OLIVEIRA HASHIMOTO et al., 2016; PINTO-ZEVALLOS; VÄNNINEN, 2013).

Os óleos essenciais podem ser encontrados em diversos órgãos das plantas, como folhas, rizomas, flores, frutos e cascas, sendo utilizadas diversas técnicas para sua extração, entre as quais as principais são por arraste a vapor, por solventes, com fluido supercrítico e a hidrodestilação (DE BARROS FERNANDES et al., 2014; DO AMARAL et al., 2018).

A utilização de óleos essenciais na medicina popular para o tratamento, prevenção e cura de algumas doenças ocorre há diversos anos. Atualmente são mundialmente utilizados em indústrias alimentícias e farmacêuticas, perfumarias, fabricação de cosméticos, inseticidas, dentre outros (BRITO et al., 2021; LAWAL; OGUNWANDE, 2013; OOTANI et al., 2013).

Os óleos essenciais apresentam diversas propriedades biológicas como a atividade antimicrobiana, antioxidante, anti-inflamatória, antitumoral, antifúngica, analgésica, larvicida, inseticida, dentre outras (AIDI WANNES et al., 2010; JUNG et al., 2013; MIRAGHAZADEH; SHAFAROODI; ASGARPANAH, 2015; RAJKUMAR; JEBANESAN, 2010; SLIMANE et al., 2014; VALERIANO et al., 2012; YAMADA et al., 2013). São constituídos principalmente por terpenos (monoterpenos, sesquiterpenos), fenilpropanóides e outros compostos oxigenados (VALERIANO et al., 2012; OLIVEIRA et al., 2011; DE OLIVEIRA HASHIMOTO et al., 2016). Os terpenóides apresentam, dentre diversas atividades biológicas, a ação antimicrobiana, anti-alérgica, anti-espasmódica, anti-hiperglicêmica, anti-inflamatória, antifúngica, antiparasitária, antiviral e propriedade imunomoduladora, sendo um dos responsáveis pelas propriedades biológicas dos óleos essenciais (PADUCH et al., 2007).

Os óleos essenciais podem apresentar em sua constituição, substâncias com concentrações e quantidades variadas, podendo conter de 20 a 60 compostos diferentes, determinando sua propriedade biológica e os seus aspectos benéficos ou prejudiciais. As composições dos óleos essenciais podem sofrer modificações de acordo com a espécie e subespécie da planta, a localização geográfica, método de extração usado e tempo de colheita (BAKKALI et al., 2008; SLIMANE et al., 2014).

Por serem compostos lipofílicos, eles tendem a penetrar a parede celular e membrana citoplasmática, rompendo diferentes estruturas das camadas fosfolipídicas, polissacarídeos e ácidos graxos encontrados nestas regiões causando danos consideráveis à membrana (CARSON; MEE; RILEY, 2002). Em bactérias, a

permeabilização das membranas está associada à perda de íons e redução do potencial de membrana, colapso da bomba de prótons e depleção de ATP (DEBONNE et al., 2018; DI PASQUA et al., 2006; KNOBLOCH et al., 2011; MAURYA et al., 2021). Em células eucarióticas, os óleos essenciais podem levar a uma despolarização da membrana plasmática e das membranas mitocondriais, diminuindo o potencial de membrana e afetando o ciclo iônico do Ca^{++} e outros canais iônicos, onde reduzem o pH, afetando a bomba de prótons, mecanismo parecido com o que ocorre em bactérias (CARSON; MEE; RILEY, 2002; RICHTER; SCHLEGEL, 1993).

As atividades citotóxicas de óleos essenciais ou seus principais componentes, às vezes ativados pela luz, também foram demonstradas em células de mamíferos *in vitro* por ensaios de viabilidade de curto prazo usando coloração celular específica ou corantes fluorescentes, incluindo o teste NRU e teste MTT e demonstraram que a citotoxicidade dos óleos essenciais em células de mamíferos é causada pela indução de apoptose e necrose (CHUNG et al., 2007; SÖDERBERG; JOHANSSON; GREF, 1996).

Quanto à propriedade antimutagênica dos óleos essenciais, alguns estudos demonstram que esta atividade pode estar envolvida com a inibição da entrada de agentes mutagênicos nas células, inativação desses agentes, na captura de radicais livres ou na ativação de enzimas antioxidantes (CARSON; MEE; RILEY, 2002; IPEK et al., 2005; SHANKEL et al., 1993).

Segundo Sacchetti e colaboradores (2005), a citotoxicidade dos óleos essenciais ocorre principalmente pela presença das funções aldeído, álcool e fenol nos óleos essenciais. Grecco e colaboradores (2014) avaliaram a atividade citotóxica do óleo essencial extraído das folhas de *Nectandra leucantha* Nees & Mart., e indicaram que este óleo possui atividade citotóxica significativa em células de melanomas murinos com IC 50 de 33.0$\pm$ 1.0 µg/ml, câncer cervical humano (IC 50 de 194.9 ± 0.1 µg/ml) e glioblastoma humano (IC50 de 75.95 ± 0.03 µg/ml).

A capacidade citotóxica dos óleos essenciais e sua atividade antioxidante podem torná-los excelentes agentes na composição de novos fármacos. Outra grande vantagem dos óleos essenciais é o fato de serem geralmente isentos de riscos genotóxicos a longo prazo. Além disso, alguns deles podem mostrar uma capacidade antimutagênica importante que pode estar ligada a uma atividade anticarcinogênica. Logo, estudos que consigam analisar com mais clareza essas propriedades são bem-

vindos, uma vez que pequenas doses podem ser combinadas e usadas para diversos tratamentos contra o câncer.

Composição dos óleos essenciais e aplicabilidade

Os óleos essenciais são produtos obtidos a partir de partes vegetais e podem apresentar grande importância na economia devido a sua grande variedade de aplicações em diversas indústrias como as de alimentos, química e farmacêutica (ARCE et al., 2005; CHÁFER et al., 2005).

No que se refere a composição química, os óleos essenciais são compostos basicamente por uma mistura de hidrocarbonetos terpênicos e seus derivados oxigenados, os quais são responsáveis pelas principais características aromatizantes (ARIDOĞAN et al., 2002) e geralmente apresentam as melhores propriedades sensoriais (ARCE et al., 2005) sendo, portanto, os preferidos pela indústria. Os hidrocarbonetos terpênicos tendem a se decompor na presença de calor e oxigênio, gerando odores desagradáveis, os quais contribuem para a perda de qualidade do óleo (GIRONI; MASCHIETTI, 2012).

Terpenos, hidrocarbonetos e derivados oxigenados terpenóides são os principais constituintes dos óleos essenciais. Esta classe fitoquímica é ampla, porém somente os mono e sesquiterpenos estão presentes nos óleos essenciais. Os terpenos, de forma geral, são formados por unidades do isopreno (05 carbonos). Os monoterpenos são compostos por duas unidades do isopreno (10 carbonos), os sesquiterpenos, por sua vez, são compostos por três unidades do isopreno (15 carbonos), os diterpenos por 20 unidades de carbonos, os triterpenos por 30 unidades de carbono e os tetraterpenos por 40 unidades de carbono (BRUNETON, 1991). Neste conjunto, os terpenos são a principal classe, sendo o D-limoneno, um monoterpeno presente na maioria dos óleos essenciais conhecidos (LANÇAS; CAVICCHIOLI, 1990).

Os mono e sesquiterpenos podem ser divididos em três grupos: acíclicos, monocíclicos e bicíclicos. Em cada um desses subgrupos há ainda outras classificações (quanto à função dos grupamentos): hidrocarbonetos insaturados (por exemplo, o D-limoneno), alcoóis (linalol), aldeídos (geranial) ou cetonas, lactonas e tropolonas. As variações estruturais dos sesquiterpenos são da mesma natureza que as precedentes, podendo ser acíclicos (nerol), monocíclicos ou bicíclicos (β-selineno) ou lactonas sesquiterpênicas (Santana et al., 2014).

Devido à sua abundância nos tecidos vegetais e suas funções importantes para diversos fins, os monoterpenos são largamente utilizados em processos farmacêuticos e na indústria cosmética, além de possuir ação antitumoral já conhecida pela literatura (SOBRAL et al., 2014).

Alguns desses terpenoides se destacam na atividade citotóxica para prevenção do câncer, por meio de diversos mecanismos que possam levar à morte celular, como o Limoneno, Geraniol, Citral, Citronelol e outros terpenos existentes na composição geral da maioria dos óleos essenciais (MACHADO et al., 2022).

Os efeitos do limoneno foram recentemente demonstrados em células de câncer de bexiga humana apresentando um IC50 de 9*μ* M. O trabalho apresentou capacidade antitumoral de indução da parada do ciclo celular, supressão da migração e invasão celular e apoptose com observação de fragmentação nuclear, condensação da cromatina, divisão do núcleo, aumento de Bax e caspase-3 e diminuição da expressão de Bcl-2 (YE et al., 2020). d-Limoneno mostrou atividade antitumoral pulmonar *in vivo* e *in vitro* prevenindo o crescimento de células cancerígenas do pulmão e induzindo a apoptose por mecanismos que envolvem a autofagia. Houve aumento de Bax e PARP clivado durante o tratamento, o que pode estar relacionado à indução da morte das células cancerígenas do pulmão. Aumentos também foram encontrados em Atg-5, presumindo que a sobrecarga de Atg5 pode estar parcialmente envolvida na apoptose induzida por d-limoneno (YU et al., 2018).

O geraniol induz a apoptose com uma alta regulação de Bax e uma baixa regulação da expressões de Bcl-2, dano ao DNA e parada do ciclo celular em células de câncer de cólon Colo-205, apresentando um IC50 de 20 e 30 μM (MADANKUMAR et al., 2017). No entanto, o geraniol apresenta atividade antitumoral por diversos outros mecanismos, como observado nos últimos anos. Em um modelo de carcinogênese oral usando uma dose de 200 mg/kg, o geraniol regula negativamente a ativação de NF- *κB* , reduzindo a expressão de TNF - *α* , IL-1β *,* COX-2 e iNOS (MADANKUMAR et al., 2017). Em células de câncer endometrial de Ishikawa, com IC50 de 140,929 μM *,* o geraniol induz apoptose com envolvimento da via mitocondrial, observada por diminuição de Bcl-2 e aumento de coloração Bax e células TUNEL-positivas, além de aumento de os níveis de mRNA de Bax, caspase-3 e -8, citocromo C e Fas e uma diminuição no gene Bcl-2 (KUZU et al., 2020).

O citral demonstrou um efeito antiproliferativo em várias células cancerosas. A apoptose foi observada em células de câncer de estômago humano tratadas com

citral a 5 µg/mL que apresentaram diminuição no número de colônias e indução de morte celular (BALUSAMY et al., 2019); em células de câncer de próstata (células PC3 e PC-3M) com concentração de 10 e 12,5 *µg* /ml, respectivamente, de maneira dose dependente pela regulação positiva de BAX e regulação negativa da expressão de Bcl-2 (BALUSAMY et al., 2020); e em HCT116 e HT29 (linhagens celulares de câncer colorretal), nas quais induziu apoptose mediada por mitocôndrias via aumento de ROS intracelular e fosforilação da proteína p53, expressão de Bax e diminuição da expressão de Bcl-2 e Bcl-xL que promoveu a clivagem de caspase-3 (SHEIKH et al., 2017); também mostrou citotoxicidade na linhagem de células de linfoma de Burkitt humano e aumentou aditivamente os efeitos citotóxicos e apoptóticos da doxorrubicina (THOMAS et al., 2016). A combinação de citral e doxorrubicina aumentou a expressão da proteína pró-apoptótica BAK, mas diminuiu a expressão da proteína antiapoptótica BCL-XL em comparação com células tratadas apenas com doxorrubicina (DANGKONG; LIMPANASITHIKUL, 2015).

O citronelol vem sendo descrito com atividade antitumoral contra câncer de pulmão (YU et al., 2019) e de mama (RAJENDRAN; PACHAIAPPAN; THANGARASU, 2020), induzindo necroptose e apoptose, respectivamente. Para o câncer de pulmão, o IC50 encontrado foi de 49,74 *µg* /ml e a necroptose foi confirmada por uma regulação positiva da via TNF- *α* e regulação negativa das atividades de caspase-3 e -8. Além disso, o citronelol na dose de 50 mg/kg inibiu 80% do crescimento tumoral subcutâneo previamente induzido por injeção intraperitoneal de NCI-H1299 em camundongos. Para câncer de mama, IC50 foi encontrado entre 35 e 80 *µ*M/ml, e a apoptose foi validada pela perda de viabilidade celular, aumento na geração de ROS, potencial de membrana mitocondrial alterado, dano aumentado ao DNA e modulação da expressão de proteínas apoptóticas (inibição de Bcl-2 com upregulation de Bax e caspase-9 e -7) em células MCF-7 e MDA-MB-231 (RAJENDRAN; PACHAIAPPAN; THANGARASU, 2020).

Atividade quimiopreventiva e quinona redutase

As espécies reativas de oxigênio causam estresse oxidativo, resultando em dano celular ou, eventualmente, alterando o material genético de uma célula normal em uma transformada. A ativação de enzimas desintoxicantes também pode combater a carcinogênese e proteger as células dos efeitos dos carcinógenos

finais. Vários agentes quimiopreventivos naturais e sintéticos são utilizados nos casos em que há um risco elevado de desenvolver câncer ou para prevenir a recorrência do câncer após o tratamento (AHMAD et al., 2022).

O câncer é um processo crônico determinado pelo crescimento descontrolado de células anormais, possuindo subdivisões de vários tipos, variando conforme o tecido do órgão originário e o mecanismo de desenvolvimento da doença (SPORN; SUH, 2000). Com os avanços na compreensão do processo carcinogênico em nível celular e molecular feitos nas últimas décadas levaram ao desenvolvimento de uma nova abordagem promissora para a prevenção do câncer, denominada "quimioprevenção" (GERHÄUSER et al., 2003).

A prevenção da doença em estágios primários e secundários apresenta relação direta com a diminuição da mortalidade. A prevenção primária consiste em evitar os precursores da carcinogênese, como a radiação e substâncias químicas (SPORN; SUH, 2000).

A prevenção secundária consiste na chamada quimioprevenção, onde os compostos carcinogênicos são impedidos de reagirem em alvos teciduais pelo que pode ser denominado de "agentes bloqueadores" (*bloking agents*) (SPORN; SUH, 2000). Um agente quimiopreventivo apresenta efeitos colaterais reduzidos e, consequentemente, baixa toxicidade (SPORN; SUH, 2000). A classificação dos agentes terapêuticos ocorre de acordo com seu mecanismo de ação no estágio da carcinogênese, classificados como bloqueadores ou supressores (WATTENBERG, 1985). O potencial quimiopreventivo é realizado por meio de ensaios de citotoxicidade em cultura de células tumorais, onde os resultados são uma análise conjunta com os ensaios de quimioprevenção. As substâncias de boa atividade quimopreventiva não deverão ser citotóxicas, porém, caso as amostras apresentem resultados esperados de citotoxicidade, é possível que possuam atividade antitumoral, com mecanismos diferentes dos pesquisados (PAN; CHAI; KINGHORN, 2012).

A quimioprevenção do câncer pode ser alcançada pela eliminação dos efeitos dos carcinógenos pela inibição ou regulação negativa de enzimas, como aromatase e óxido nítrico sintase induzível (iNOS), que são capazes de gerar espécies cancerígenas (AGGARWAL; SHISHODIA, 2006). Por outro lado, a quimioprevenção do câncer também pode ser alcançada pela ativação ou regulação positiva de enzimas anticarcinogênicas, que incluem enzimas citoprotetoras de processamento de eletrófilos, como glutationa S-transferases, bem como superóxido dismutase e

NAD(P)H: quinona redutase (QR1) (PROCHASKA; TALALAY, 1988; ROSS et al., 2000).

Dentre as estratégias para proteger as células dos eventos iniciadores do câncer, incluem-se a diminuição das enzimas metabólicas responsáveis pela geração de espécies reativas (enzimas da fase I) e o aumento das enzimas da fase II, que podem desativar radicais e eletrófilos conhecidos por interceder nos processos celulares normais. A redução de quinonas eletrofílicas por Quinona Redutase é uma importante via de desintoxicação, que converte quinonas em hidroquinonas e reduz o ciclo oxidativo (CUENDET et al., 2006; PROCHASKA; TALALAY, 1988).

NAD(P)H:quinona redutase 1 (QR1) é uma enzima antioxidante que pertence à família NAD(P)H desidrogenase (quinona). É uma redutase de dois elétrons essencial que pode usar NADH ou NADPH como cofator redutor (RAHMAN; LIN, 2018). Contudo, a enzima antioxidante endógena NQO1 é um dos genes mais induzidos de forma consistente e robusta em se tratando de membros da família de proteínas citoprotetoras contra o estresse oxidativo no geral (DINKOVA-KOSTOVA; TALALAY, 2010).

Seu mecanismo de ação envolve a enzima glicose-6-fosfato-desidrogenase que ao atuar sobre a glicose-6-fosfato transfere dois elétrons e um próton para o NADP gerando in loco NADPH. Este transfere o hidreto para o complexo FAD-QR, com subseqüente redução à $FADH_2$ pela ação da enzima QR1. A quinona redutase é uma enzima homodimérica contendo FAD, que catalisa reduções obrigatórias de dois elétrons dependentes de NAD(P)H de quinonas e protege as células contra os efeitos tóxicos e neoplásicos dos radicais livres e espécies reativas de oxigênio decorrentes da redução de um elétron (LI et al.,1992).

Plantas medicinais são potentes candidatas na produção de compostos com atividades quimiopreventivas. Diversas espécies são utilizadas como aliadas nesta busca por compostos que podem ser utilizadas como potenciais medicamentos. Estudos recentes mostraram que alguns compostos naturais tem capacidade quimiopreventiva potentes (AHMAD et al., 2022; EL-HALAWANY et al., 2018). Metabólitos secundários de plantas, por exemplo, flavonóides e triterpenóides são mediadores bem conhecidos de QR1 com papéis apreciáveis na quimioprevenção do câncer (CHENG et al., 2010; FAHEY; STEPHENSON, 2002).

Docking Molecular

As metodologias de *docking* molecular são de grande importância no desenvolvimento de novos fármacos. Esse método visa prever o modo de ligação experimental e a afinidade de uma pequena molécula dentro do sítio de ligação do receptor alvo de interesse (GUEDES; DE MAGALHÃES; DARDENNE, 2014).

Esta metodologia explora o comportamento de pequenas moléculas no sítio de ligação de uma proteína alvo. À medida que mais estruturas de proteínas são determinadas experimentalmente usando cristalografia de raios X ou espectroscopia de ressonância magnética nuclear (RMN), o *docking* molecular é cada vez mais usado como uma ferramenta na descoberta de novos compostos (PAGADALA; SYED; TUSZYNSKI, 2017).

Com as estratégias de *docking,* o potencial dos compostos e sua especificidade em relação a um alvo específico podem ser calculadas para outros processos de otimização de processos (SHOICHET; KUNTZ, 1991). Além disso, considerando os recentes desenvolvimentos da tecnologia da computação e o rápido aumento de dados estruturais, químicos e biológicos disponíveis em um número crescente de alvos terapêuticos, é facilmente compreensível como o uso de abordagens *in silico* aumentou significativamente nas últimas décadas o que permite a triagem virtual de milhões de compostos em um tempo acessível, reduzindo assim os custos iniciais de identificação e melhora as chances de descobrimento de novos fármacos (AGOSTINO et al., 2013; MACALINO et al., 2015; SONG et al., 2011).

Rodrigues e colaboradores (2012) corroboram e manifestam que os custos computacionais com esses estudos são bem menores, se comparados aos gastos laboratoriais despendidos ao sintetizar e testar farmacologicamente várias substâncias. Esta importante ferramenta tem sido usada para filtrar compostos que não servem para serem designados como alvo, e desenhar os possíveis candidatos que apresentariam uma boa interação com o sítio ativo do receptor.

Os métodos de *docking* molecular, quando aplicados a uma grande biblioteca de compostos, devem ser capazes de distinguir entre moléculas que, provavelmente, não se ligariam ao receptor e classificar os compostos com maior afinidade. Entre as ferramentas básicas para os métodos de *docking* estão o algoritmo de busca conformacional e a função escore de energia antes mesmo que esses sejam sintetizados (GUEDES; DE MAGALHÃES; DARDENNE, 2014)

Os algoritmos de busca exploram o perfil de energia livre para encontrar o melhor modo de ligação (posicionamento) do ligante dentro do sítio ativo do receptor, enquanto as funções de escore avaliam a qualidade do modo de ligação e selecionam as conformações mais relevantes. Atualmente, há diversas metodologias e pacotes de software disponíveis para o *docking* automatizado que fornecem predições aliadas a bom desempenho e rapidez com baixo custo computacional (GUEDES et al.,2014).

O *docking* ou ancoragem molecular pode ser realizado considerando o ligante totalmente flexível ou até, além do ligante, alguns aminoácidos da enzima ou proteína flexíveis, tendo em vista que no meio biológico tanto o receptor como o ligante são flexíveis. Assim, com a evolução e a implementação de novos algoritmos nos programas de ancoramento molecular, é possível prever o comportamento de um sistema biológico no computador (PIETRALONGA et al., 2015).

Para avaliação do ancoramento molecular é usada a equação geral da energia, E docking = E inter + E intra , na qual o primeiro termo (E inter) se refere à energia de interação entre o receptor e o ligante baseada nas interações de van der Waals e eletrostáticas, enquanto que o segundo termo (E intra) está relacionado, principalmente, com os graus de liberdade dos ligantes. Deste modo, quanto menor a energia de docking, melhor será o ancoramento molecular e melhor será a atividade biológica (PIETRALONGA et al., 2015)

A partir da ancoragem molecular, são obtidas diferentes conformações espaciais do ligante, possibilitando ao analista identificar qual dentre estas é a mais provável na interação ligante alvo. A partir de cada conformação espacial, são obtidas energias livres de ligação (entre ligante e alvo), onde a menor energia é considerada a mais provável para justificar a conformação da interação (KITCHEN et al., 2004).

O preparo das estruturas químicas a serem testadas, compreende uma das partes mais importantes nas propostas de novos ligantes, pois as moléculas devem ser projetadas com cuidado, mantendo disposições parecidas dos átomos da molécula primária. Pela varredura conformacional e alinhamento tridimensional com o ligante original podem ser propostas moléculas melhoradas (GOODARZI et al., 2009).

Atualmente, várias técnicas de modelagem molecular estão disponíveis para facilitar tarefas de descoberta de drogas, sendo a maioria classificadas em abordagens baseadas nas estruturas e à base de ligantes (SLIWOSKI et al., 2014).

O AutoDock utiliza um de método de rede (grid) para a busca no espaço conformacional disponível para o ligante próximo a uma proteína, o qual permite uma avaliação eficaz da energia de ligação entre conformações. Neste método, atribui-se uma rede que contém a proteína alvo. Em seguida, um átomo de teste é colocado em cada ponto da rede, a energia de interação entre o átomo e a rede é calculada, e o valor é estocado na rede. Essa rede de energias pode então ser utilizada como uma tabela de referência durante o processo de *docking.* O principal método para busca conformacional neste pacote é o algoritmo genético Lamarckiano (PERRYMAN et al., 2014; SANTOS-MARTINS et al., 2019).

Neste método, uma população de conformações de teste é criada, e então, em gerações sucessivas, são mutadas, trocam parâmetros conformacionais e competem de maneira análoga à evolução biológica, selecionando moléculas com a energia de ligação mais baixa.

Para predizer as energias livres de ligação de pequenas moléculas em alvos macromoleculares, o AutoDock utiliza um campo de força de energia livre semiempírico, descrito e testado por HUEY e colaboradores (2006). O campo de força é baseado em um modelo termodinâmico que permite incorporação de interações intramoleculares na energia livre de ligação. Isto é feito avaliando energias por ambos estados, ligado e não ligado. Este método também incorpora um conjunto próprio de tipos de átomos e cargas. Estas metodologias representam grande impacto no planejamento e design de novas drogas (TROTT; OLSON, 2010).

Desta forma, utilizando-se o docking molecular como preditivo para ação farmacológica de compostos bioativos pertencentes ás partes isoladas da planta *Labramia bojeri*, pertencente à família Sapotaceae, que atuam de forma quimiopreventiva de câncer consubstanciada por intermédio de ensaios para identificação de sua composição química, efeito antioxidante, efeito citotóxico em células, ensaios *in vitro* de indução da NAD(P)H:quinona redutase, inibição de NFK-b. Ademais, os óleos essências demonstram possuir substâncias que também atuam com atividade quimiopreventiva de câncer pela indução da enzima NAD(P)H:quinona redutase Quinona e ratificadas por análises in sílico de *docking* molecular.

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HIPÓTESES

HIPÓTESE CAPÍTULO 1

As partes isoladas da planta *Labramia bojeri* possuem atividade quimiopreventiva de câncer consubstanciada por ensaios para identificação de sua composição química, efeito antioxidante, efeito citotóxico em células, ensaios in vitro de indução da NAD(P)H:quinona redutase, inibição de NFK-b e estudos *in sílico* de *docking* molecular.

HIPÓTESE CAPÍTULO 2

As substâncias isoladas de óleos essenciais apresentam atividade quimiopreventiva de câncer consubstanciada por ensaios *in vitro* de indução da NAD(P)H: quinona redutase e estudos *in sílico* de docking molecular.

OBJETIVOS

OBJETIVOS CAPÍTULO 1

Objetivo Geral

Realizar o estudo químico e avaliação das atividades biológicas de *Labramia bojeri*: composição química, antioxidante e efeito na quimioprevenção de câncer.

Objetivo Específicos

- Avaliar a atividade antioxidante dos extratos hexânicos e etanólicos das partes de *Labramia bojeri*.
- Avaliar a citotoxicidade dos extratos hexânicos e etanólicos das partes de *Labramia Bojeri* empregando-se ensaio em cultura de células.
- Avaliar o potencial quimiopreventivo de câncer do extrato hexânico e etanólico das partes da planta *Labramia bojeri* empregando-se ensaios em cultura celular de inibição de NF-κB e de indução da quinona redutase;
- Realizar o fracionamento biomonitorado do extrato hexânico das folhas de *Labramia bojeri*, visando isolar as substâncias responsáveis pela atividade inibitória da enzima Quinona Redutase;
- Elucidar a estrutura química dos fitoconstituintes isolados de folhas de *Labramia Bojeri*.
- Avaliar o *docking* molecular entre a substância isolada e o acoplamento com a enzima Quinona Redutase.
- Predizer a atividade farmacocinética e toxicológica do composto isolado do extrato ativo.

OBJETIVOS CAPÍTULO 2

Objetivo Geral

Realizar o estudo da atividade quimiopreventiva de câncer *in silico* e *in vitro* de substâncias isoladas de óleos essenciais.

Objetivo Específicos

- Avaliar o potencial quimiopreventivo de câncer das substâncias isoladas de óleos essenciais empregando-se o ensaio de indução da quinona redutase;
- Avaliar o *docking* molecular entre as substâncias isoladas de óleos essenciais e o acoplamento com a enzima Quinona Redutase.
- Predizer a atividade farmacocinética e toxicológica das substâncias presentes nos óleos essenciais.

CAPÍTULO 1

MANUSCRITO CIENTÍFICO

Chemopreventive activity and chemical and nutritional composition of *Labramia bojeri*

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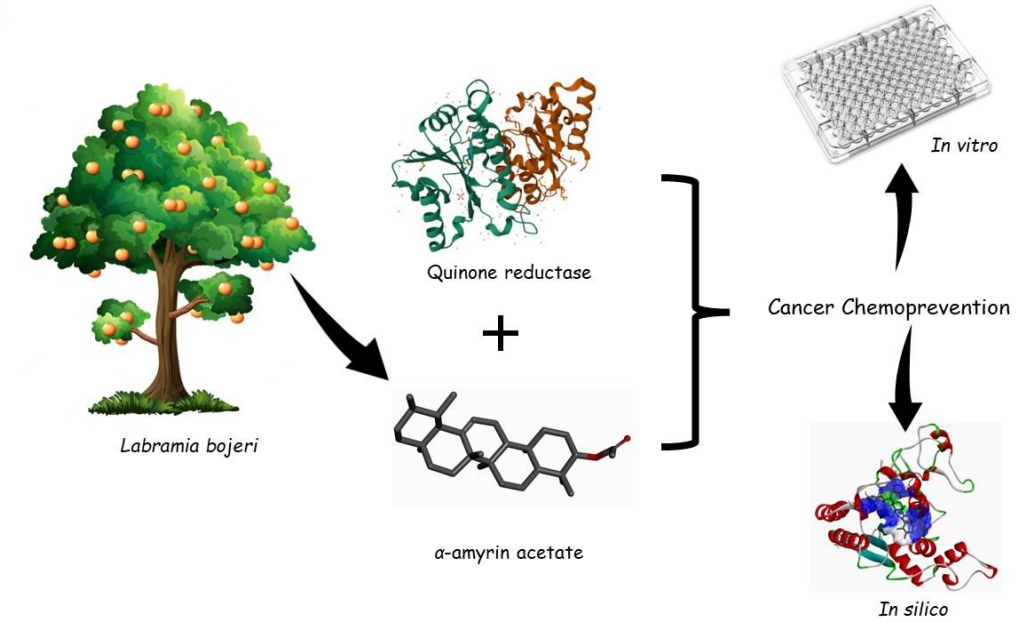
GRAPHICAL ABSTRACT



ABSTRACT

Natural products have been a reservoir for novel drug discovery for centuries, particularly in combating infectious diseases, cancer, and neurodegenerative disorders. One approach that harnesses natural products for cellular protection involves mitigating the production of metabolic enzymes that generate reactive oxygen species. These species play a crucial role in activating carcinogens, known as phase 1 enzymes, while simultaneously stimulating phase 2 enzymes. The latter is instrumental in the body's detoxification process against carcinogens, aiding in neutralizing radicals and electrophiles implicated in cellular activities. The Sapotaceae family has garnered attention due to the antioxidant, chemopreventive, and cytotoxic activities many of its species exhibit. This study evaluated the antioxidant, anti-inflammatory, and cancer chemoprevention properties of *Labramia bojeri* extracts, followed by biofractionation and structure elucidation, which led to the isolation of a-amyrin acetate. The findings indicate that *L. bojeri* leaf extracts displayed antioxidant activities in both solvents used in the study. However, these extracts showed cytotoxic activity in almost extracts tested in macrophage cells. The hexane leaf extract showed cancer chemopreventive activity in Hepa1c1c7 cells. Additionally, its anti-inflammatory activity was affirmed through an NF-κB inhibitory activity assay. This study proposes that these effects are likely attributed to the a-amyrin acetate isolated from the hexane extract of the plant's leaves. Further, the chemopreventive properties of this compound might stem from the induction of the enzyme quinone reductase, as inferred from in silico modeling.

Key Words: Labramia bojeri, Cancer chemopreventive activity, Quinone Reductase

INTRODUCTION

Natural products have served as rich sources of new drugs for centuries, playing a pivotal role in the unearthing of remedies for infectious diseases, cancer, and a spectrum of neurodegenerative disorders, a role they continue to fulfill to this day (Atasanov et al. 2015; Newman and Cragg 2016). Plant extracts, comprising a medley of compounds (secondary metabolites) synthesized from primary metabolites, are derived from various plant parts and act as the basis for a plethora of existing medications (Mushtaq et al. 2018). Remarkably, it is posited that roughly 50% of the drugs formulated between 1981 and 2010 can trace their origins to natural products, prominently featuring among them are cancer therapeutics (Buxani et al. 2014).

There is a compelling body of evidence pointing to the efficacy of certain compounds, derived from plant extracts, as potent anti-inflammatory agents. These compounds work by stifling the constitutive activation of NF-κB and promoting the production of reactive oxygen species (ROS), including superoxide. This triggers oxidative stress and apoptosis processes, significantly supporting cancer prevention (Abdellatef et al. 2022; Fakhurudin et al. 2014; Kiemer et al. 2003).

NF-κB activity fuels tumor cell proliferation, represses apoptosis, and sparks angiogenesis. It further induces epithelial-mesenchymal transition, facilitating metastasis. Under certain conditions, NF-κB activation may also re-engineer local metabolism and the immune system to support tumor growth. Hence, suppressing NF-κB in tumor cells often triggers tumor regression, underscoring the potential of the NF-κB pathway as a promising therapeutic target (Xia et al. 2014).

The Sapotaceae family has distinguished itself with several species exhibiting antioxidant, chemopreventive, and cytotoxic activities (Baky et al. 2016; Tapondjou et al. 2011). This family of flowering plants, belonging to the order Ericales and divided into five tribes with 53 genera and roughly 1250 species, is known for its broad spectrum of chemical constituents, including saponins, flavonoids, and polyphenolic compounds (Baky et al. 2016). The most remarkable species diversity is distributed globally in the tropical and subtropical regions of Asia, South America, and Africa (Govaertis et al. 2021; Swenson and Anderberg 2005). Numerous species yield edible fruits, both with and without economic value, notable among them being Manilkara (*Sapodilla sapota*), *Chrysophyllum cainite*, and *Planchonia careya* (Baky et al. 2016).

Labramia bojeri, a member of the Sapotaceae family and an evergreen native to Madagascar, can reach up to 10 meters in height with a diameter of up to 90 cm. This plant extensively uses Brazilian beach afforestation (Lorenzi 2003). A study conducted by Macedo et al (2004) characterized a protein from *L. bojeri,* dubbed Labramine, which exhibited homology in the NH2 terminal sequence with Kunitz-type inhibitors without demonstrating trypsin inhibitory activity. Instead, it displayed an activity akin to lectin. Numerous proteins in this group that bind to chitin are associated with plant defense mechanisms against organisms that contain this polysaccharide in their structures.

Therefore, preliminary evidence hints at the potential of extracts from this plant for functional characteristics, positioning them as a promising source of potential pharmaceuticals. Nevertheless, the scientific evidence is currently insufficient to affirm the activity of extracts or isolated molecules from *Labramia bojeri* (Sapotaceae) as future drugs. Consequently, this study aims to assess the chemical composition, in vitro antioxidant, and anti-inflammatory activity of *Labramia bojeri* extracts, in addition to conducting the structural elucidation of the substances present in the plant extracts and predicting their pharmacokinetic and pharmacological actions in silico."

MATERIAL AND METHODS

Plant material

Labramia bojeri leaves and fruits were collected in the municipality of Vila Velha - Espírito Santo - Brazil (latitude -20.509722 and longitude -40.361944). Sample specimens were deposited in the herbarium of the Federal University of Espírito Santo under the number VIES 45625 (*Labramia bojeri*) Figure 1. After collection, the plant material was selected, and leaves and fruits contaminated by insects or fungi were discarded. Then the leaves were washed and dried in a ventilated oven at 40°C for 96 hours.

Figure 1 *Labramia bojeri* B.C. VIES 45625 Collection: Coutinho, SCG Source: UFES virtual herbarium.

Sample preparation

After drying, the samples (leaves and fruits separately) were ground using a knife mill. Samples were then degreased with 100% hexane and then with 75% ethanol in three times of 30 minutes each solvent in an ultrasonic bath following the ratio of 1 g of plant material to 20 ml of solvent. At the end, the extracts were filtered through a Buchner funnel coupled to a suction pump and evaporated to dryness. Then the extracts were rotaevaporated under vacuum at 40°C and lyophilized (Oliveira VB et al.,2016).

Centesimal composition

Moisture matter, protein and lipid content were individually determined for fruit peel, seed, pulp, leaf and kernel. Moisture content was determined gravimetrically in a drying oven at 105°C until a stable weight was reached. The results were presented in grams of moisture per 100 g of sample. The samples were subjected to direct extraction with hexane for 6 hours in Soxhlet to determine total fatty acids. The total ash content was determined by incineration of dried samples in a muffle furnace at 550°C (Instituto Adolfo Lutz 2008). The results were expressed in grams of total ash per 100 g of sample. All analyzes were performed in triplicate.

Total Proteins

Leaf, fruit skin, pulp, seed skin, and almond samples of L. bojeri were subjected to protein quantification by the Kjeldahl method. For the Kjeldahl method, approximately 500 mg of each sample was digested with 7 mL of concentrated sulfuric acid and 2 g of catalytic mixture. Digestion was carried out in tubes at 380ºC for 4 hours. The tubes were transferred to a nitrogen distiller, and this procedure was performed after adding 20 mL of 10 mol/L sodium hydroxide solution.

The ammonia released in the procedure was retained in a 2 % boric acid solution containing methyl red and bromocresol green indicators. The solution was then titrated with 0.1 mol/L hydrochloric acid solution. The volume spent on the titration was proportional to the amount of nitrogen in the sample. For the conversion of the total nitrogen value to the protein value, the conversion factor available in the literature for fruits was used (Instituto Adolfo Lutz 2008). All analyzes were performed in triplicate.

Chemical composition

Determination of flavonoids, total phenolics and tannins

The total content of flavonoids was determined by the spectrophotometric method after a reaction with aluminum chloride (10 % w/v), according to (Asem et al, 2019). The quantification was made from constructing a standard curve of quercetin (100 - 800 µg/ml) and determined by reading the absorbances in a spectrophotometer (Molecular Devices Spectra MAX 190) at 415 nm. The total flavonoid content of hexanic and ethanolic extracts of *Labramia bojeri* was expressed in g of quercetin

equivalent (QE)/100 g of dry extract. The experiments were performed in triplicate on different days.

Total phenolics and tannins were quantified using the Folin-Ciocalteau method, as described by (Krepsky et al. 2012). To determine total phenolics, an analytical curve was prepared with gallic acid (6.2 - 70 µg/mL). The total phenolic and tannin contents were determined by reading the absorbances performed in a spectrophotometer (Molecular Devices Spectra MAX 190) at 715 nm . The tannin content was estimated by the difference between the total phenolic and non-phenolic content in the extracts based on the precipitation of polyvinylpolypyrrolidone (PVPP). The content of total phenolics and tannins of hexanic and ethanolic extracts of *Labramia bojeri* was expressed in g gallic acid equivalents (GAE)/100 g crude extract. The experiments were performed in triplicate on different days.

Antioxidant activity

The antioxidant activity of hexanic and ethanolic extracts of *L. bojeri* were determined by the ability to scavenge the organic radicals $ABTS^{+}$ 2,2-azinobis (3-ethylbenzothiazole-6-sulfonate) and DPPH 2,2-diphenyl-1-picrylhydrazyl (Blois 1958; Re et al, 1999). The antioxidant activity of the extracts was compared with the action of quercetin. The results were expressed in IC_{50} (µg/mL), representing the required sample concentration for a 50% reduction of free radicals. The experiments were performed in triplicate on different days (Pulido et al.,2000)

Biological activity

Cytotoxicity assessment

To evaluate the cytotoxicity of *Labramia bojeri* extracts and in the products resulting from its fractionation, the MTT colorimetric method was used (Mosmann 1983). Macrophage cell line (RAW 264.7) were seeded in 96-well plates at a concentration of 4×10^3 cells per well for adhesion for 24 hours at 37 ºC in a humidified atmosphere (5% CO^2). After adhesion, the cells were treated with the extracts (100 µg/ml) and then incubated for another 24 hours. Camptothecin (10 µM) was used as a positive control. After this period, the culture was incubated with MTT for 2 hours and then 100 µL of dimethylsulfoxide (DMSO) was added, for the dissolution of the

formazan crystals, the plate was placed on an orbital shaker until obtaining homogeneous color. The reaction absorbance reading was performed in a spectrophotometer (Molecular Devices Spectra MAX 190) at 595 nm. Results were expressed as percentage of cytotoxicity and analyzes were performed in triplicate.

NAD(P)H induction assay: quinone reductase

The Quinone Reductase (QR) induction assay was performed according to the method described by Pezzuto et al.(2005). The QR inducing activity was expressed as DC (twice the concentration required for the specific QR activity). The results were presented as average. Samples with a DC value greater than 2 were considered active. Analyzes were performed in triplicate.

NF- κB inhibitory activity assay

NF-κB inhibition assay was performed as described by (Homhual et al, 2006). The 293-NF-κB cell line (293 cell line derived from human kidney, 293, 12-PTA-5554) was used for the assay, transfected with the NF- κB reporter gene luciferase , were seeded in a 96-well plate at a density of 1 x 10^4 cells for 48 h. The extracts were tested at a concentration of 100 µg/ ml. After treatment, cells were incubated for another 6 h with TNF-α (5 ng/mL). Next, the luciferase assay was performed using the Promega ® Luc Assay System according to the manufacturer's instructions. Luciferase activity was monitored using the microplate reader, with absorption at 515 nm . Results were expressed as a percentage of NF- κB inhibitory activity. Natosyl - L-phenylalanine chloromethyl ketone (TPCK) was used as a negative control.

Hexane extract from *Labramia bojeri* leaves Biofractionation

All chromatographic fractionations were monitored using different eluents by silica gel thin layer chromatography (TLC). The chromatograms were observed under visible and ultraviolet light (254 and 365 nm), before and after development with the developing solution of Sulfuric Anisaldehyde. The fractions were pooled according to their profiles, concentrated on a rotary evaporator at 40-60 °C, transferred to previously tared flasks, and kept in a desiccator, under vacuum, to eliminate the solvent for at least 48 h.

Chromatographic conditions and obtaining chromatographic profiles

UV detectors were used according to the characteristics of the analyzed sample, such as polarity and the presence of chromophores. For the preparation of the chromatographic column, silica gel 70-230 Mesh was used 60 batch 1922500614 Marcherey brand Nagel. Approximately 1 liter of hexane reagent was used for packing, which resulted in a column 17 cm high and 3 cm in diameter. The proportion of 1.5 g of material for each 17 g of silica was followed.

A cotton mass was adhered to the bottom of the column and gradually added silica already dissolved in hexane. Hexane was collected and replaced to complete column packing. To continue the preparation of the column, a mixture was made with the hexane extract and the silica with the aid of a mortar and pestle and placed on the packed silica finished with cotton on its surface. The isolated substances were grouped according to the degree of similarity of the compounds with the aid of silica thin layer chromatography plates.

Nuclear Magnetic Resonance Spectroscopy

13C and 1H NMR spectra were obtained with the Avance III 500 MHz NMR instrument, 5mm BBO probe at 25 °C.To prepare the sample, approximately 700 µL of deuterated chloroform were used, filtered with a cotton pad and a Pasteur pipette. Then, it was transferred to the NMR tube and sent for 1H and 13C NMR analysis to elucidate its structure.

Molecular docking

Quinone Reductase dimer crystal structure and the isolated *a*-amyrin chemical structure were used in this study as targets for theoretical activity and were retrieved from the PDB file (Berman et al. 2000). The Enzyme dimer was saved as PDB entry for the docking study molecular.

As molecular docking is a stochastic technique, all calculations were performed 10 times, with the extraction of the best results for ligand. Of the conformers per ligand, with the lowest interaction ΔG value was selected. That is, the more negative,

the ΔG value, more favorable the receptor-ligand interaction in the thermodynamic aspect (Pietralonga et al., 2015; Gonçalves et al., 2020).

The Grid, a three-dimensional point array centered on the enzyme's active site under consideration, delineates the protein region for analysis when the ligand-macromolecule interaction occurs (Huey et al., 1996; Lokesh and Krishnan, 2016). The grid dimensions, designed to accommodate the full active site of the protein, were adapted from the crystallized ligand structure for docking calculations. Thus, all needed parameters for carrying out the molecular docking were added to a text file (conf.txt), according to the following description: receptor = monomer_FAD.pdbqt; ligand= ligand.pdbqt; center_x = 2.777; center_y = -3.439; center_z = 9.333; size_x = 32; size_y = 26; size_z = 28; cpu = 8; num_modes = 20.

The systems were treated with bonded atoms approximation in which the nonpolar hydrogens are bonded to the bonded atoms. The degrees of freedom of the ligands, defined by the ADT program (Morris et al., 2009), were used to consider the flexibility of the compounds. A three-dimensional grid was created by ADT (Morris et al. 2009) to calculate the docking energy between selected ligands and quinone reductase. Docking calculations were performed using the AutoDock program (Trott and Olson, 2010).

Ten rounds of docking were performed for each system. The choice of the best docked ligand was based on the lowest docking energy and its theoretically estimated inhibition constant (Ki), calculated using Equation 1, where ΔG binding (binding free energy) was the sum of inter and intramolecular enthalpies, R was the universal gas constant, T was the temperature in Kelvin and lnKi the natural logarithm of Ki, as follows:

$$\Delta G = R.T.lnKi$$

Equation 1 : Result of the energy variation of the system

Autodock calculates the interactions between the ligand and the macromolecule by predicting the binding free energies.

In silico pharmacokinetic analysis of the isolated substance

The pharmacokinetic analysis of the isolated substance a - amyrin acetate was carried out using the tool available on the Internet, SwissADME , which offers free access to a set of rapid predictive models for physicochemical properties, pharmacokinetics, drug-likeness and medicinal chemistry, which allows to quickly predict key parameters of several molecules and support efforts in drug discovery (Daina et al. 2017). It was necessary to convert the pdbqt files to smiles using the Open Babel program, available online.

Absorption, Distribution, Metabolism and Excretion parameters (ADME) can be evaluated and estimate probable failures related to the pharmacokinetics of new drugs(Hay et al. 2014), constituting a valid alternative, which uses computational models, for experimental procedures parameter prediction for ADME, especially in the initial stages (Dahlin and Walters 2015).

In silico Toxicity Analysis of the isolated substance

To analyze the toxicity of the isolated substance, the tool available on the Internet ProTox -II was used, which analyzes the molecular similarity, groups pharmacophores, and predicts types of toxicity such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity and immunotoxicity from a two-dimensional chemical structure as input or Smiles code.(BAMERJEE et al. 2018)

Statistical analysis

All data were analyzed by variance (ANOVA). The significance of the difference between means was determined by Tukey 's posthoc test adjusted for multiple comparisons; $p<0.05$ was considered statistically significant. Statistical analyzes were performed using GraphPad 8 software (GraphPad Software Inc., San Diego, CA).

RESULTS AND DISCUSSION

Centesimal Composition Analysis

The centesimal analysis reveals that the plant's polycarp retains significantly more moisture (60.74%) than the other sections, with the leaf exhibiting the least moisture content (7.63%). Interestingly, even though the pulp has a higher moisture percentage, its ash analysis shows a 10% difference in mass loss compared to the leaves. This discrepancy does not differ significantly from other examined parts, such as the bark and almond. As for lipid percentages, a substantial concentration is found in the leaves (11.1%). However, the remaining parts do not present much variation, and there is not a statistically significant difference among them (Table 1).

Table 1 *Labramia bojeri`s* centesimal composition

	Sheet	Pulp	Bark	Almond
Moisture (%)	7.63 ± 0.67^{b}	60.74 ± 2.63^{a}	52.72 ± 2.53^{a}	49.99 ± 1.41^{a}
Ashes (%)	11.30 ± 0.21^{ab}	1.41 ± 463.65^{a}	0.98 ± 0.32^{b}	1.19 ± 0.09^{b}
Lipids (%)	11.10 ± 0.96^{a}	4.30 ± 0.90^{a}	6.05 ± 0.35^{a}	4.25 ± 0.65^{a}

*Different letters on the same line correspond to significant differences between samples ($p < 0.05$). Means were submitted to analysis of variance (ANOVA) and the significance of the difference between them was determined by *post-hoc test,* Tukey 's method. Tests were performed in triplicate and expressed as mean ± standard deviation.

Protein Content

When assessing the total protein count across all components of the L. bojeri fruit, we find the leaves and almond yield the highest protein values (12.53 and 2.33, respectively). On the contrary, the fruit skin (0.8), pulp (0.73), and seed skin (0.6) provide the lowest protein content (Table 2).

Table 2 Labramia bojeri's fruit Total Proteins

Total Proteins

Leaves	12,53[a] ± 1,52
Peel	0,8[b] ± 0,10
Polycarch	0,73[b] ± 0,05
Seed	0,6[b] ± 0
Almond	2,33[c] ± 0,15

* Different letters in the same column correspond to significant differences between samples ($p < 0.05$). Means were submitted to analysis of variance (ANOVA) and the significance of the difference between them was determined by post-hoc test, Tukey's method. Tests were performed in triplicate and expressed as mean ± standard deviation

Antioxidant Capacity

The antioxidant properties of *L. bojeri* were measured using ABTS, FRAP, and DPPH methods from two distinct ethanol extracts - leaves extract and seed extract. For the ABTS test, both extracts showed a significant contrast. While the ethanolic seed extract demonstrated an IC_{50} of 422.2 µg/ml, the leaf extract yielded an IC_{50} of 115.1 µg/ml. The FRAP method also revealed a significant difference between the two extracts. However, we could not calculate the IC 50 values for DPPH for the ethanolic seed extract, making it unfeasible to compare with the leaf extract (Table 3).

Table 3 Antioxidant activity of *Labramia bojeri* extracts.

Antioxidant activity (IC_{50} µg/mL)

Sample	ABTS*	FRAP*	DPPH
EA	ND#	ND#	ND#
ES	422.20 ± 10.00 [c]	304.80 ± 1.43 [c]	ND#
EP	ND#	1126.26 ± 0.07[d]	ND#
EP1	ND#	5963.19 ± 0.01	ND#
EL	115.10 ± 2.81[b]	68.05 ± 4.20 [b]	376.40 ± 56.40[b]
HA	ND#	1822.60 ± 0.04[e]	ND#
HP	ND#	1716.06 ± 0.04[e]	ND#
HP1	ND#	1209.20 ± 0.06[d]	ND#
HL	ND#	1127.30 ± 0.07[d]	ND#
HS	ND#	ND#	ND#
Quercetin	6.86 ± 1.10 [a]	3.43 ± 0.80 [a]	6.03 ± 1.90[a]

Extracts: EA (ethanolic almond), ES (ethanol seeds), EP (ethanol polycarp), EP1 (ethanolic peel), EL (ethanolic leaves), HA (hexane almond), HS (hexane seeds), HL (hexane leaves), HP (hexane Polycarp), HP1 (hexane peel), ND# (not detected)..

* Different letters in the same column correspond to significant differences between samples ($p < 0.05$). Means were submitted to analysis of variance (ANOVA) and the significance of the difference between them was determined by post-hoc test, Tukey's method. Tests were performed in triplicate and expressed as mean ± standard deviation. # IC_{50} was not detected at concentrations up to 1 mg/ml.

Flavonoids, Total Phenolics, and Tannins Determination

The quantitative analysis of hexane and ethanolic extracts of *L. bojeri* is presented in Table 4. The Leaf Ethanol Extract (EL) and Seed Ethanol Extract (ES) exhibit the highest mean values for total polyphenols, at 16.3 g EAG/100g and 13.2 g EAG/100g, respectively. The same extracts also display a remarkable amount of total flavonoids and tannins, where EL and ES yielded values of 4.2 g EQ/100g and 1.2 g EQ/100g for flavonoids, and 13.1 g/100g and 10.6 g/100g for tannins, respectively.

Table 4 Quantification of total polyphenols, total flavonoids and tannins in *Labramia bojeri* extracts.

Sample	Total Polyphenols* (g GAE/100g)	Total Flavonoids* (g EQ/100g)	Tannins (g/100g)
HL	2.10 ± 0,40 ^{c}	1.10 ± 0.20 ^{a}	ND$^{\#}$
EL	16.30 ± 0,60 ^{a}	4.20 ± 3.20 ^{a}	13.10 ± 0.80 ^{a}
HS	0.50± 0.08 ^{d}	0.20 ± 0.10 ^{b}	ND$^{\#}$
ES	13.20 ± 0.30 ^{b}	1.20 ± 0.50 ^{a}	10.60 ± 0.30 ^{b}
HP1	ND$^{\#}$	0.05 ± 0.14 ^{b,c}	ND$^{\#}$
EP1	1.50 ± 0.40 ^{c}	0.0008 ± 0.03 ^{c}	0.36+0.38 ^{b}
HP	0.20 ± 0.08 ^{e}	0.13 ± 0.12 ^{b,c}	ND$^{\#}$
EP	0.33± 0.17 ^{d,e}	0.09 ± 0.01 ^{b}	ND$^{\#}$
HA	0.55+0.08 ^{d,e}	0.23 ±0.12 ^{b,c}	ND$^{\#}$
EA	ND$^{\#}$	0.30 ±0.16 ^{b}	ND$^{\#}$

Extracts: HL (hexan leaves); EL (ethanolic leaves); HP1 (hexane peel); EP1 (ethanolic peel); HP (hexane Polycarp); EP (ethanol polycarp); HS (hexane seed); ES (ethanol seed); HA (hexan almond);EA (ethanol almond). EQ: Quercetin equivalent; EAG: Gallic acid equivalent. Different letters in the same column correspond to $p<0.05$ differences. Tests were performed in triplicate and expressed as mean ± SD. *Results are expressed as g quercetin/gallic acid equivalents per 100g dry extract. #Not detected.

Antioxidant activity is a critical characteristic when identifying species with medicinal potential. Antioxidants are crucial in preventing diseases and premature cellular aging due to their inherent association with active oxygen (Ahmed et al. 2018). L. bojeri extracts displayed significant antioxidant activity in all three tests conducted. Notably, with its high antioxidant activity, the leaves extract exhibits a robust capacity to act as an antioxidant agent, reducing radicals, donating hydrogen and electrons, and inhibiting oxygen action. Therefore, the compounds within this extract can mitigate and even inhibit potential oxidative stress (Birasuren et al. 2013). Generally, the Sapotaceae family exhibits antioxidant activity in various tissues, such as leaves, seeds, and fruits obtained from alcoholic extracts, a finding consistent with this study (Baky et al. 2016; De Souza Dias et al. 2010).

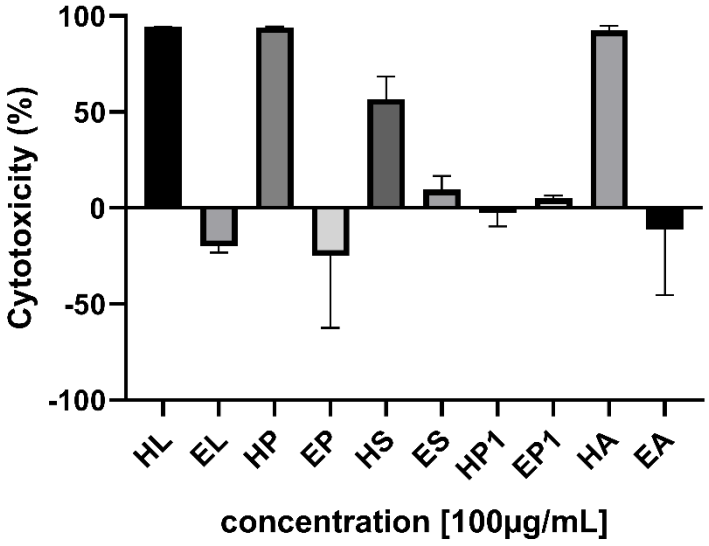
Phenolic compounds, including polyphenols, flavonoids, and tannins, are renowned components across various plant species offering diverse beneficial effects, encompassing anti-inflammatory, anticancer, diabetes, and cardiovascular disease

treatments (Koksal et al. 2016). These compounds were found in almost all extracts analyzed in this study, with the ethanolic extracts of leaves and seeds displaying the highest values. These variations in composition can be attributed to the solvents and extract concentrations used in this study. Different solvent combinations have been utilized to efficiently extract polyphenols from various plant tissues (ENECHI et al. 2013). Notably, Kumatia and Appiah-Opong (2021) discovered large amounts of these compounds in the ethanolic extracts of Tieghemella heckelii. This research corroborates their findings, as we found similar values in the ethanolic extract of L. bojeri leaves.

The positive effect on anti-inflammatory processes by the ethanolic extracts of L. bojeri leaves might be attributed to their rich concentration of polyphenols, flavonoids, and total tannins. Polyphenols are prominent among natural compounds potentially beneficial for cancer treatment due to their exceptional antioxidant properties, as demonstrated in a study by Montane et al. (2020). Oxidation and the release of free radicals are integral to cellular defense processes. However, excessive production may lead to significant cellular damage and potentially provoke a variety of pathologies in different tissue types, as suggested by Sugimoto et al. (2016) and Xu et al. (2019). The inflammatory response, involving various leukocyte cells like macrophages, plays a vital role in several tissues (Abdulkhaleq et al. 2018). Under stress, these cells release multiple inflammatory mediators such as nitric oxide, superoxide anion, cytokines, and specific transcription factors (Abdulkhaleq et al. 2018; Arulselvan et al. 2016).

An in vitro cytotoxicity test (MTT) was conducted to assess cell viability and chemopreventive activity. Different L. bojeri extracts were tested on macrophage strains (RAW 264.7) at a concentration of 100µg/ml in macrophage cell. It was observed that just leaves, polycarp, seeds and almond hexanic extracts of L. bojeri proved cytotoxic at this concentration on macrophage cell test. In addition, seeds and peels ethanolic extracts showed cytotoxicity too. (Figure 2).

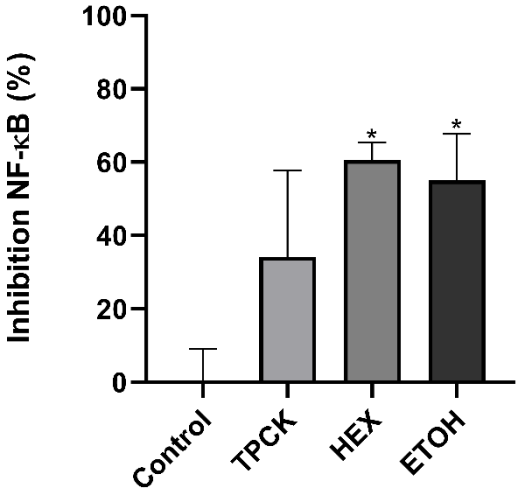
Figure 2 Cytotoxic effects of hexane and ethanolic extracts of leaves (HL and EL), polycarch (HP and EP), seed (HS and ES), peel (HP1 and EP1) and almond seed (HA and EA) of *L. bojeri* on macrophage strains (RAW 264.7).



The potential of *L. bojeri* leaves extract to induce quinone reductase, a phase II chemoprotective enzyme, was evaluated in vitro. The outcomes for the two leaf extracts revealed an average DC of 2.6 ± 0,1 for the hexane extract and an average DC of 0.2± 0,1 for the ethanol extract, indicating the hexane extract's quinone reductase induction activity with 20 µg /ml.

In evaluating the inhibition of NF-κB activation, the mean values found for the L. bojeri leaves extracts ranged from 55% (ethanolic extract) to 60.5% (hexane extract). However, these differences were insignificant, demonstrating both extracts' potential anti-inflammatory activity (Figure 3). 293 HEK human embryonic kidney cells were stimulated with TNF-α in 100 µg /ml and after 24 h of incubation the supernatant was collected and the NF-κB activity determined using the Luciferase assay kit from Promega.

Figure 3 Results were expressed as mean ± SD of three independent experiments. * $p<0.05$ compared to the control group (baseline) (analysis performed by one-way ANOVA). HX: leaves hexane extract , ETOH: leaves ethanolic extract .



This study establishes that *L. bojeri* extracts show significant induction of quinone reductase and inhibition of NF-kB, signifying the species' considerable chemopreventive potential. High-polyphenol concentration extracts are crucial for combating toxicity, thus preventing severe oxidative damage to cells through quinone reductase induction (Ahoua et al. 2019; Jan and Khan 2016). NF-kB activity is directly associated with inflammatory processes and is a critical modulator of COX-2 and inflammatory cytokines (Tang et al. 2021). NF-kB is renowned for its regulatory role in Nitric Oxide signaling proteins. Under normal circumstances, its activation prevents cell death in primary cells through TNF-α regulation (De Aquino et al. 2017; Han et al. 2009). Thus, L. bojeri extracts appear to inhibit these inflammatory mediators through a process partly dependent on NF-Kb activation.

NMR obtained spectra from sample isolated revealed seven singlets, two methyl doublets, one of which at 2.02, suggesting the presence of acetate and confirming the carbonyl carbon at 171.18. The presence of 2 methyl doublets hints at a bear-type triterpenoid. The compound also shows one olefenic proton at 5.10 for H-12 and an oxygenated proton at 4.48 for H-3. The acquired spectra allowed a comparison of the experimental Carbon 13 data with literature data (Table 5), confirming the compound as an impure a-amyrin acetate (Figure 4). The impurities are

believed to be fatty due to the weak signals at 29 characteristics of several CH 2. The processed spectra are provided in the appendix.

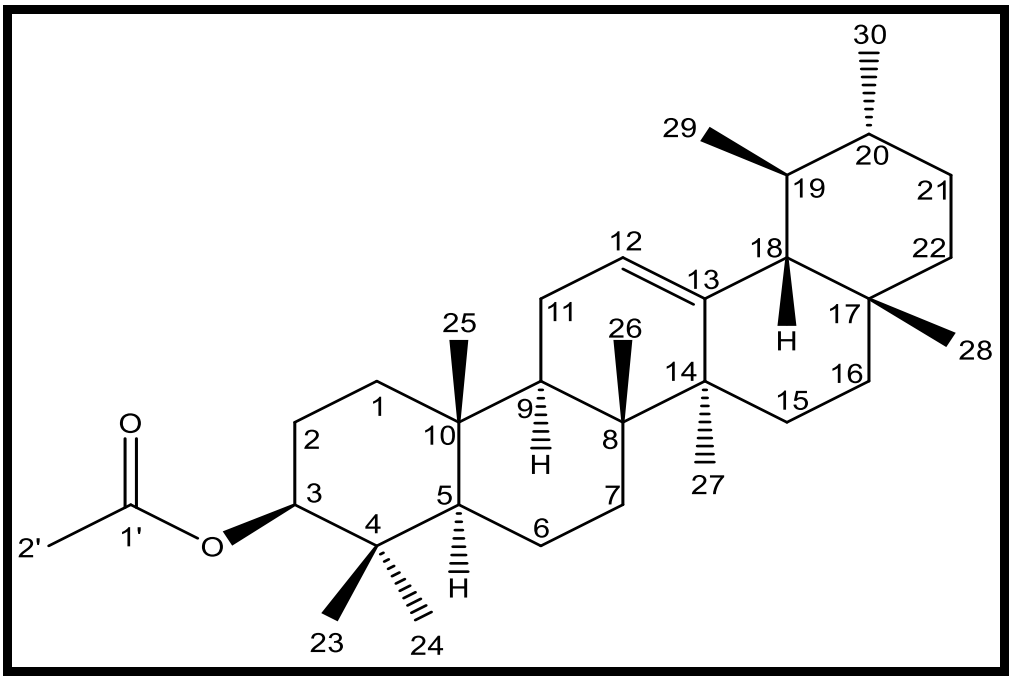
Table 5 α-amyrin acetate 13C NMR data and literature data (OKOYE et al, 2014)

Nº	Carbon type	δ_C* α-amirin acetate	δ_C* (OKOYE *et al*., 2014)
1	CH_2	38.58	38.60
2	CH_2	23.71	23.80
3	CH	81.10	81.18
4	C	37.82	37.90
5	CH	55.37	55.46
6	CH_2	18.36	18.45
7	CH_2	32.98	33.09
8	C	40.14	40.20
9	CH	47.76	47.84
10	C	36.90	36.99
11	CH_2	23.48	23.60
12	CH	124.43	124.50
13	C	139.74	139.80
14	C	42.18	42.40
15	CH_2	28.21	28.29
16	CH_2	26.71	26.80
17	C	33.86	33.95
18	CH	59.17	59.26
19	CH	39.76	39.81
20	CH	39.72	39.84
21	CH_2	31.36	31.47
22	CH_2	41.65	41.70
23	CH_3	28.18	28.27
24	CH_3	16.86	16.95
25	CH_3	15.85	15.96
26	CH_3	16.98	17.71
27	CH_3	23.34	23.43
28	CH_3	28.87	29.06
29	CH_3	17.62	17.02
30	CH_3	21.52	21.64
1’	CH_3	21.43	21.53

2’	C	171.18	171.53

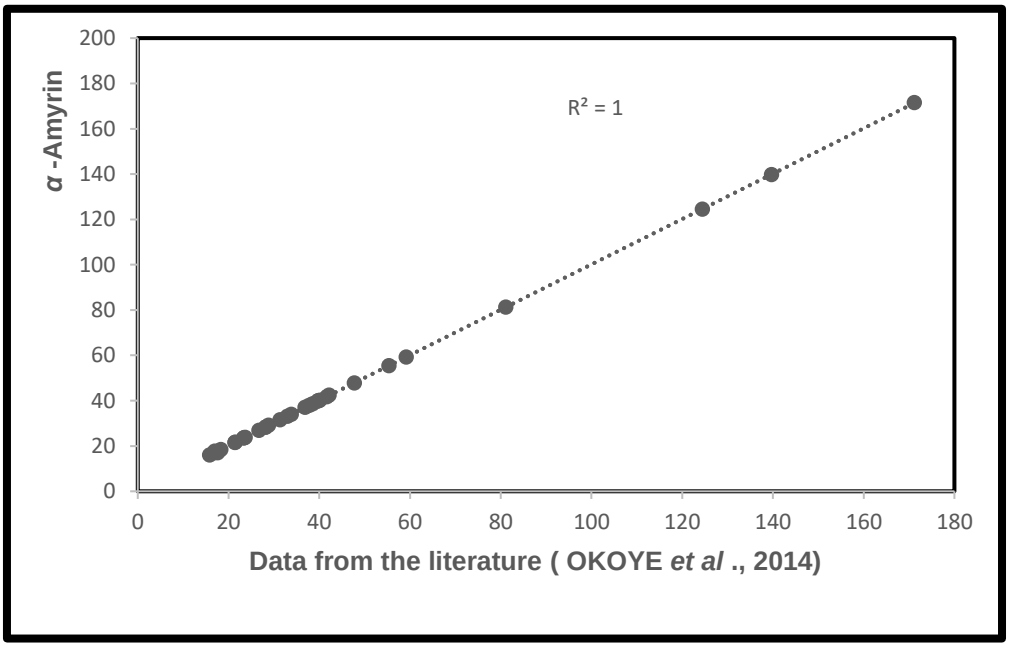
(*$CDCl_3$)

Figure 4 *a* - amyrin acetate Structure – Source: Pubchem



The data comparison on displacement trends was rigorously analyzed, yielding a positive correlation between our experimental findings and previously published literature (Figure 5).

Figure 5 Correlation between alfa- amyrin acetate and 13 C NMR data and literature data.



In a pioneering discovery within scientific research, we isolated and characterized a molecule from the hexane extract of L. bojeri leaves following biofractionation. Utilizing Nuclear Magnetic Resonance for structural elucidation, we determined that the molecule is the acetylated pentacyclic triterpene, α-amyrin acetate.

Our analysis resonates with a past study that discovered profound anti-inflammatory properties of this compound, extracted from the stem bark of Alstonia boonei. This study demonstrated the ability of α-amyrin acetate to inhibit egg albumin-induced paw edema in laboratory mice, lower total leukocyte counts, and suppress neutrophil infiltration (Okoye et al. 2014).

Romero et al. (2022) have previously established α-amyrin derivatives as potent inhibitors of the Cycloxygenase-2 (COX-2) enzyme, while their impact on Cycloxygenase-1 is limited. Another critical study found that α-amyrins could serve as highly selective COX/5-LOX inhibitors, making them safer than traditional non-steroidal anti-inflammatory drugs. Considering the strong association between inflammation and cancer, these compounds might emerge as promising candidates for cancer therapy (Ranjibar et al. 2016).

Moreover, as most evaluated cancers express the lipoxygenase receptor, dual 5-LOX/COX inhibitors hold potential as new therapeutic agents, given their role in preventing the formation of prostaglandins and leukotrienes (Ranjibar et al. 2016).

Studies on the cytotoxic properties of α-amyrin acetate against human cancer cells are still in their infancy. Nevertheless, notable investigations have shown promising results, such as the antiproliferative activities of a Ficus dichloromethane extract, containing α-amyrin acetate, against various cancer cell lines (Tsay et al. 2012). Neto et al. (2021) also demonstrated a potential selective cytotoxic effect in acute myeloid leukemia cases with α-amyrin derivatives administered via nanocapsules. The lipophilic extract of M. sinaica, comprising α-amyrin, demonstrated cytotoxic properties against human liver cancer cells (Aly et al. 2023).

Given these findings, we propose that α-amyrin acetate present in the hexanic extract of Labramia Bojeri leaves could be responsible for its anti-inflammatory and cancer chemopreventive activity.

Molecular *Docking*

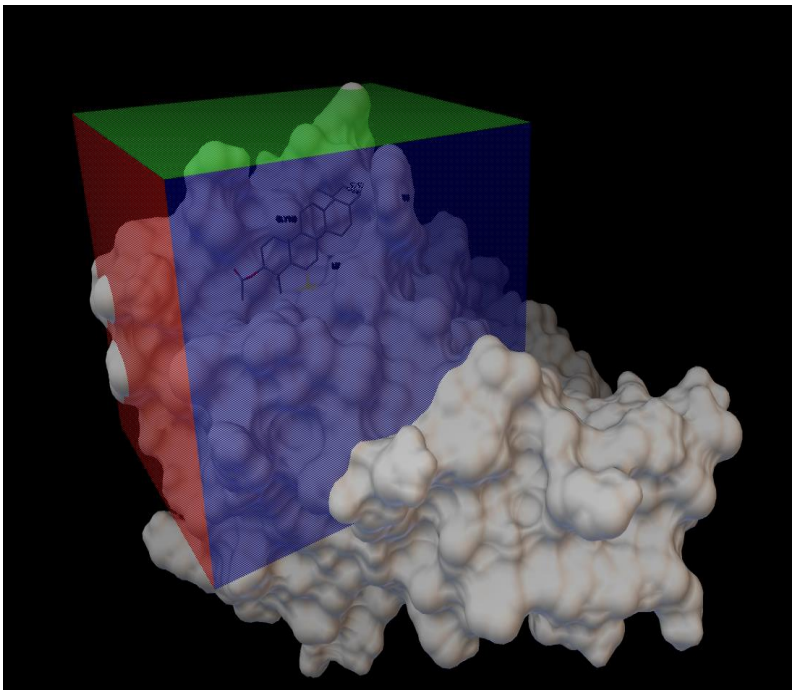
We evaluated the quinone reductase inducing activity of α-amyrin acetate in silico, both in the presence and absence of FAD. Results are detailed in Table 6.

Table 6 Docking energy values in Kcal/mol inhibition of the enzyme quinone reductase for *a* - amyrin acetate and Inhibition Constant

Molecular formula	**Molecular Weight**	**Docking Power (With FAD)**	**Docking Power (Without FAD)**	**Ki(M)**
$C_{32}H_{52}O_2$	468.8 g/mol	-6.8	-7.7	1.62336E-05

A Grid (a three-dimensional set of regularly spaced points) was centered on the enzyme's active site, marking the protein region to be analyzed for ligand and macromolecule interaction (Huey et al. 1996; Lokesh and Krishnan 2016)(Figure 6).

Figure 6 Three-dimensional structure of the Quinone reductase and FAD Grid.



We employed Discovery Studio Viewer v21.1.0.20298 for graphical presentations, which automatically generates comprehensive graphs and diagrams of binding protein interactions (Laskowski and Swindells 2011). As depicted in Figures 7 and 8, it displays patterns of hydrogen bonds and hydrophobic contact interaction between ligands and protein elements.

Figure 7 Two-dimensional structure of *a*- amyrin acetate and its interactions with FAD and Quinone reductase

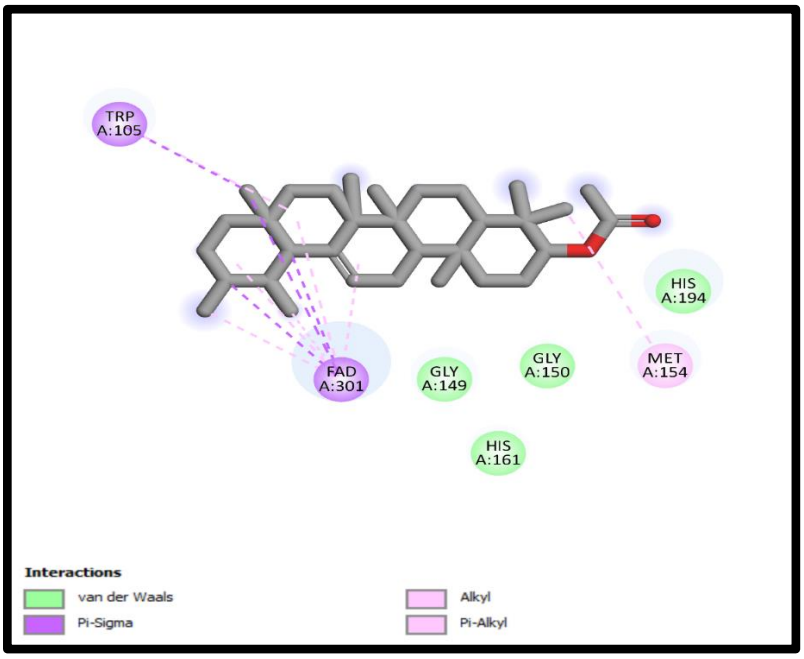
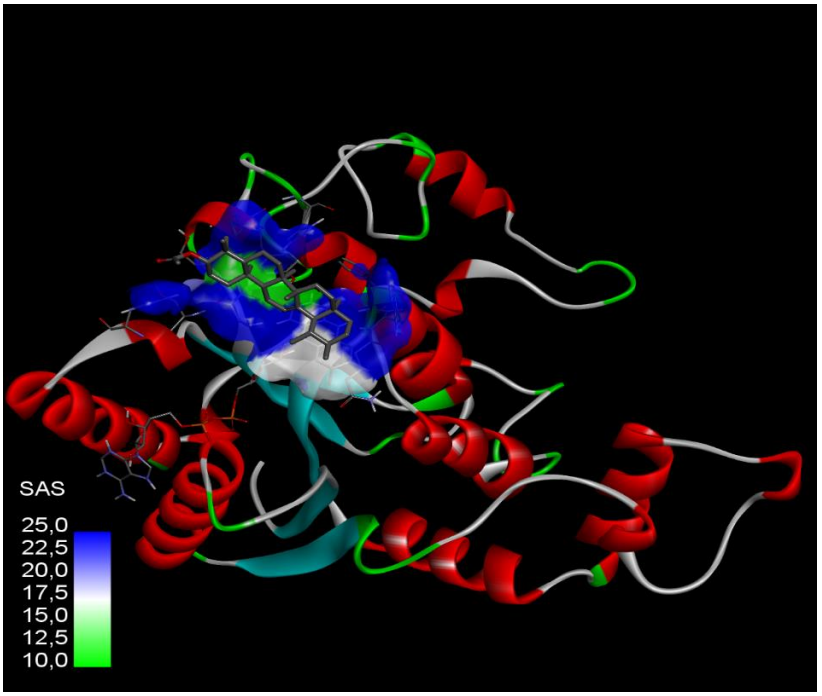


Figure 8 Three-dimensional structure of the interaction between a - amyrin acetate , quinone reductase and FAD.



The Discovery Studio Visualizer enabled us to scrutinize the interaction types between the quinone reductase enzyme, FAD, and α-amyrin acetate, as outlined in Table 7.

Table 7 Chemical interactions between *a* - amyrin acetate , FAD and quinone reductase.

Amino acid / Molecule	*a*-amyrin Acetate
FAD301	
Gly149	
Gly150	
His161	
Ileu167	
Met154	
Trp105	
Alkyl	
Pi- Alkyl	
Van der Walls	
Pi sigma	

In vitro experimental findings were corroborated by in silico analysis, a popular tool in drug discovery and elucidating medicinal plant compounds' bodily effects (Geysen et al. 2003; Morris et al. 2009). Docking analysis indicated multiple Alkyl, Pi-Alkyl, and Pi-sigma interactions between the quinone reductase enzyme, FAD, and α-amyrin acetate. This interaction pattern, demonstrated in our group's unpublished analyses of 33 molecules, suggests the pattern is consistent for tested molecules.

A key observation was the Van der Waals interaction with the amino acid Glycine 149, which enhances intermolecular interaction and consequently boost quinone reductase activity. From our yet-to-be-published data, molecules with lower docking energies, Eugenol and Valencene, displayed this type of interaction.

In the ten rounds of molecular docking, the selected conformation exhibited the lowest interaction energy at -6.8 kcal/mol, indicating a higher degree of enzyme inhibition within the FAD, Quinone reductase, and α-amyrin acetate complex. Docking rounds excluding FAD yielded a lower energy value of -7.7 kcal/mol, signifying better

coupling and stability. This suggests that FAD may increase electron transfer between FAD and quinone reductase and could deter other substances from binding to the enzyme's active site. Therefore, lower molecular docking energy corresponds to increased FAD inhibition and higher enzymatic activity, suggesting that α-amyrin acetate is a potent inducer of the detoxification enzyme quinone reductase (Table 6).

Without FAD, the compound infiltrates the enzymatic binding site, ensuring better complex stability and more favorable interaction, as evidenced by the lower docking energy and visualization program images. However, these hypotheses require quantum calculations for confirmation.

Enhanced Pharmacokinetic Analysis of an Isolated Substance In Silico

The pharmacokinetic profile of α-amyrin acetate was extensively examined via the online resource, SwissADME. Variables such as Gastrointestinal Absorption (AGI), Blood-Brain Barrier Permeation (PBHE), Lipinsky Rule Violations (VL), Water Solubility (AS), Octanol/Water Partition Coefficient (CP), and Induction of Cytochrome P450 family enzymes were analyzed. The substance demonstrated strong gastrointestinal absorption and penetration through the blood-brain barrier. Lipinski's rule indicated a violation, and the compound showed poor water solubility, a partition coefficient of 8.6, and inhibition of the CYP2C9 enzyme(Table 8).

Table 8 Pharmacokinetic analysis of a - amyrin acetate

Substance	**AGI**	**PBHE**	***VL***	***ICYP***
a -amyrin acetate	High	Yes	Yes MLOGP>4.15	CYP2C9

In Silico Toxicological Analysis of the Isolated Substance

Toxicity analysis was performed using the online resource ProTox-II, facilitating the determination of the lethal dose of α-amyrin acetate in mg/kg, its toxicity class, and potential types of toxicity. The compound showed a lethal dose of 3460 mg/kg and was classified under toxicity class 5, posing a risk when ingested in concentrations greater than 200 and less than 5000mg/kg.

The in silico and synergistic in vitro properties of α-amyrin acetate suggest its potential as a drug candidate, contingent on predicting pharmacokinetic and toxicological criteria. Therefore, various mechanisms, including Lipinski's Rule of Five, were used to predict these properties. This rule has been employed to determine if a chemically synthesized compound with specific pharmacological or biological activity could be applied as an orally administered medication. The rule outlines molecular properties crucial to a drug's pharmacokinetics in the human body, including its absorption, distribution, metabolism, and excretion (Lipinski et al. 2001).

Lipinski's rule proposes that a compound qualifies as an orally administered drug if it satisfies specific criteria: a maximum of 5 hydrogen bond donors per molecule, a maximum of 10 hydrogen bond acceptors per molecule, a molecular mass less than 500 Daltons, and an octanol-water partition coefficient log P not exceeding 5 ($logP \leq 5$) or MlogP >4.15. Any compound with more than one Lipinsky rule violation should be excluded from the study due to bioavailability issues (Lipinski et al, 2001). According to in silico analyses, α-amyrin acetate complies with Lipinski's rule, with a single violation corresponding to MLOGP exceeding 4.15. This suggests that a formulation containing this substance could have good oral bioavailability, despite its poor water solubility. Moreover, the compound exhibits high gastrointestinal absorption, confirming its conformity to Lipinski's rule.

α-amyrin acetate demonstrated the capability to penetrate the blood-brain barrier (BBB), an essential communication component between the central nervous

system and peripheral tissues, functioning as an interface that regulates substance exchange between the blood and the central nervous system (Banks 2009). There is a growing demand for drugs that can reach specific brain regions, as the brain's adequate protection against exogenous substances often presents challenges.

The isolated molecule inhibits the cytochrome P450 2C9 enzyme, not other CYP450 isoforms. This isoform plays a critical role in the oxidation of xenobiotic and endogenous compounds, predominantly expressed in the liver. It is responsible for the metabolic clearance of approximately 15-20% of all drugs undergoing phase I metabolism (Van et al. 2010).

There are no existing in silico studies regarding the toxic activity of α-amyrin acetate, which underscores this research's pioneering nature and importance. The analysis classified the substance under category V for acute oral toxicity, with an LD50 of 3460mg/kg. This implies a large dose is required to cause toxic effects (Oliveira 2018). LD50 values are often given as toxic doses in mg/kg of body weight, representing the dose that causes 50% of test subjects to die after exposure to a compound.

CONCLUSION

For the first time, our study proposes the anti-inflammatory and chemopreventive properties of L. bojeri. These extracts hindered the activities of quinone reductase in cancer cells (H1C1C7) and NF-kB in kidney cells and also displayed the capacity to eliminate free radicals through their antioxidant activity, potentially reducing oxidative stress. These biological activities may be directly linked to the concentration of flavonoids, polyphenols, and tannins, especially in leaf tissues. α-amyrin acetate, identified in the hexane leaf extract, appears responsible for inducing quinone reductase activity, supported by in silico studies and scientific literature. Therefore, these preliminary in vitro data are considered exceptionally promising for future biological and phytochemical studies to isolate and identify new active principles, providing new scientific evidence for using L. bojeri and contributing to developing new drugs.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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CAPÍTULO 2

MANUSCRITO CIENTÍFICO

Molecular Docking Analysis and Quinone Reductase Enzyme-Inducing Activity: Evaluating the Chemopreventive Potential of Monoterpenes and Sesquiterpenes

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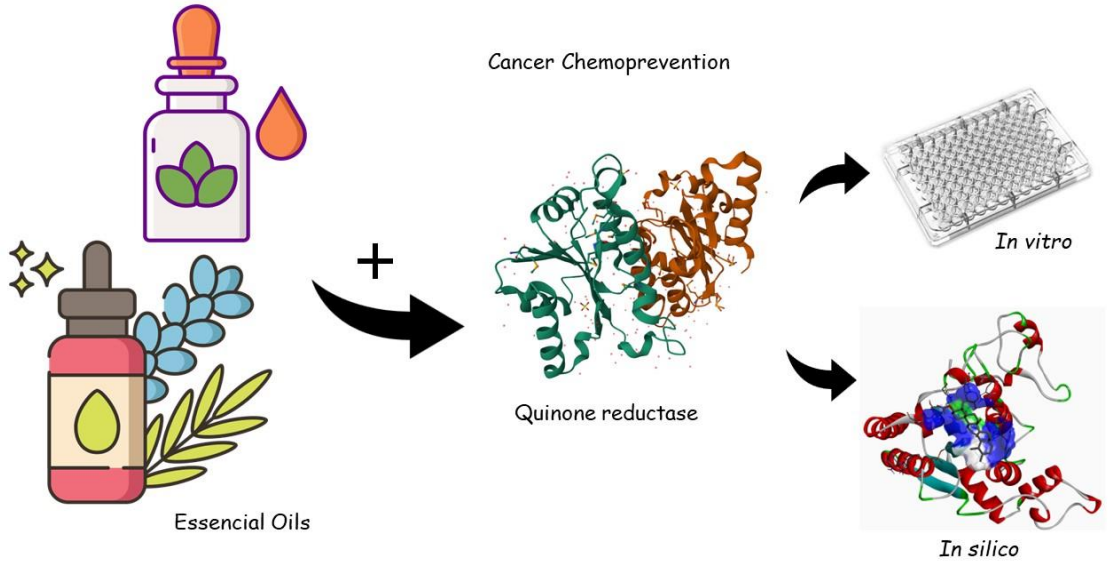
GRAPHICAL ABSTRACT



ABSTRACT

Essential oils are mainly constitute by complex mixture of mono and sesqueternes. The use of essential oils in folk medicine for prevention and cure of some diseases has been going on for several years. Cancer chemoprevention can be achieved by activating or upregulating anticarcinogenic enzymes, which include cytoprotective electrophile processing enzymes such as NAD(P)H:quinone reductase. Its induction represents an important cancer chemopreventive factor and can be analyzed both experimentally in vitro and in silico through methodologies using molecular docking. Thus, this work aimed to evaluate the induction capacity of the enzyme quinone reductase in vitro and in silico of the main substances present in essential oils. The results showed that of the 33 substances tested, 15 showed chemopreventive activity against cancer resulting from in vitro quinone reductase induction, corroborated by the in silico molecular docking analysis of the binding between the Quinone Reductase enzyme complex, FAD and the substances isolated from essential oils. The isolated substances that showed in vitro quinone reductase activity and the lowest docking energy were valencene, eugenol and (+)carvone. This work demonstrates for the first time that the chemopreventive activity of these substances occurs due to the induction of quinone reductase. The in silico analysis of prediction of the pharmacokinetic and toxicological activity of the 33 substances was carried out and demonstrates that all of them meet Lipinski's rule and would present good oral bioavailability however , p-cymene and γ-terpinene are fatal if ingested.

Key Words: Essential oils, Cancer chemopreventive activity, Quinone Reductase

INTRODUCTION

Essential oils, the volatile secondary metabolites synthesized by aromatic plants, are often colorless, have an oily texture, and emit a strong aroma. Characterized by their limited solubility in water and low stability in environments with light, air, and heat, they play a crucial role in plant defense against threats Iike parasites, viruses, predators, insects, and environmental changes, and even assist in attracting pollinators (Asbahani et al. 2015; Bakkali et al. 2008; De Oliveira Hashimoto et al. 2016; Pinto-Zevallos and Vaninnen 2013).

They can be sourced from various plant parts, such as leaves, rhizomes, flowers, fruits, and bark. The principal extraction techniques include steam distillation, solvents, supercritical fluid, and hydrodistillation (De Barros Fernandes et al. 2014; Do Amaral et al. 2018).

Essential oils exhibit many biological properties, including antimicrobial, antioxidant, anti-inflammatory, antitumor, antifungal, analgesic, larvicidal, and insecticidal activity. They chiefly comprise terpenes, phenylpropanoids, and other oxygenated compounds. Terpenoids specifically manifest a variety of biological activities such as antimicrobial, anti-allergic, anti-spasmodic, antihyperglycemic, anti-inflammatory, antifungal, antiparasitic, antiviral, immunomodulatory, and cytotoxic, hence considerably contributing to the biological properties of essential oils (Padush et al. 2007).

Research has demonstrated the cytotoxic activities of essential oils, which are sometimes activated by light. They have been shown to induce apoptosis and necrosis in mammalian cells in vitro. Essential oils also possess an antimutagenic property, which might be involved in inhibiting the penetration of mutagenic agents into cells, inactivating these agents, neutralizing free radicals, or triggering antioxidant enzymes (Carson et al. 2002; Ipek et al.2005; Shankel et al.1993).

The cytotoxic capability of essential oils presents the potential for them to be effective agents in new drug compositions. Some even exhibit substantial antimutagenic capacity, which could correlate with anticarcinogenic activity. Hence, more in-depth studies analyzing these properties could lead to potential new cancer treatments (Bakkali et al. 2008).

Cancer chemoprevention strategies often involve reducing metabolic enzymes that generate reactive species and boosting phase II enzymes that neutralize known radicals and electrophiles, thereby protecting cells from cancer-initiating events. An essential two-electron reductase, NAD(P)H: quinone reductase 1 (QR1), plays an integral role in cancer chemoprevention. It can be analyzed in vitro and in silico through molecular docking methodologies (Rahman and Lin 2018).

Molecular docking methodologies predict a small molecule's experimental binding mode and affinity within a target receptor's binding site. These computational strategies can optimize the effectiveness of potential compounds for a specific target. Moreover, due to advancements in computer technology and the surge in available structural, chemical, and biological data, the use of in silico approaches for virtual screening of potential compounds has significantly increased, improving the chances of new drug discovery while reducing initial identification costs (Guedes et al. 2014; Hassan et al. 2017; Agostino et al. 2013; Macalino et al. 2015; Song et al. 2011).

In silico molecular docking allows the comparison of bonding strengths between groups or derivatives of compounds, proving invaluable in rational drug design. It facilitates the evaluation of protein-ligand interactions, which is crucial for successfully discovering and planning new drugs. Binding affinity and specificity between a protein and a ligand, determined by various intermolecular interactions, form a stable protein-ligand complex (De Almeida et al. 2016; Guryanov et al., 2016).

Potential bioactive molecules can be screened for a given molecular target to determine the best fit for the target's active site. The binding energy required for the

proposed bioactive molecule to bind to the active site of the selected molecular target illustrates the molecule's potential biological activity (Barros 2015).

It becomes critical to evaluate pharmacokinetic parameters related to absorption, distribution, metabolism, and excretion earlier in the discovery process. In this context, computational models emerge as valid experiment alternatives (Daina 2017).

In pursuing new drug development, the early assessment of pharmacokinetic parameters involving absorption, distribution, metabolism, and excretion is crucial. Within this framework, computational models have emerged as valid alternatives to traditional experiments (Daina 2017).

Evaluating parameters related to absorption, distribution, metabolism, and excretion allows for estimating probable pharmacokinetic failures in developing new drugs (Hay et al. 2014). Computational models, thus, serve as effective alternatives to traditional experimental procedures for predicting these pharmacokinetic parameters, especially in the early stages (Dahlin et al. 2015).

Lipinski's Rule has been employed to ascertain the likelihood of a drug possessing favorable characteristics for oral absorption (LIPINSKI, 2004). This principle, also known as the "Rule of Five," stipulates that a bioactive molecule intended for passive diffusion absorption should have a partition coefficient (miLogP) under 5.0, a molecular mass (MM) that does not exceed 500 Daltons, and no more than 5 donor functional groups and 10 hydrogen bonding acceptor groups (Lipinski 2004). If a compound violates more than one of these guidelines, it should be excluded from consideration as it likely lacks the necessary traits for effective oral administration (Ranjibar et al. 2016).

Computational prediction also extends to determining toxic doses, typically given as LD50 values in mg/kg of body weight. The LD50 represents the median lethal dose, i.e., the dose that results in 50% mortality in test subjects following exposure to a compound. The median lethal dose defines toxicity classes in the following manner

[mg/kg]: Class I: fatal if swallowed (LD50 ≤ 5), Class II: fatal if swallowed (5 < LD50 ≤ 50), Class III: toxic if swallowed (50 < LD50 ≤ 300), Class IV: harmful if swallowed (300 < LD50 ≤ 2000), Class V: potentially harmful if ingested (2000 < LD50 ≤ 5000), and Class VI: non-toxic (LD50 > 5000) (Banerjee et al. 2018; Drwall et al. 2014).

Subsequently, evidence suggests that primary substances derived from essential oils may possess functional characteristics, indicating a promising source of potential chemopreventive drugs against cancer. However, the current scientific evidence needs to be more comprehensive to substantiate the efficacy of these substances as prospective drugs. Hence, this study aims to evaluate their chemopreventive activity against cancer both in vitro and in silico and predict their pharmacokinetic and toxicological impacts in silico.

MATERIAL AND METHODS

Plant Material

The pure substances isolated from essential oils, a total of 33, were purchased from Sigma Aldrich with a high level of superior purity, kept at room temperature. The following were selected: (−)- borneol, (+)-borneol, (−)-α-bisabolol, (+)-carvone, (+)-α-Pinene, (+)-β-Pinene, (±) -Citronellal, (R)-(+)-Limonene, Camphene, Camphor, Carvacrol, Citral, Eucalyptol, Eugenol, Geraniol, Guayene, L-Carveol, L-Carvone, Linalool, m-Cymene, Myrcene, Ocimene, p-Cymene , Sabinene, Terpineol, Terpinolene, Thymol, Valencene, α-Humulene, α-Phelandrene, β-Caryophyllene, β-Citronellol, γ-Terpinene.

Molecular docking

Quinone Reductase crystal structures dimer and the isolated substances chemical structures were used in this study as a target for theoretical activity. The three-dimensional structures of quinone reductase and substances isolated from essential oils (Protein Data Bank -PDB) were retrieved from the PDB file (Berman et

al. 2000). The three-dimensional models were verified and repaired using the WHAT IF program (Vriend 1990). The dimer of this enzyme was saved as a PDB entry for the molecular docking study.

As molecular docking is a stochastic technique, all calculations were performed 10 times, with extraction of the best results for ligand. Of the conformers per ligand, with the lowest interaction ΔG value was selected. That is, the more negative ΔG value, more favorable the receptor-ligand interaction in thermodynamic aspect (Pietralonga et al. 2015).

The Grid, a three-dimensional point array centered on the enzyme's active site under consideration, delineates the protein region for analysis when the ligand-macromolecule interaction occurs (Huey et al. 1996; Lokesh and Krishnan 2016). The grid dimensions, designed to accommodate the full active site of the protein, were adapted from the crystallized ligand structure for docking calculations. Thus, all needed parameters for carrying out the molecular docking were added to a text file (conf.txt), according to the following description: receptor = monomer_FAD.pdbqt; ligand= ligand.pdbqt; center_x = 2.777; center_y = -3.439; center_z = 9.333; size_x = 32; size_y = 26; size_z = 28; cpu = 8; num_modes = 20.

The systems were treated with bonded atoms approximation in which the nonpolar hydrogens are bonded to the bonded atoms. The degrees of freedom of the ligands, defined by the ADT program (Morris et al. 2009), were used to consider the flexibility of the compounds. A three-dimensional grid was created by ADT (Morris et al., 2009) to calculate the docking energy between selected ligands and quinone reductase. Docking calculations were performed using the AutoDock program (Trott and Olson 2010).

The docking calculations between selected ligands and quinone reductase were performed using the AutoDock program (Trott and Olson 2010). Ten rounds of docking were performed for each system. The choice of the best-docked ligand was based on the lowest docking energy and its theoretically estimated inhibition constant (Ki), calculated using Equation 1, where ΔG binding (binding free energy) was the sum of inter and intramolecular enthalpies R, was the universal gas constant, T was the temperature in Kelvin and lnKi is the natural logarithm of Ki, as follows:

$$\Delta G = R.T.\ln Ki$$

Equation 1 : Result of the energy variation of the system

In vitro quinone reductase induction assay

Quinone reductase induction assay was performed according to the method described by Pezzuto, 2005. QR-inducing activity was expressed as DC (twice the concentration required for the specific QR activity). The results were presented as average. Samples with a DC value greater than 2 were considered active. Analyzes were performed in triplicates.

In silico Pharmacokinetic Analysis of the substances

The pharmacokinetic analysis of the substances was carried out using the tool available on the Internet, SwissADME, through the SMILES code obtained by the OPEN Babel server, which offers free access to a set of rapid predictive models for physicochemical properties, pharmacokinetics, similarity to drugs and medicinal chemistry, which allows to quickly predict key parameters of several molecules and support efforts in drug discovery (Daina et al. 2017).

In silico Toxicity Analysis of Substances

For toxicity analysis, the tool available on the Internet, ProTox-II was used, which analyzes molecular similarity, pharmacophoric groups, and interaction between fragments to predict toxicity, such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, and immunotoxicity from the two-dimensional chemical structure as input (Bamerjee et al. 2018).

Statistical analysis

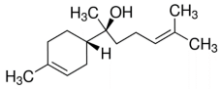
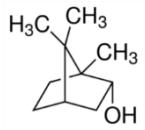
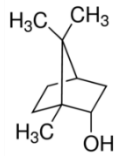
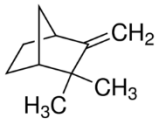
All data were analyzed by variance (ANOVA). Statistical analyzes were performed using GraphPad 8 software (GraphPad Software Inc., San Diego, CA).

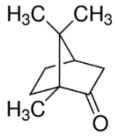
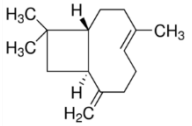
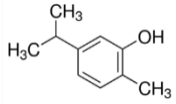
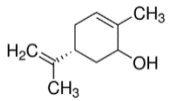
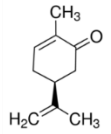
RESULTS AND DISCUSSION

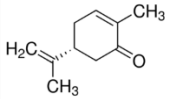
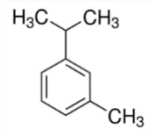
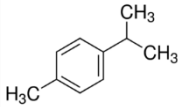
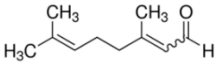
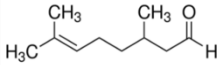
Various substances isolated from essential oils, encompassing a spectrum of classes, have been identified as potential inducers of the enzyme quinone reductase. These include flavonoids, terpenes, and terpenoids, as observed by Kang and Pezzuto in 2004. In the context of this research, 33 primary substances found in essential oils were evaluated for their chemopreventive potential by examining their ability to induce quinone reductase using both in vitro and in silico methods.

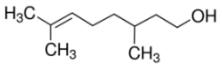
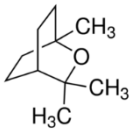
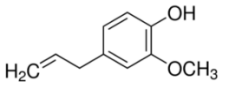
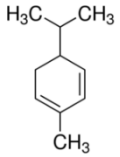
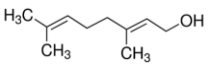
The focus of the subsequent analysis will be on the in vitro induction of quinone reductase, specifically highlighting substances that showcased an induction index surpassing 2. Table 1 illustrates these substances' in vitro quinone reductase-inducing activity, along with their respective Chemical Abstracts Service registration numbers. This information is accompanied by their molecular formulas, molecular weights, chemical classifications, and structures

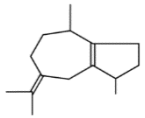
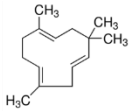
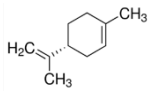
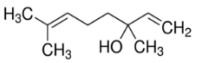
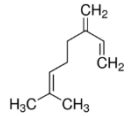
Table 1 Quinone reductase induction values for mono and sesquiterpenes.

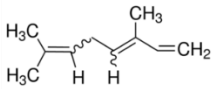
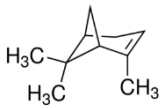
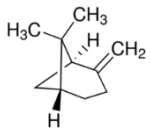
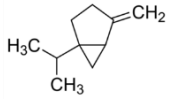
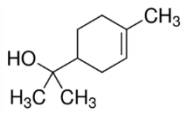
NOME	CAS nº	Molecular Formula	Molecular Weight	Chemical Class	Structure	Reductase Induction Index (1 mM)
D1 Bisabolol (−)-α	23089-26-1	C15H26O	222.37	Alcoholic Monoterpene		0,3±0,06
D2 Borneol (−)	464-45-9	$C_{10}H_{18}O$	154.24	Alcoholic Monoterpene		2,7±0,44
D3 Borneol (+)	• 464-43-7	$C_{10}H_{18}O$	154.25	Alcoholic Monoterpene		3,0±0,36
D4 Camphene	79-92-5	$C_{10}H_{16}$	136.24	Monoterpene		2,0±0,71

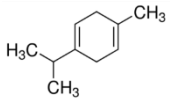
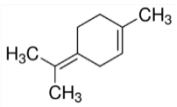
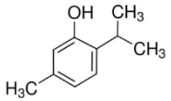
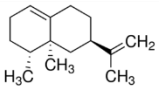
NOME	CAS nº	Molecular Formula	Molecular Weight	Chemical Class	Structure	Reductase Induction Index (1 mM)
D5 Camphor	76-22-2	$C_{10}H_{16}O$	152.23	Monoterpene		2,6±1,02
D6 Caryophyllene(beta)	87-44-5	C15H24	204.35	Sesquiterpene		1,7±0,5
D7 Carvacrol	499-75-2	C10H14O	150.22	Monoterpene		0,4±0,16
D8 Carveol (L)	99-48-9	C10H16O	152.23	Alcoholic Monoterpene		2,7±088
D9 Carvone (+)	2244-16-8	C10H14O	150.22	Ketone monoterpene		2,9±0,99

NOME	CAS nº	Molecular Formula	Molecular Weight	Chemical Class	Structure	Reductase Induction Index (1 mM)
D10 Carvone (L)	6485-40	C10H14O	150.22	Ketone monoterpene		4,1±1,95
D11 Cimenian (m)	535-77-3	C10H14	134,22	Monoterpene		0,6±0,35
D12 Cimenian (p)	99-87-6	C10H14	134.22	Monoterpene		0,7±0,29
D13 Citral	5392-40-5	C10H16O	152.23	Monoterpene aldehyde		1,7±0,76
D14 Citronellal (±)	106-23-0	C10H18O	154.25	Monoterpene aldehyde		0,6±0,03

NOME	CAS nº	Molecular Formula	Molecular Weight	Chemical Class	Structure	Reductase Induction Index (1 mM)
D15 Citronellol (beta)	106-22-9	C10H20-O	156.27	Alcoholic Monoterpene		0,9±0,68
D16 Eucalyptol	470-82-6	C10H18O	154.25	Monoterpene		3,0±0,10
D17 Eugenol	97-53-0	C10H17O2	164.20	Phenolic monoterpene		2,5±0,13
D18 Phellandrene (alfa)	99-83-2	C10H16	136.23	Monoterpene		1,1±0,77
D19 Geraniol	106-24-1	C10H18O	154.25	Alcoholic Monoterpene		2,6±0,23

NOME	CAS nº	Molecular Formula	Molecular Weight	Chemical Class	Structure	Reductase Induction Index (1 mM)
D20 Guayeno	92724-67-9	C15H24	204.35	Sesquiterpene		1,7±0,85
D21 Humulen (alpha)	6753-98-6	C15H24	204.35	Sesquiterpene		0,4±0,10
D22 (R) Limonen	5989-27-5	C10H16	136.23	Monoterpene		2,1±0,01
D23 Linalool	78-70-6	C10H18O	154.24	Monoterpene		2,1±0,43
D24 Myrcene	123-35-3	C10H16	136.23	Monoterpene		0,9±0,12

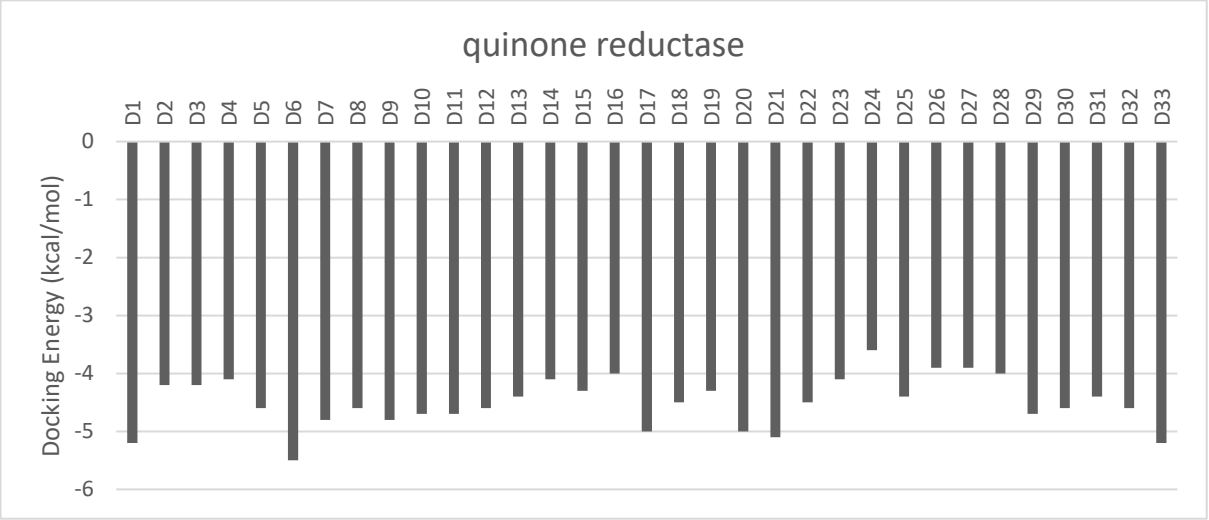
NOME	CAS nº	Molecular Formula	Molecular Weight	Chemical Class	Structure	Reductase Induction Index (1 mM)
D25 Ocimene	13877-91-3	C10H16	136.23	Monoterpene		1,0±0,23
D26 Pinene (alpha)(+)	7785-70-8	C10H16	136.23	Bicycle monoterpene		1,7±0,29
D27 Pinene(beta)(+)	19902-08-0	C10H16	136.23	Monoterpene		2,2±1,00
D28 Sabinene	3387-41-5	C10H16	136.23	Bicycle monoterpene		0,6±0,13
D29 Terpineol	8000-41-7	C10H18O	154.249	Alcoholic Monoterpene		1,5±0,48

NOME	CAS nº	Molecular Formula	Molecular Weight	Chemical Class	Structure	Reductase Induction Index (1 mM)
D30 Terpinene (gama)	99-85-4	C10H16	136.23	Alkene monoterpeno		0,8±0,04
D31 Terpinolene	586-62-9	C10H16	136.23	Monoterpene		2,8±0,11
D32 Tymol	89-83-8	C10H14O	150.217	Phenolic monoterpene		0,8±0,34
D33 Valencene	4630-07-3	C15H24	204.35	Sesquiterpene		2,8±0,33

Among the 33 compounds examined, only 14 exhibited a quinone reductase induction index surpassing 2. Notably, substances such as (+)-borneol (3.0), L-Carvone (4.1), Eucalyptol (3.0), Terpinolene (2.8), and Valencene (2.8) demonstrated activity indices exceeding 2.6.

Figure 1 graphically represents the docking energies derived from various molecular docking analyses. It indicates that the most favorable docking energies were associated with Bisabolol (−)-α, Caryophyllene (beta), Eugenol, Guaiene, Humulene (alpha), and Valencene.

Figure 1. 33 tested compounds Molecular *docking* energy values



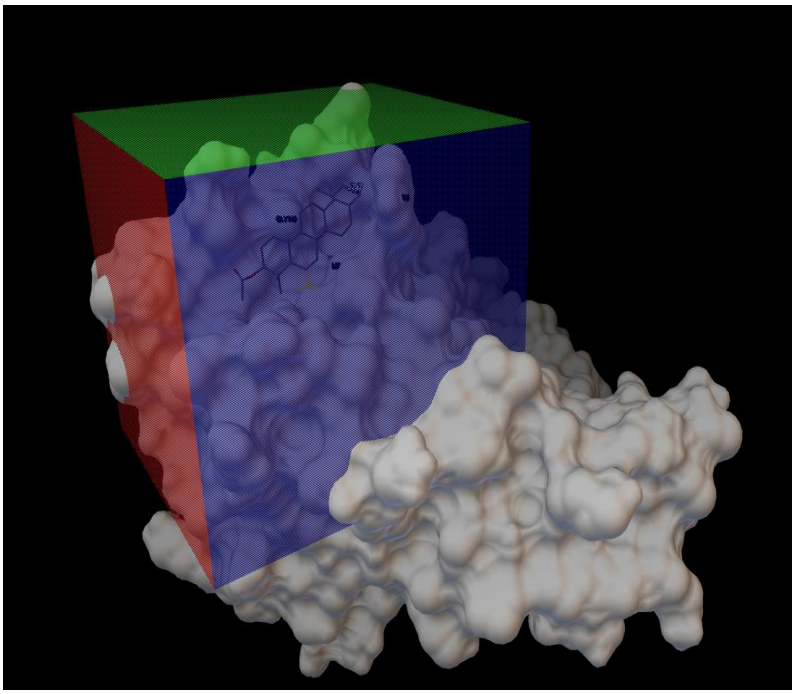
For each substrate, ten three-dimensional assessments were conducted to determine the most effective and lowest interaction energy, thereby achieving the optimal fit between the FAD-Quinone Reductase complex and the previously identified compound. The molecular docking energies (in kcal/mol), along with the enzyme inhibition constants of the quinone reductase, both in Molar and milliMolar units, are presented in Table 2. This includes data both with and without the presence of FAD.

Table 2. Molecular Docking Energy Values, Inhibition Constant and experimental induction index of Quinone reductase

Substance	Docking Energy with FAD (Kcall/mol)	Docking Energy without FAD (Kcall/mol)	Ki(M)	Ki(mM)	Experimental Induction Index
D1	-5.2	-5.4	0.000217459	0.21745879	0.3
D2	-4.2	-5.4	0.001100848	1.10084848	2.7
D3	-4.2	-5.4	0.001100848	1.10084848	3
D4	-4.1	-4.7	0.00129468	1.2946805	2.0
D5	-4.6	-5.4	0.000575423	0.57542313	2.6
D6	-5.5	-5.8	0.000133682	0.13368165	1.7
D7	-4.8	-5.1	0.000416023	0.41602286	0.4
D8	-4.6	-5.0	0.000575423	0.57542313	2.7
D9	-4.8	-5.0	0.000416023	0.41602286	2.9
D10	-4.7	-5.1	0.000489274	0.48927413	4.1
D11	-4.7	-4.7	0.000489274	0.48927413	0.6
D12	-4.6	-4.6	0.000575423	0.57542313	0.7
D13	-4.4	-4.7	0.000795898	0.79589803	1.7
D14	-4.1	-4.7	0.00129468	1.2946805	0.6
D15	-4.3	-4.5	0.000936036	0.93603586	0.9
D16	-4.0	-5.1	0.001522642	1.5226415	3
D17	-5.0	-5.0	0.000300779	0.3007787	2.5
D18	-4.5	-4.7	0.000676741	0.67674082	1.1
D19	-4.3	-4.9	0.000936036	0.93603586	2.6
D20	-5.0	-6.0	0.000300779	0.3007787	1.7
D21	-5.1	-5.7	0.000255748	0.25574787	0.4
D22	-4.5	-4.6	0.000676741	0.67674082	2.1
D23	-4.1	-4.6	0.00129468	1.2946805	2.1
D24	-3.6	-4.5	0.002912983	2.91298265	0.9
D25	-4.4	-4.3	0.000795898	0.79589803	1
D26	-3.9	-4.9	0.001790741	1.79074077	1.7
D27	-3.9	-4.9	0.001790741	1.79074077	2.2
D28	-4.0	-4.8	0.001522642	1.5226415	0.6
D29	-4.7	-5.0	0.000489274	0.48927413	1.5
D30	-4.6	-4.6	0.000575423	0.57542313	0.8
D31	-4.4	-4.4	0.000795898	0.79589803	2.8
D32	-4.6	-5.0	0.000575423	0.57542313	0.8
D33	-5.2	-5.6	0.000217459	0.21745879	2.8

The Grid, a three-dimensional point array centered on the enzyme's active site under consideration, delineates the protein region for analysis when the ligand-macromolecule interaction occurs (Huey et al., 1996; Lokesh and Krishnan, 2016). The grid dimensions, designed to accommodate the full active site of the protein, were adapted from the crystallized ligand structure for docking calculations. Thus, the docking operation was performed with grid dimensions of 32x26x28 Å along the X, Y, and Z axes, and the grid center coordinates on the X, Y, and Z axes were identified as 2.77, -3.439, 9.333, respectively (Figure 2).

Figure 2 Three-dimensional structure of the Quinone reductase and FAD Grid.



The 33 tethered complexes are depicted using two- and three-dimensional graphics, visualized via the Discovery Studio viewer v21.1.0.20298. These visual representations can be found in the appendix of this study. The software creates an automated system for graph generation, illustrating two-dimensional diagrams of binding protein interactions derived from three-dimensional coordinates. The visualizations encapsulate the interaction patterns of hydrogen bonds and hydrophobic contacts between ligands and primary or secondary protein structures (Laskowski and Swindells 2011). Figures 3 and 4 illustrate the Quinone Reductase Complex's two- and three-dimensional models, with FAD, Eugenol, and Valencene, respectively.

Figure 3 Three-dimensional and two-dimensional structure of the Quinone Reductase, FAD and Eugenol complex

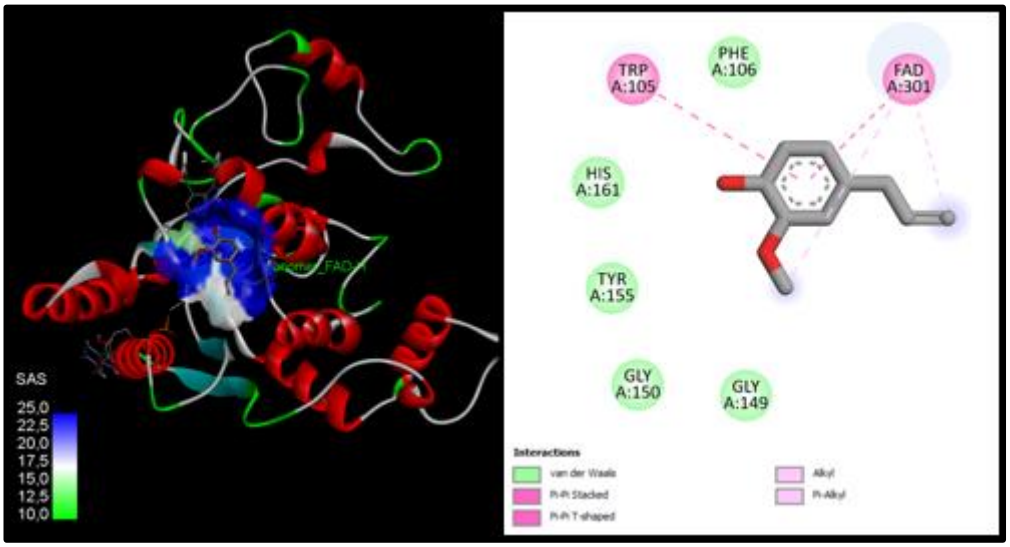
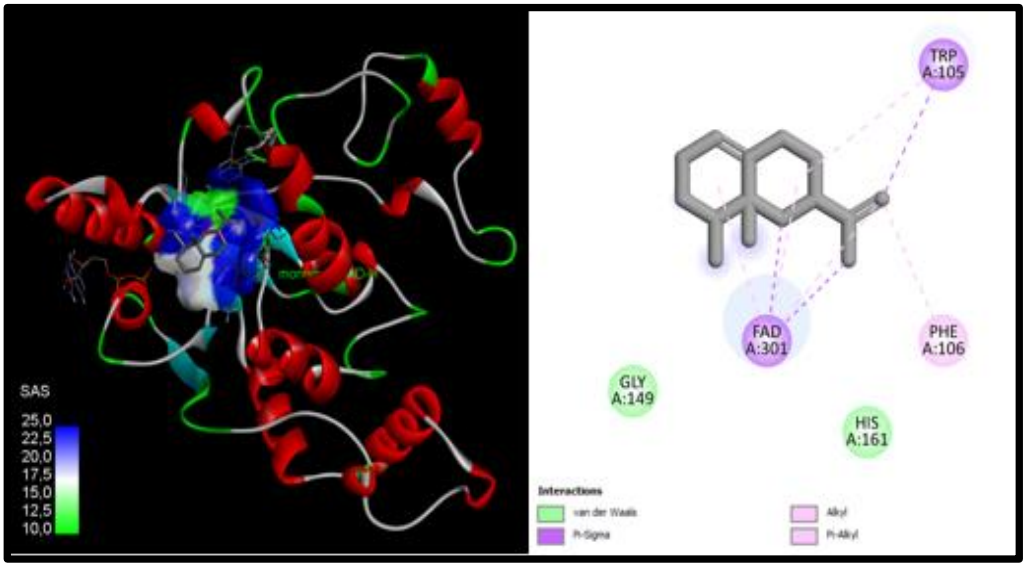


Figure 4 Three-dimensional and two-dimensional structure of the Quinone Reductase, FAD and Valencene complex



The intermolecular interactions between the Quinone reductase enzyme, FAD, and the tested compounds are detailed in tables 4 and 5, cataloging substances 1 to 16 and 17 to 33, respectively. The formation of a protein-ligand complex arises from a specific interaction between a protein and a particular ligand, influenced by their binding affinity and specificity. These affinities and specificities arise from intermolecular interactions such as Van der Waals forces, hydrophobic interactions, π-π, ionic or electrostatic interactions, hydrogen bonds, and covalent bonds (Guryanov et al. 2016).

Table 3 Types of interaction between amino acids, FAD and Quinone reductase enzyme.

Aminoacid/Residue	Binder															
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16
FAD301	2 1	2 1	1 2	1 2	1 1	3 1	3 1 1	1 2 2	1 3	2 2	2 1 1	1 1 2	2 1	1 3	1 2	2 1
Gly107	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Gly149	X	X	X	X	X	1	X	X	X	X	X	X	1	X	1	X
Gly150	X	1	X	X	X	X	X	X	X	X	X	X	X	1	1	X
His161	1	1 1	1	1	1	1	1	1	1	1	X	1	1	1	1	2
His194	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Ileu167	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Ileu171	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Ileu175	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lys113	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Met154	X	X	X	X	X	X	X	X	X	X	X	X	X	1	X	X
Phe106	2	1	1	X	X	1	1	X	1	1	1	1	1	1	1	1
Pro170	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Trp105	3	2	2	2	2	1	1	2	2	2	1 2	1 1	2	2	1 2	2
Tyr155	X	1	X	X	X	X	X	X	X	X	X	X	1	X	1	X
Val108	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Val166	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Alkyl																
Pi- Alkyl																
Van der Walls																
Pi-Sigma																
Conventional Hidrogen Bond																
Pi-Pi Stacked																
Pi Pi T Shaped																
Unfavorable Donor Donor																

Table 4 Types of interaction between amino acids, FAD and Quinone reductase enzyme.

Aminoacid/Residue	Binder																
	D17	D18	D19	D20	D21	D22	D23	D24	D25	D26	D27	D28	D29	D30	D31	D32	D33
FAD301	1 2	X	4	2 2	1 2	3 2	4 1	1 3	2 2	X	2	1 4	1 1	1 2 1	X	1 2	2 3
Gly107	X	X	X	X	X	X	X	X	X	1	X	X	X	X	X	X	X
Gly149	1	X	X	1	1	X	X	1	X	X	X	X	X	X	X	1	1
Gly150	1	X	1	1	X	X	X	X	X	X	X	X	X	X	X	X	X
His161	1	1	1	X	1	1	X	2	1	X	2	1	2	X	1	X	1
His194	X	X	X	1	X	X	X	X	X	1	X	X	X	X	X	X	X
Ileu167	X	2	X	X	X	X	X	X	X	1	X	X	X	X	2	X	X
Ileu171	X	X	X	X	X	X	X	X	X	1	X	X	X	X	X	X	X
Ileu175	X	X	X	X	X	X	X	X	X	3	X	X	X	X	X	X	X
Lys113	X	X	X	X	X	X	X	X	X	2	X	X	X	X	X	X	X
Met154	X	X	X	X	1	X	X	X	X	X	X	X	X	X	X	X	X
Phe106	1	1 1	X	1	1	1	1	1	1		2	1	1	1	1 1	1	1
Pro170	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X
Trp105	1	X	1	1 2	1	2	3	2	1 3	X	4	4	1	1 1	X	1 1	1 1
Tyr155		X	1	X	X	X	X	X	X	X	X	X	1	X	X	X	X
Val108	X	X	X	X	X	X	X	X	X	1	X	X	X	X	X	X	X
Val166	X	1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Alkyl																	
Pi- Alkyl																	
Van der Walls																	
Pi-Sigma																	
Conventional Hidrogen Bond																	
Pi-Pi Stacked																	
Pi Pi T Shaped																	
Unfavorable Donor Donor																	

Among the compounds tested for in vitro quinone reductase activity, almost all demonstrated Alkyl and Pi-Alkyl type hydrophobic interactions with Tryptophan-105, apart from Terpinolene and Eugenol. Terpinolene showed no interaction, while eugenol displayed a Pi-Alkyl interaction, suggesting a potential for improved coupling with FAD and Quinone reductase due to its low docking energy of -5.0kcal/mol.

Experimental data in this study affirm eugenol's reported role as an apoptotic inducer in human promyelocytic leukemia cells through a mechanism dependent on reactive oxygen species and mitochondria. This influences cancer cells by acting as an antioxidant, preventing mutation, modifying signaling pathways, and inducing cancer cell death. Furthermore, it inhibits NF-κB activation, reducing prostaglandin synthesis by lowering cyclooxygenase-2 activity and promoting S phase mitotic cycle interruption leading to apoptotic cell death (Fangjun and Zhijia 2018; Fathy et al. 2019; Ulanowska and Olas 2021).

Regarding Histidine-161, all active compounds in the in vitro quinone reductase test, except for linalool, demonstrated Van der Waals-type interactions. Several compounds, including Valencene and Eugenol, demonstrated Alkyl and Pi-Alkyl hydrophobic interactions, while others showed hydrogen bonds, such as (−)- borneol and Camphor. However, Geraniol exhibited an unfavorable interaction, potentially affecting the stability of the complex due to the presence of repulsive forces (Dhorajiwala et al. 2019). Interestingly, Geraniol formed a Hydrogen bond with Glycine 150, potentially improving intermolecular interaction and contributing to its in vitro and in silico activity. Hydrogen bonds are crucial for maintaining protein structures, as covalent bonds in biological systems carry a high energy cost and are infrequently broken (Barreiro et al. 2015).

Published studies indicate the cytotoxic effect of Geraniol, found in essential oils of aromatic plants such as Cinnamomum tenuipilum and Valeriana officinalis, among others. These findings suggest its potential use in cancer treatment and in reducing

patient mortality (Lei et al.2019). The specific mechanism by which these effects are elicited has not been linked with quinone reductase induction, making this study pioneering in this domain.

The amino acid Glycine 150 exhibited Van der Waals Interactions with both (−)-borneol and eugenol. Borneol, classified as a monoterpenoid, has previously exhibited antiproliferative properties in HepG2 cells, inducing apoptosis and activating the intrinsic apoptotic pathway. It achieves this by modulating the pro-survival and pro-apoptotic proteins of the Bcl-2 family, according to the research conducted by Su et al. (2013). This study provides further evidence of borneol's chemopreventive capacity, demonstrating its ability to induce the Quinone Reductase enzyme. This conclusion aligns with the in silico results of our molecular docking studies.

Concerning the amino acid Glycine 149, hydrophobic and Van der Waals interactions were exclusively observed with Eugenol and Valencene. These substances demonstrated lower docking energies of -5.0 and -5.2 kcal/mol, respectively. These results suggest that these interactions are crucial in decreasing the complex's docking energy, enhancing the intermolecular interaction, and subsequently boosting quinone reductase activity.

Valencene has been found to exhibit cytotoxic and antiproliferative effects on various cancer types, including doxorubicin-sensitive ovarian cancer cell lines and lymphoblastic cancer cell lines (Ambroz et al. 2015, 2017; Tomko et al. 2020).Although the exact mechanisms remain unknown, the findings of cytotoxicity assays corroborate this study's focus on chemoprevention. This study is the first to suggest that Valencene's chemopreventive activity could be linked to the induction pathway of the Quinone Reductase enzyme, as evidenced by both in vitro and in silico studies.

Regarding the FAD molecule, Valencene and Eugenol exhibited Alkyl and Pi-alkyl interactions. Eugenol, however, presented additional hydrophobic and Pi Pi stacked interactions, while Valencene exhibited Pi Pi sigma interactions. This interaction pattern with the FAD molecule is consistent for 30 of the 33 substances

tested, except for Phelandrene, (+)-α-Pinene and Terpinolene. Only L-Carveol displayed hydrogen bonding with FAD, which didn't ensure higher complex stability. The docking energy was similar to other substances that did not exhibit this interaction.

Existing research on Terpinolene suggests it has a wide range of potential pharmacological applications, but the mechanisms underlying its cellular and molecular effects remain unclear (Menezes et al. 2021). This study establishes that Terpinolene can induce quinone reductase, suggesting a chemopreventive activity.

Regarding the amino acid Phenylalanine 106, all substances active in the in vitro quinone reductase test demonstrated Van der Waals, Alkyl, Pi Alkyl, and Pi sigma type interactions, except for Camphene, Camphor, L-Carveol and Geraniol. This result indicates that these interactions are not essential for enhancing quinone-reductase activity. This corroborates existing literature, which verifies the anti-cancer and cytotoxic capacities of Camphor and Menthol against different cancer cell lines (Sinh et al. 2023).

Camphor and Eucalyptol's chemopreventive effects have been established in bacteria and mammalian cells, supported by alkaline comet assay, Escherichia coli reversion test, and cytotoxicity assay data. This provides corroboration with the findings of our study (Nikolic et al. 2015). Furthermore, Carveol, for which the literature does not associate with chemopreventive activity, is being recognized for the first time in this study for its potential to induce the enzyme quinone reductase.

In addition, camphene activity against different cancer cells was demonstrated, and its mechanism of action was investigated in vitro and in vivo in murine melanoma through the induction of apoptosis by the intrinsic pathway in melanoma cells, mainly by causing stress in the endoplasmic reticulum with Calcium release, loss of mitochondrial membrane potential and increased activity of caspase-3 (Girolla et al. 2015). As for Carveol, the literature does not describe it with chemopreventive activity,

and this is the first study to describe it with chemopreventive activity by inducing the enzyme quinone reductase.

(+)carvone, L-Carvone and (+)β-Pinene demonstrated a similar interaction pattern with the amino acids Histidine 161, Phenylalanine 160, and Tyrosine 155. Notably, (+)β-Pinene exhibited double the number of hydrophobic interactions with the last two amino acids, although this does not necessarily suggest superior induction of the quinone reductase enzyme. (+)β-Pinene has been shown to inhibit cell proliferation and induce apoptosis and cell cycle arrest of Hela cells in the G0/G1 phase in a dose-dependent manner. This study is the first to suggest that (+)β-Pinene 's antiproliferative activity could be linked to the induction of the quinone reductase enzyme (Wang et al. 2019).

Eucalyptol exhibits a deviation from the interaction pattern shown by , (+)carvone, L-Carvone and (+)β-Pinene through an additional Van der Waals interaction with Phenylalanine 106. Previous research associated the treatment with eucalyptol with the induction of caspase-3, triggering cell apoptosis in human colon cancer cell lines (Murata et al. 2013). However, the potential of eucalyptol to induce Quinone reductase has not yet been proven. Similarly, (R)Limonene, known for halting tumor proliferation, was shown in this study to induce quinone reductase. Linalool, sharing the same interaction pattern as (R)Limonene, exhibits concentration and time-dependent apoptotic and antiproliferative properties in breast cancer cells (Elbe et al., 2022).

The energy required for a molecule to bind to a specified molecular target reflects the molecule's affinity for that receptor. The stability of the complex formed between the molecule and the target is inversely proportional to the energy required for the interaction, as posited by Barros et al. (2015). Therefore, the more stable the complex, the lesser the energy required for the interaction.

In this context, molecular docking analyses were conducted in ten cycles with the Quinone reductase complex, FAD, and the substance under study. Simultaneously, the effect of FAD on the complex was scrutinized. For comprehensive

understanding, molecular docking was performed for the Quinone reductase complex and the analyzed substance without including FAD.

Out of the 33 substances tested, only cymene presented higher docking energy without the FAD in the complex, implying that its absence could promote better binding and greater stability for the other 32 substances. Therefore, including FAD could potentially destabilize the complex, enhancing its reactivity.

Consequently, the interaction between the prospective drug, the substance in question, and FAD could increase the electron transfer between FAD and Quinone reductase. This would mean that the lower the molecular docking energy, the greater the inhibition of FAD and the increased enzyme activity, resulting in the augmentation of the detoxification enzyme, Quinone reductase. This hypothesis should be verified through quantum calculations to ascertain the increase in electron transfer.

In assessing pharmacokinetic attributes, the SwissADME tool was utilized. Parameters such as Gastrointestinal Absorption (GIA), Blood-Brain Barrier Permeation (PBHE), Lipinsky's Rule Violations (VL), and inhibition of Cytochrome P450 enzymes were determined. Remarkably, all substances tested are suitable for oral administration, exhibiting no Lipinski's rule violation or only a single violation in particular derivatives, which is deemed acceptable according to Lipinski (2004) Table 5.

Table 5 Values of Gastrointestinal Absorption of substances (AGI), Permeation through the blood-brain barrier (PBHE), Violation of Lipienki's Rule (VL) and inhibition of the CYP450 family of enzymes (ICYP).

Substance	AGI	PBHE	VL	ICYP
D1	High	No	0	NO
D2	High	No	0	NO
D3	High	No	0	CYP2C9
D4	Low	No	MLOGP>4.15	CYP2D6
D5	Low	No	0	NO
D6	Low	No	MLOGP>4.15	CYP2C9
D7	High	No	0	CYP2C19
D8	Low	No	0	NO
D9	High	No	0	NO
D10	High	No	0	NO
D11	Low	No	MLOGP>4.15	NO
D12	Low	No	MLOGP>4.15	NO
D13	High	No	0	NO
D14	Low	No	0	NO
D15	Low	No	0	NO
D16	Low	No	0	NO
D17	High	No	0	NO
D18	Low	No	0	NO
D19	Low	No	0	NO
D20	Low	No	MLOGP>4.15	CYP2C9
D21	Low	No	MLOGP>4.15	NO
D22	Low	No	0	NO
D23	Low	No	0	NO
D24	Low	No	0	NO
D25	Low	No	0	NO
D26	Low	No	MLOGP>4.15	CYP2C9
D27	Low	No	MLOGP>4.15	CYP2C9
D28	Low	No	0	NO
D29	Low	No	0	NO
D30	Low	No	MLOGP>4.15	NO
D31	Low	No	0	NO
D32	High	No	0	CYP1A2
D33	Low	No	MLOGP>4.15	CYP2C9

Toxicity analysis was conducted using the ProTox-II tool, determining the median lethal dose, toxicity class, and type of toxicity for each substance. The majority of substances fell into toxicity classes 4 and 5. Only D12 and D30 presented a lethal dose of less than 5mg/kg, designating them fatal if ingested (Table 6).

Table 6 Values for Lethal dose 50 (mg/kg), Classification of degree of toxicity and type of toxicity.

Substance	DL	CT
D1	2830	5
D2	500	4
D3	500	4
D4	5000	5
D5	775	4
D6	5300	5
D7	810	4
D8	3000	5
D9	1640	4
D10	1640	4
D11	2374	5
D12	3	1
D13	500	4
D14	2420	5
D15	3450	5
D16	2480	5
D17	1930	4
D18	5700	6
D19	2100	5
D20	5000	5
D21	3650	5
D22	4400	5
D23	2200	5
D24	5000	5
D25	113	3
D26	3700	5
D27	4700	5
D28	7000	6

D29	1190	4
D30	3	1
D31	4390	5
D32	640	4
D33	5000	5

Its pharmacokinetic and toxicological properties must be established before any substance can be considered for drug application. In silico studies have emerged as viable alternatives for evaluating these properties, enhancing the development of more specific and safer drugs (Tyzack and Kirchmair 2019).

Lipinski's rule is crucial in determining a compound's potential suitability as an orally administered medication. It outlines molecular properties integral to a drug's pharmacokinetics, such as absorption, distribution, metabolism, and excretion (Lipinski et al. 2001). All 33 substances under study comply with Lipinski's rule, suggesting their bioavailability if orally administered.

Knowledge of how molecules interact with Cytochrome P450 (CYP) enzymes is crucial for predicting drug metabolism. The inhibition of these enzymes often results in toxic or other adverse effects due to reduced clearance and accumulation of the drug or its metabolites (Testa and Kraemer 2007; Di 2014; Hollemberg 2002; Huang et al. 2008). All tested substances are not permeable to the blood-brain barrier (BBB), contributing to their safety profile.

The toxicological activity of the substances from in silico studies categorized most of them in toxicity classes 4 and 5 based on their lethal doses. However, cymene (p) and Terpinene gamma were identified as lethal if ingested since they have an average lethal dose of less than 5 mg/kg. Nevertheless, studies have shown that p-cymene has low cytotoxicity at specific doses (Kummer 2015). Similarly, while γ-Terpinene is toxic upon oral exposure (Carson et al. 2006), this reaffirms the data from our study.

CONCLUSION

The present research affirms that specific substances, namely (−)-borneol, (+)-borneol, Camphene, Camphor, L-carveol, (+)-carvone, Eucalyptol, Eugenol, Geraniol, (R)Limonene, Linalool, β-pinene, Terpinolene, and Valencene, function as inducers of the quinone reductase enzyme. Consequently, they exhibit potential cancer chemopreventive effects. This assertion aligns with the data procured in silico via molecular docking, which reveals low docking energies.

Among the substances that demonstrated activity in vitro, Valencene and Eugenol displayed the most favorable docking energies, the only ones in this research to showcase docking energies lower than -5.0 kcal/mol. A hydrophobic interaction with the amino acid Glycine 149 probably enhances the complex's interaction involving these substances.

The study hypothesizes the presence of a pattern in the interactions between FAD and the quinone reductase enzyme, predominantly through Alkyl, Pi-Aquil, and Pi-Sigma interactions. Moreover, FAD enhances the electron transfer process between itself and quinone reductase. However, this hypothesis necessitates further exploration via quantum calculations for verification.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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APÊNDICE

Descrição do ensaio de indução NAD(P)H: quinona redutase

O ensaio de indução da quinona redutase foi realizado segundo método descrito por Pezzuto et al. (2005). Para a avaliação de amostras como indutores da enzima quinona redutase, foi empregada cultura de hepatoma de rato, Hepa1c1c7, ATCC CRL 2026™. As células foram semeadas em duas placas estéreis, transparentes, de 96 poços a uma densidade de 1 x 104 células. mL-1, marcadas como “Proteína” e “QR ensaio”, respectivamente. Após pré-incubação de 24 h, o meio de cultura inicial foi removido e adicionado nova alíquota (190 µL) de meio de cultura. Em cada placa foram adicionados 10 µL da solução da amostra, na concentração de 20 µg. mL-1, 10 µL das soluções da curva de diluição de 4’-bromoflavona (concentrações finais de 50,0µM a 0,4µM) (controle positivo) e 10 µL de solução de DMSO a 10% em PBS (controle negativo). As placas foram incubadas, por 48 h, em estufa de CO_2. Após a incubação, o meio de cultura foi removido da placa “Proteína”, em seguida, foi adicionado 200µL de cristal violeta a 2% em etanol e incubado à temperatura ambiente, por 10 min. Em seguida, a placa foi lavada em água corrente, com fluxo baixo, por 2 min, seca em capela e após, foi adicionado à cada poço da placa “Proteína”, 200µL de SDS a 0,5% em 50% de etanol. A incubação foi à temperatura ambiente, sob agitação, 10 ciclos/min, por 5 a 10 min. Em seguida, a absorbância foi determinada em 595nm. O valor de absorbância do controle negativo foi de 1,0. De maneira semelhante, após a 48 h de incubação, o meio de cultura foi removido da microplaca “QR ensaio” e foram adicionados 50µL de solução de digitonina a 0,8%. A placa foi incubada em estufa comum, a 37ºC, por 10 min, seguida de incubação à temperatura ambiente, sob agitação, 10 ciclos/min, por 10 min. Em 26 seguida, foi adicionada a mistura reacional (200 µL) composta por 28 ml de H_2O; 1,5 mL de Tris-HCl, 0,5 M, pH 7,4; 200 µL Tween 20 a 1,5% V/V; 20 µL FAD 7,5 mM; 200 µL G-6-P 150 mM; 18 µL NADP 50 mM; 20 mg BSA; 9 mg MTT; 60 U G-6-PH; e 30 µL Menadiona 50 mM.

A incubação foi realizada em temperatura ambiente, a placa “QR ensaio” foi agitada com 10 ciclos/min, no período de 5 min. A absorbância foi determinada em leitor de microplacas no comprimento de onda de 595 nm. A atividade indutora da QR foi expressa como DC (dobro da concentração requerida para a atividade específica da QR). Para o cálculo de DC foi aplicado, a cada valor de absorbância lido na placa “QR ensaio”, a seguinte equação: DC= [(A1 x /5) / AT] x 3.247 Onde: A1= absorbância da solução na presença do indutor, lido na placa “QR ensaio” (AAmostra - ABranco)

AT= absorbância da solução na presença do indutor, lido na placa “Proteína” (AAmostra - ABranco) O denominador 5 refere-se ao tempo de incubação à temperatura ambiente e o denominador 3,247 é a razão entre a constante de proporcionalidade do cristal violeta pelo coeficiente de extinção do MTT no comprimento de onda 595 nm.

Os resultados foram apresentados como média. Foram consideradas ativas as amostras que apresentaram valor DC superior a 2. As análises foram realizadas em triplicatas.

Biofracionamento do extrato Hexânico das folhas de *Labramia bojeri*

Após secas, as folhas foram moídas com auxílio de um moinho de facas. Em seguidas amostras foram desengorduradas com hexano 100% em três tempos de 30 minutos cada solvente em banho ultrassônico seguindo a proporção de 1 g de material vegetal para 20 ml de solvente. Ao final os extratos foram filtrados por meio de funil de buchner acoplado a uma bomba sucção e evaporados até a secura. Em seguida os extratos foram concentrado em evaporador rotatório, a 40 °C, sob pressão reduzida, até resíduo e posteriormente liofilizados totalizando 1,4303 g do extrato hexânico da *Labramia bojeri*. Todos os fracionamentos cromatográficos foram monitorados por cromatografia em camada delgada de sílica gel (CCD), utilizando-se diversos eluentes. Apresenta-se, na Figura 12, o fluxograma resumido do fracionamento do extrato hexânico das folhas de *Labramia bojeri*. Os cromatogramas foram observados sob luz visível e ultravioleta (254 e 365 nm), antes e após revelação com a solução reveladora de Anisaldeído Sulfúrico. As frações foram reunidas de acordo com seus perfis, sendo concentradas em evaporador rotatório a 40-60 °C e transferidas para frascos previamente tarados e mantidas em dessecador, sob vácuo, para completa eliminação do solvente por, no mínimo, 48 h.

Condições cromatográficas e obtenção dos perfis cromatográficos

Para obtenção dos perfis cromatográficos das amostras foram empregadas diferentes condições cromatográficas e detectores UV segundo as características da amostra analisada, como polaridade e presença de cromóforos. Para a preparação da coluna cromatográfica foi utilizada sílica gel 70-230 Mesh 60 lote 1922500614 marca Marcherey Nagel. Foi utilizado aproximadamente 1 litro do reagente hexano para empacotamento o que resultou em uma coluna de 17 cm de altura e 3 cm de diâmetro. Foi seguida a proporção de 1,5g material para cada 17g de sílica.

Uma massa de algodão foi aderida no fundo da coluna e paulatinamente adicionada sílica já dissolvida no hexano. Recolheu-se o hexano recolocando-o para conclusão do empacotamento da coluna. Para continuação da preparação da coluna, foi feita uma mistura com o extrato hexânico e a sílica com o auxílio de gral e pistilo e colocado sobre a sílica empacotada finalizada com algodão em sua superfície. As substâncias isoladas foram agrupadas de acordo com o grau de similaridade dos compostos com o auxílio das placas de cromatografia em camada delgada de sílica.

Solução reveladora de Anisaldeído Sulfúrico

Para a revelação empregada nos processos cromatográficos utlizou-se a Solução de anisaldeído sulfúrico obtida pela Mistura de 0,5 ml de anisaldeído, 10 ml de Acido acético glacial, 85 ml de álcool metílico e 5 ml de ácido sulfúrico a 98% v/v, nesta ordem, sob resfriamento. A solução foi armazenada a 4 °C, até o momento de uso e utilizada sob aquecimento de uma chapa aquecedora.

Fracionamento do extrato hexânico de folhas de *Labramia bojeri* por cromatografia em coluna aberta de sílica gel

Solvente da série eluotrópica
Hexano Puro
Hexano+ 10% Diclorometano
Hexano+ 50% Diclorometano
Diclorometano 100%
Diclorometano 90%+ 10%Acetato de Etila
Diclorometano 70%+ 30%Acetato de Etila
Diclorometano 50%+ 50%Acetato de Etila
Etanol 25% + Diclorometano 25%+ 50%Acetato de Etila
Etanol 25% + 75%Acetato de Etila
Etanol 50% + 50%Acetato de Etila
Etanol 75% + 25%Acetato de Etila
Etanol 100%
Água Deionizada 100%

A fração 359, denominada de Isolado, foi recolhida para elucidação estrutural pelo método de análise por espectroscopia de Ressonância Magnética Nuclear.

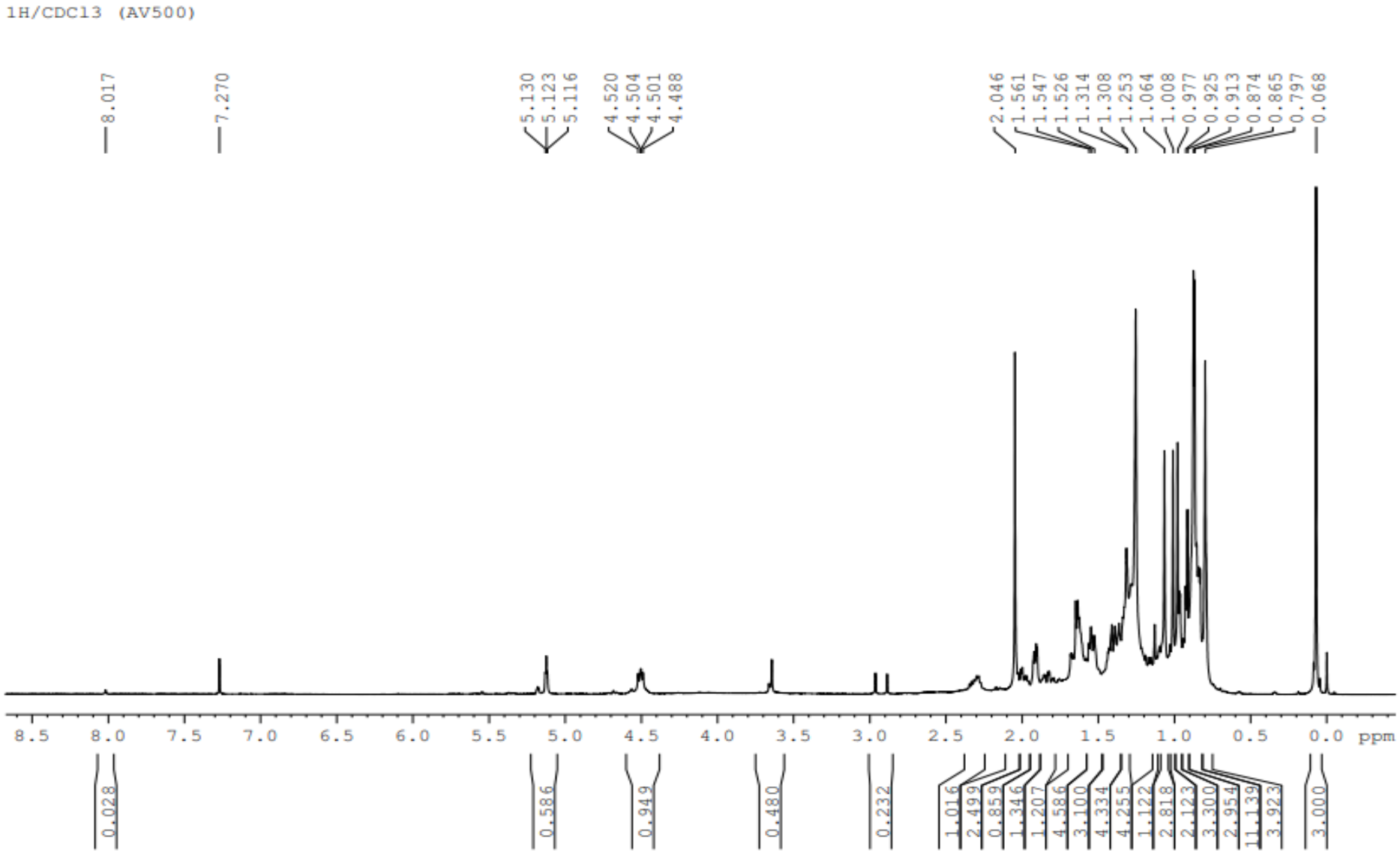


Figura 1 Espectro de RMN de ^{1}H

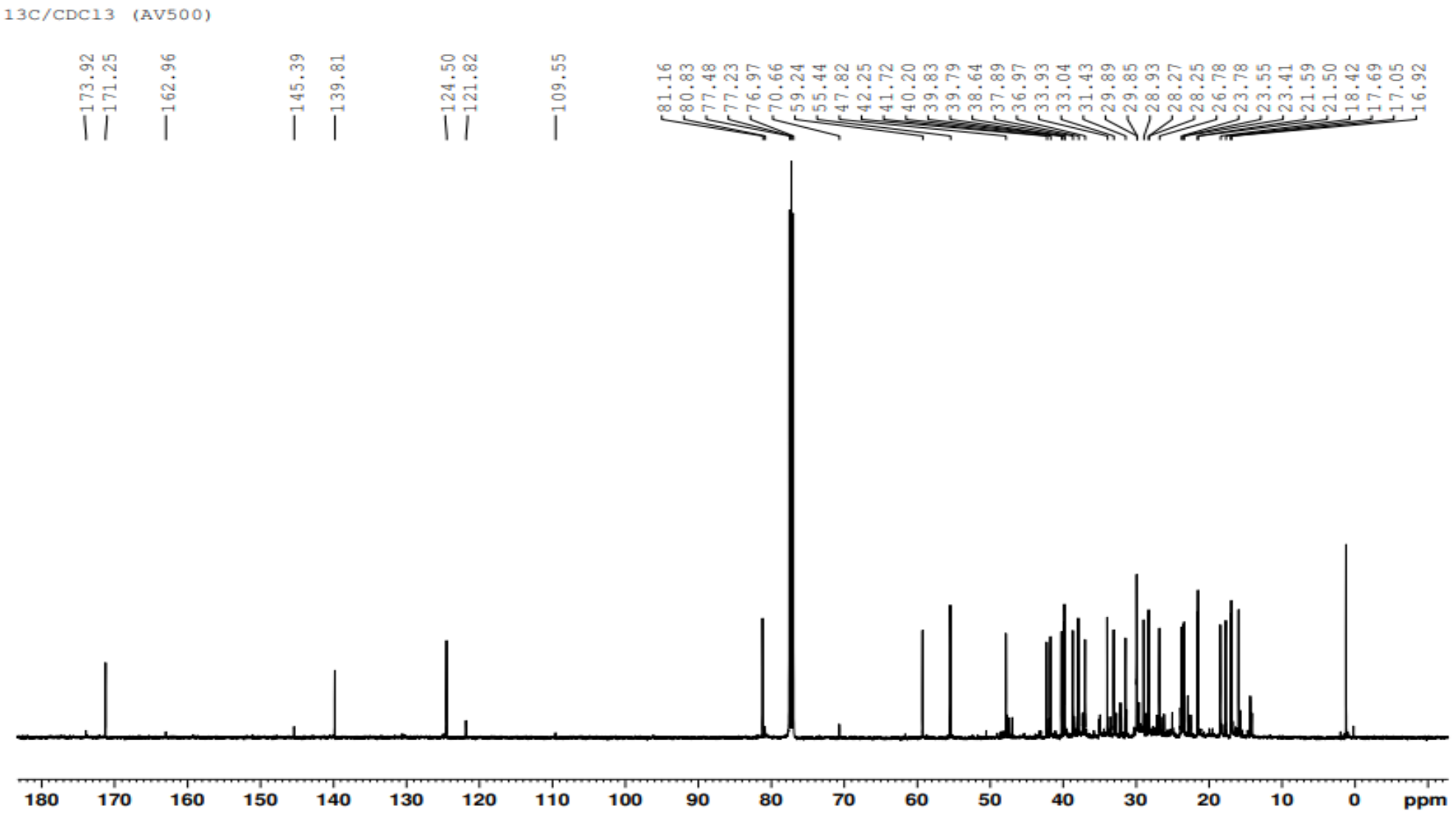


Figura 2 Espectro de RMN de ^{13}C

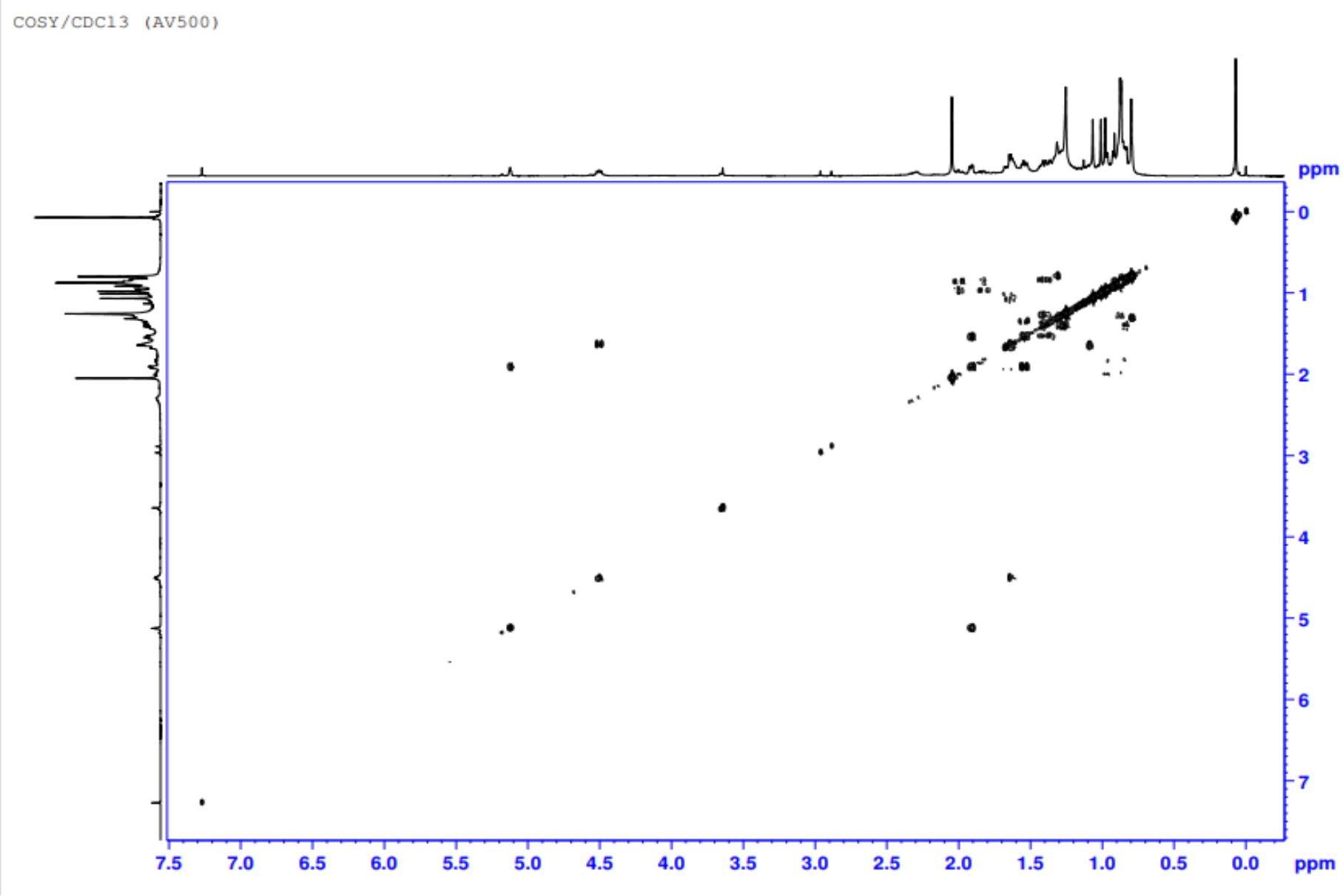


Figura 3 Espectro de RMN COSY

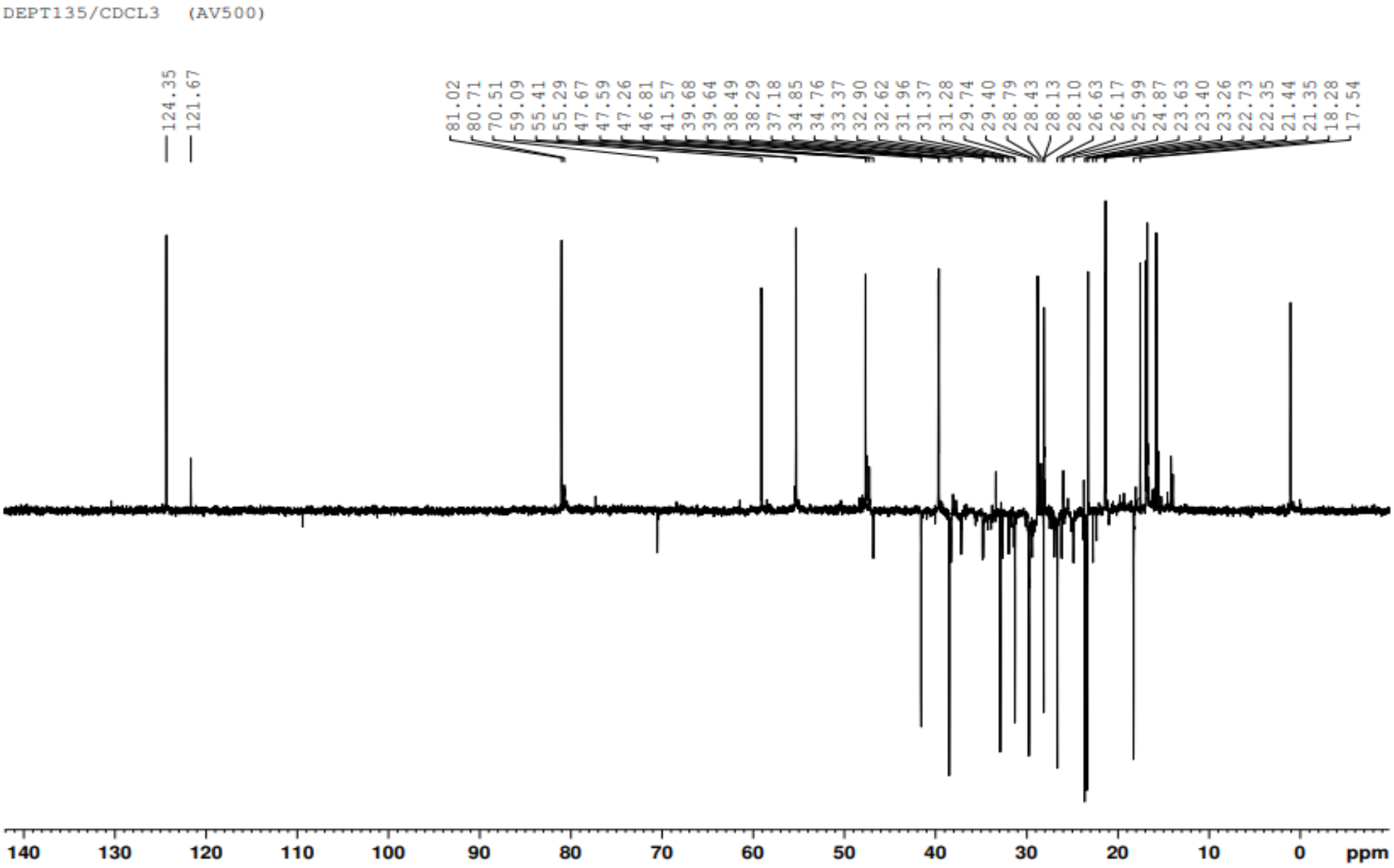


Figura 4 Espectro de RMN DEPT 135

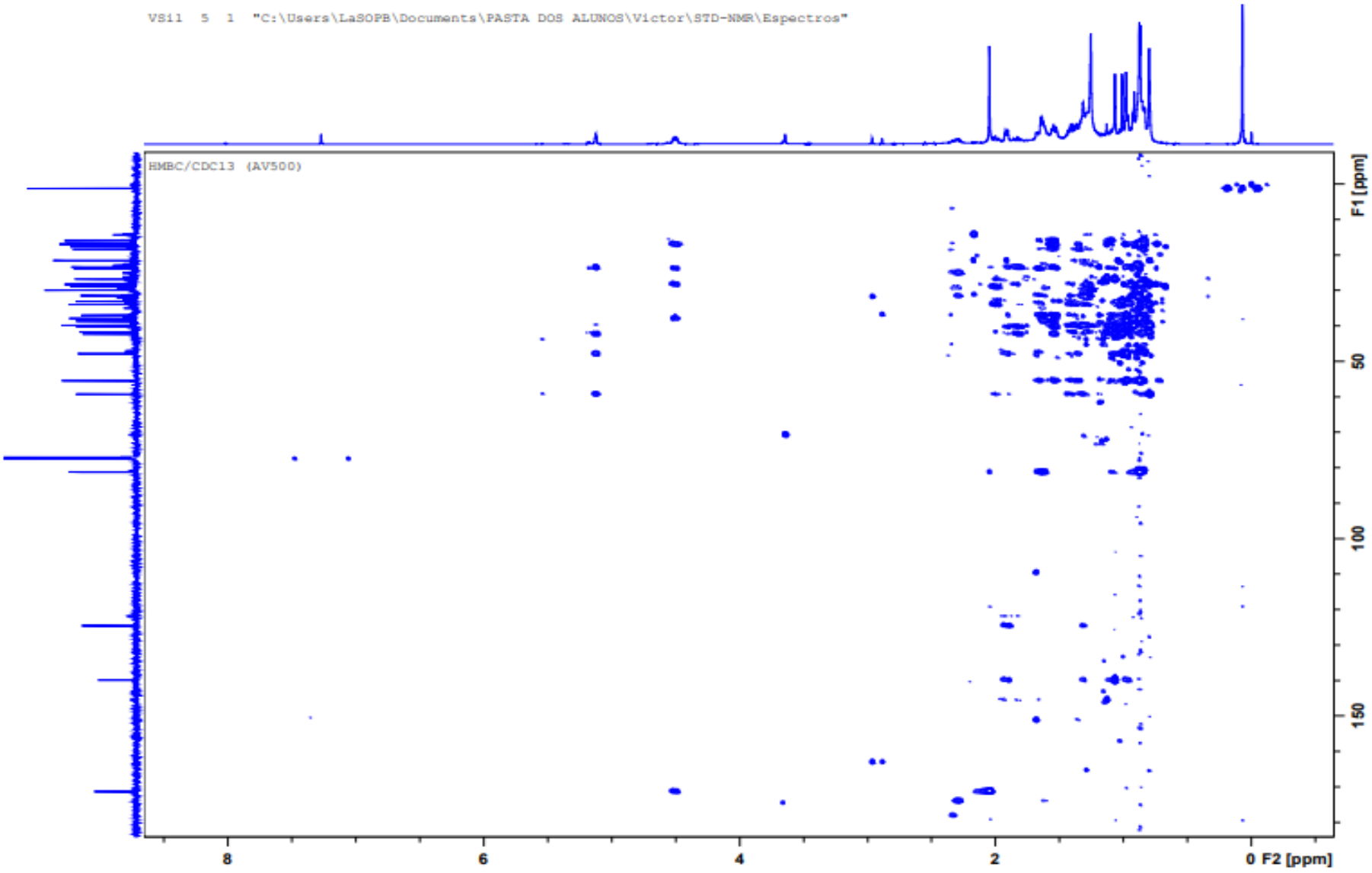


Figura 5 Espectro de RMN HMBC

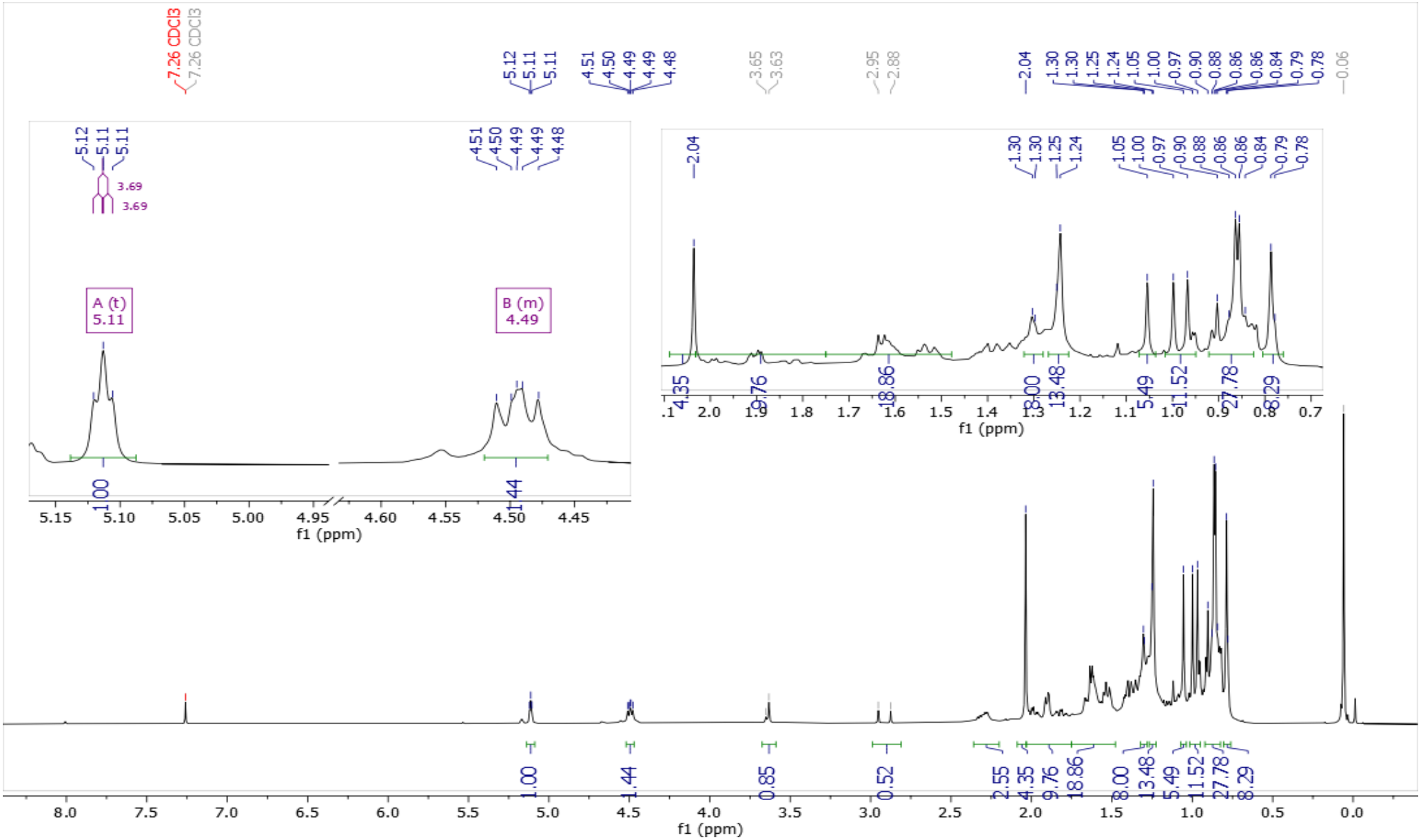


Figura 6 Espectro de RMN de ^{1}H (500 MHz, $CDCl_3$) do acetato de α-amirina

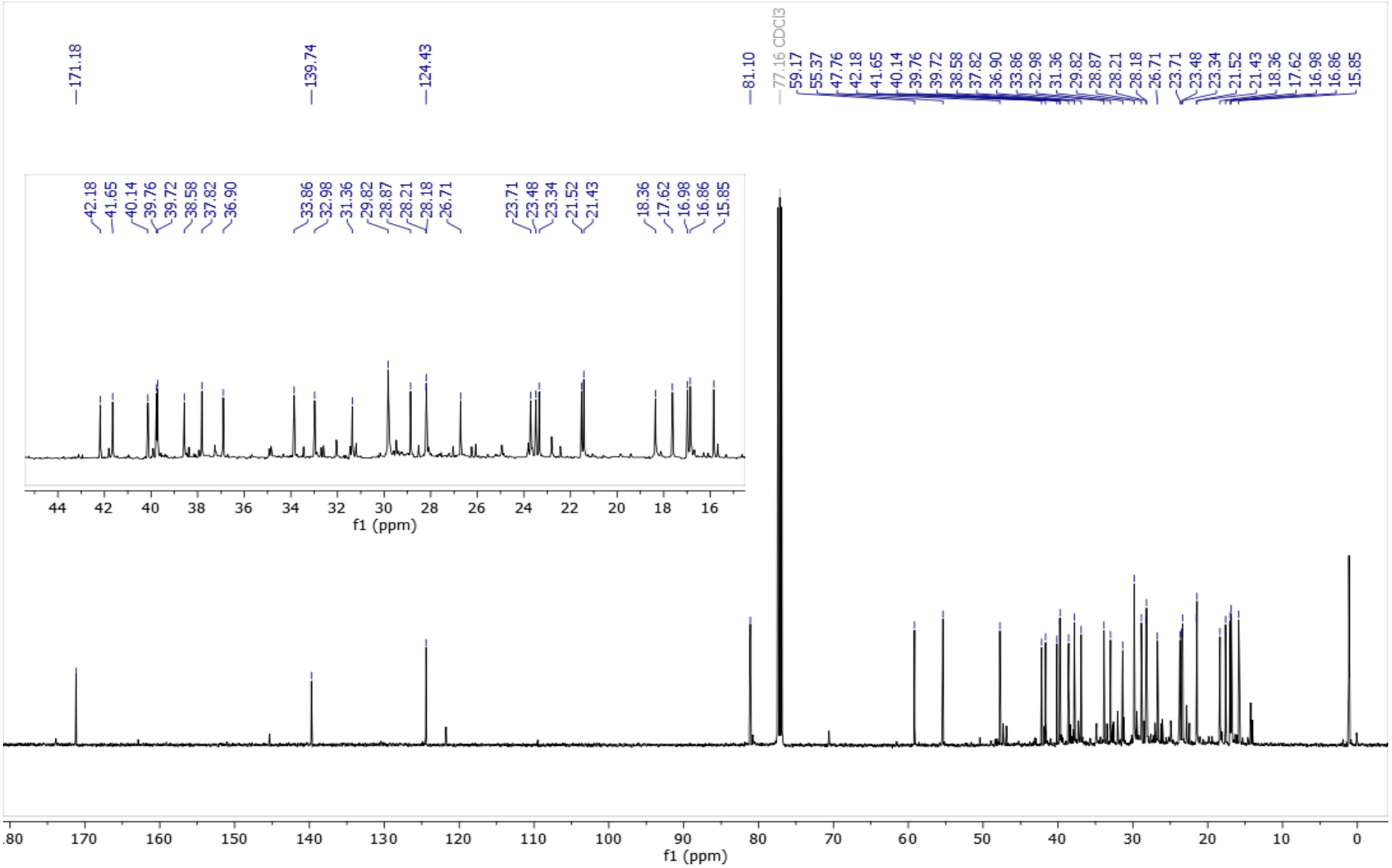


Figura 7 Espectro de RMN de ^{13}C (125 MHz, $CDCl_3$) do acetato de α-amirina

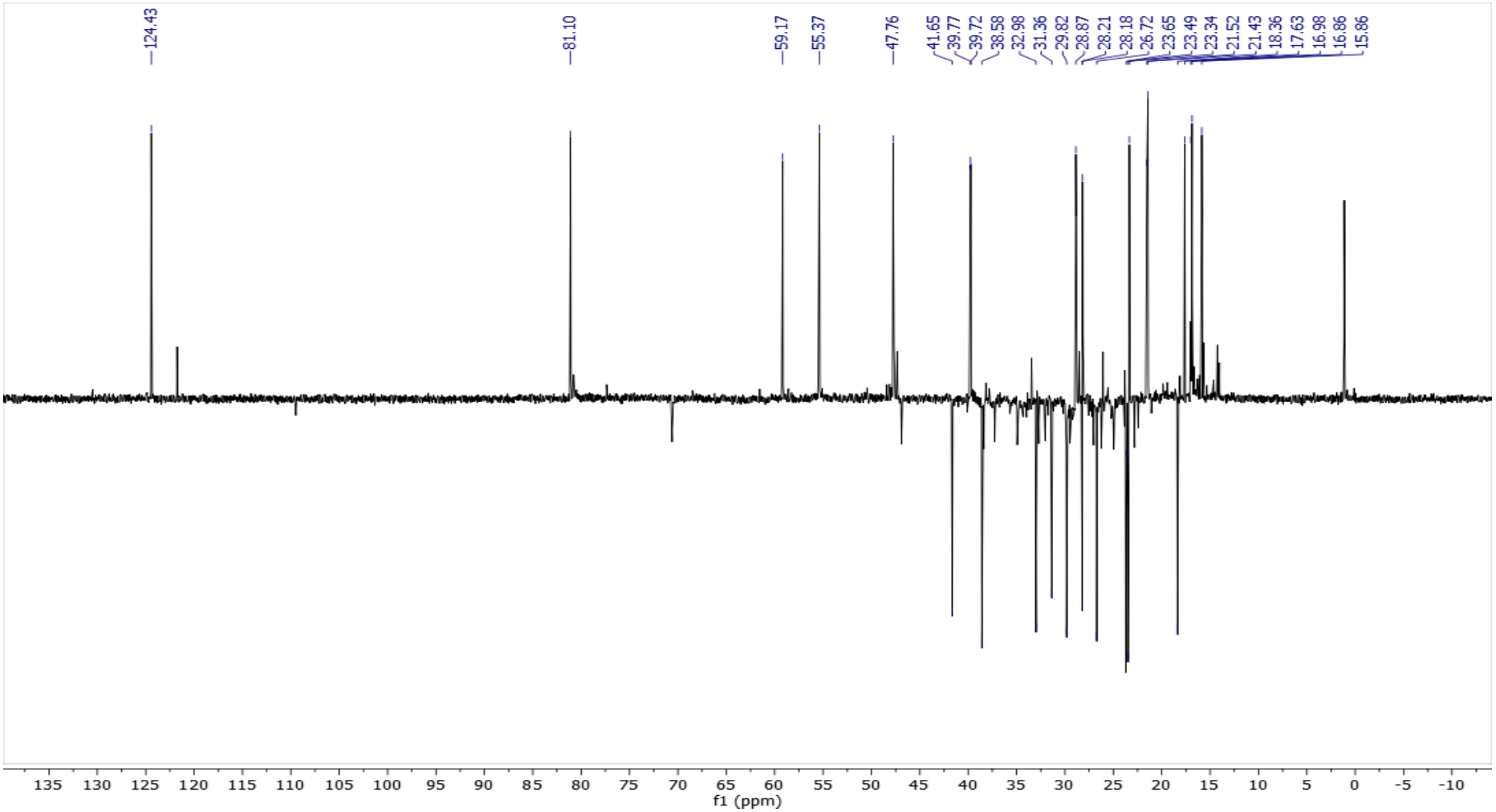
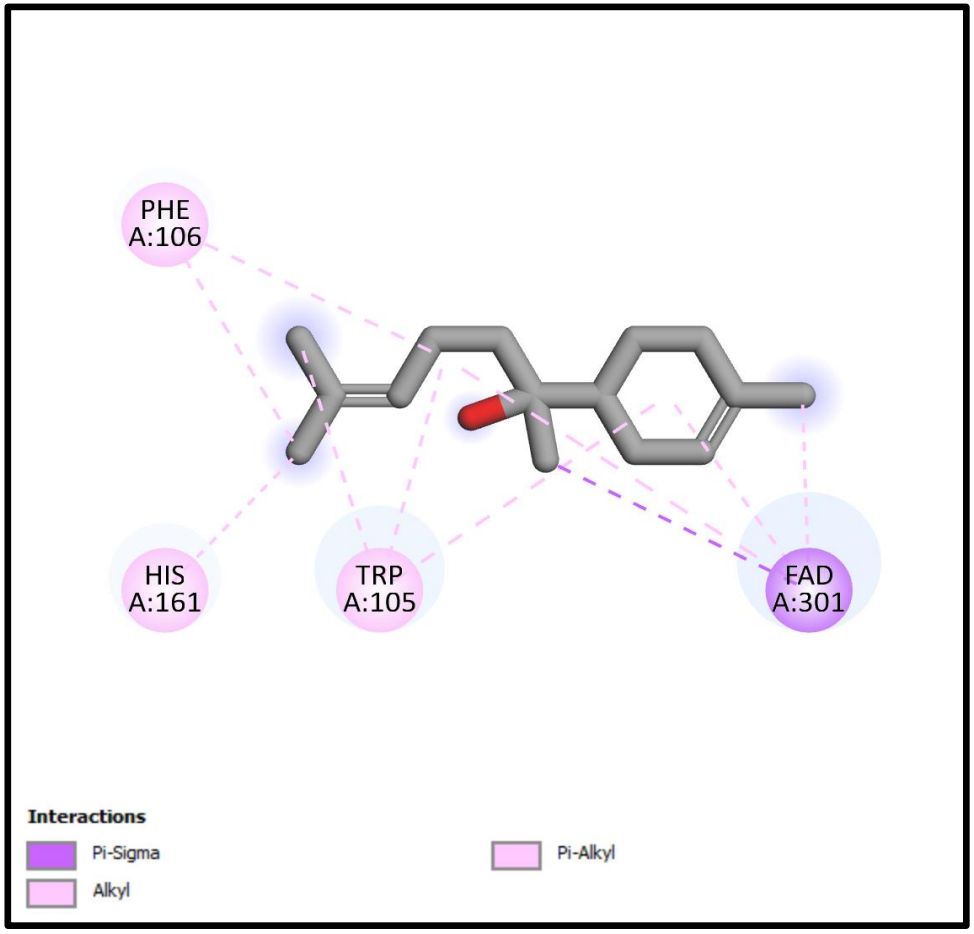


Figura 8 Espectro de RMN DEPT-135 (125 MHz, $CDCl_3$) do acetato de α-amirinaFigura



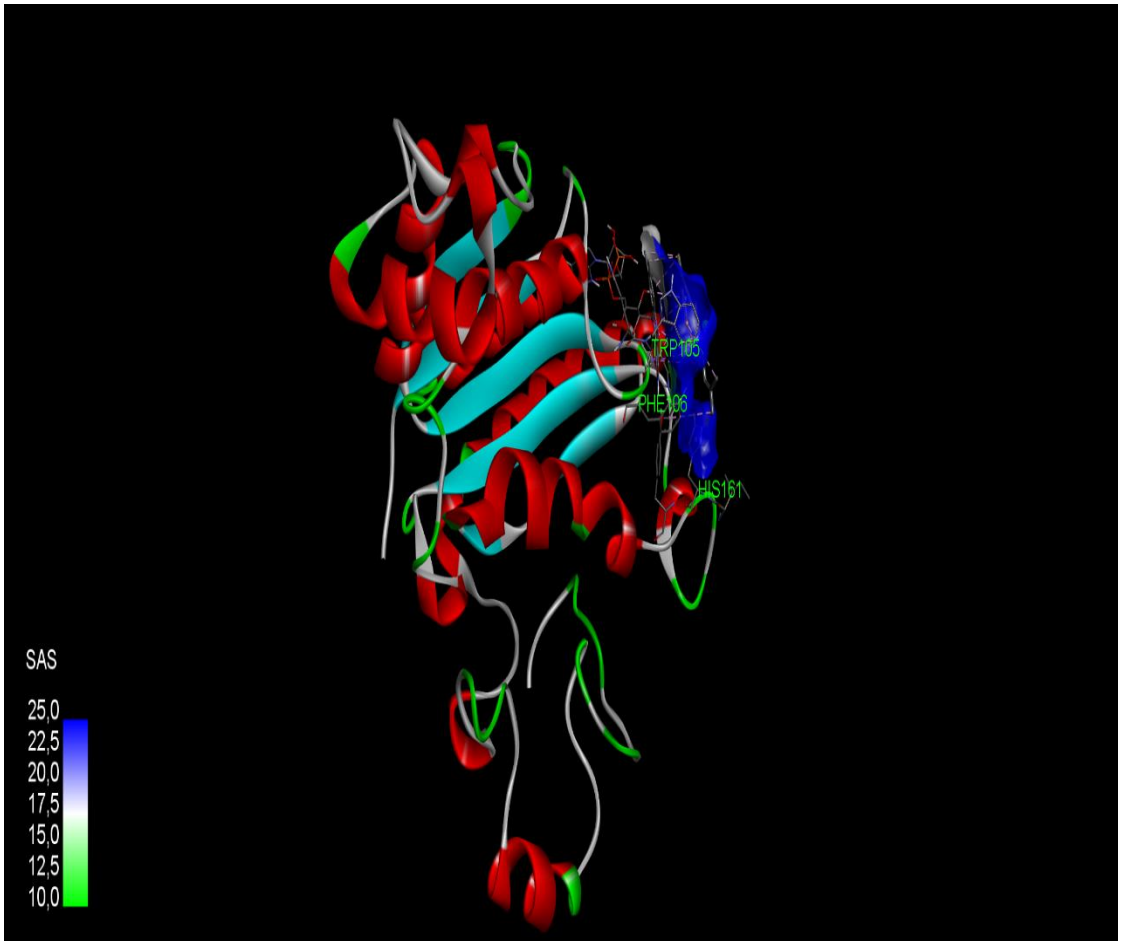


Figura 9 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Bisabolol (-)-

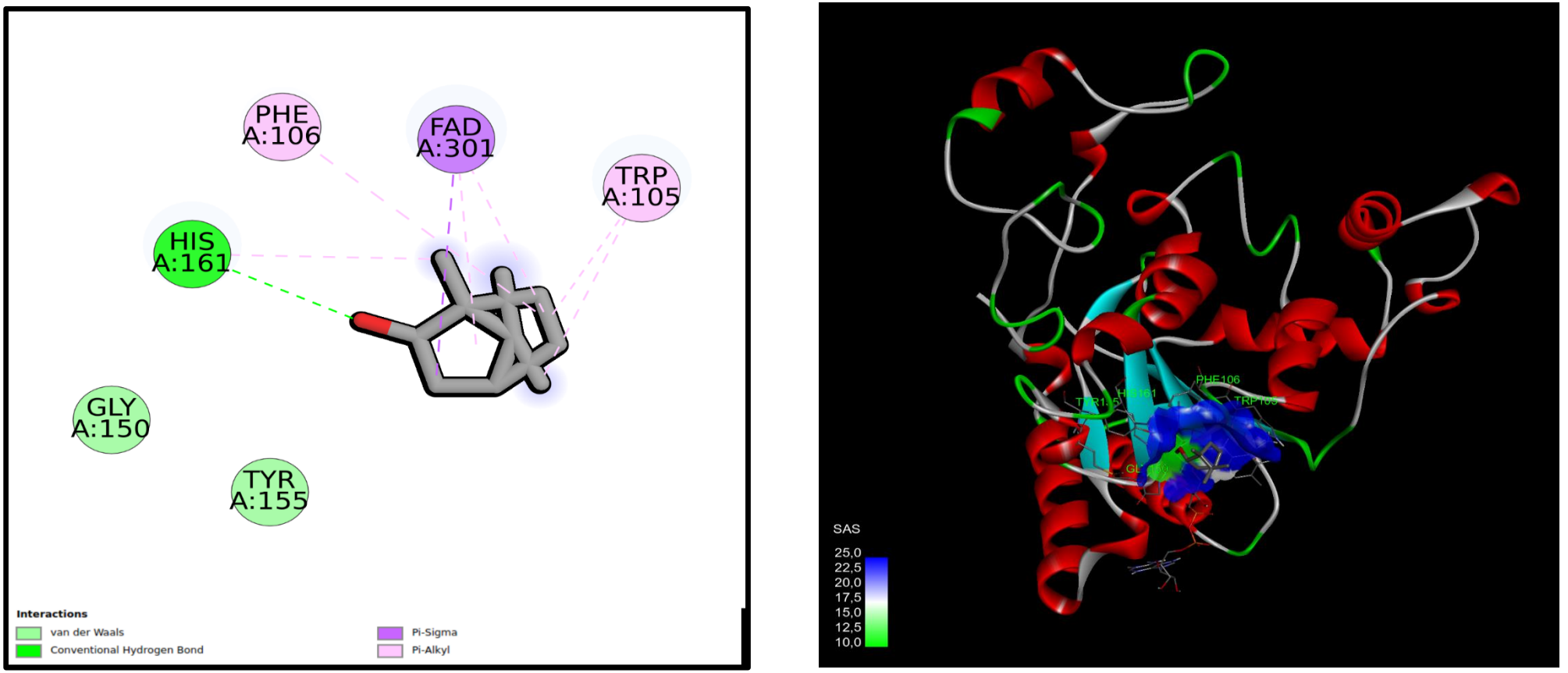


Figura 10 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Borneol(-)

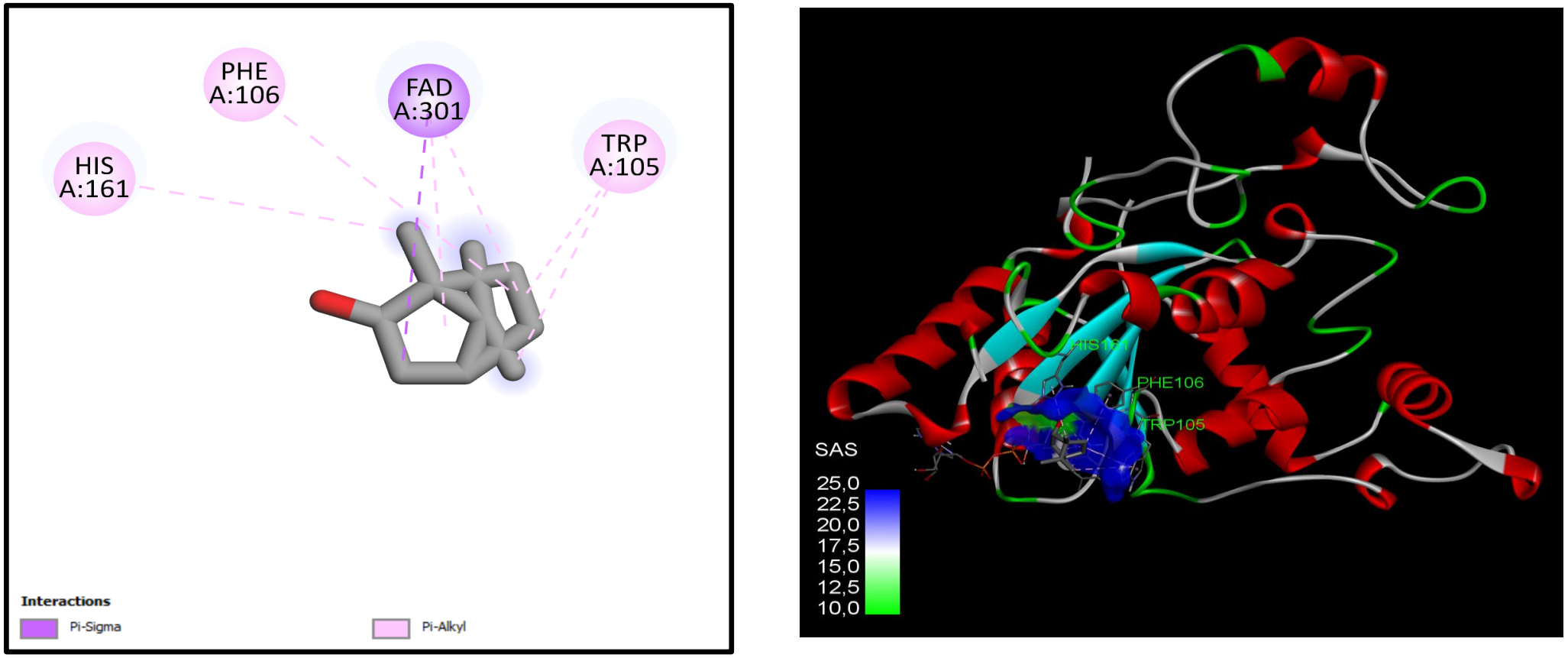
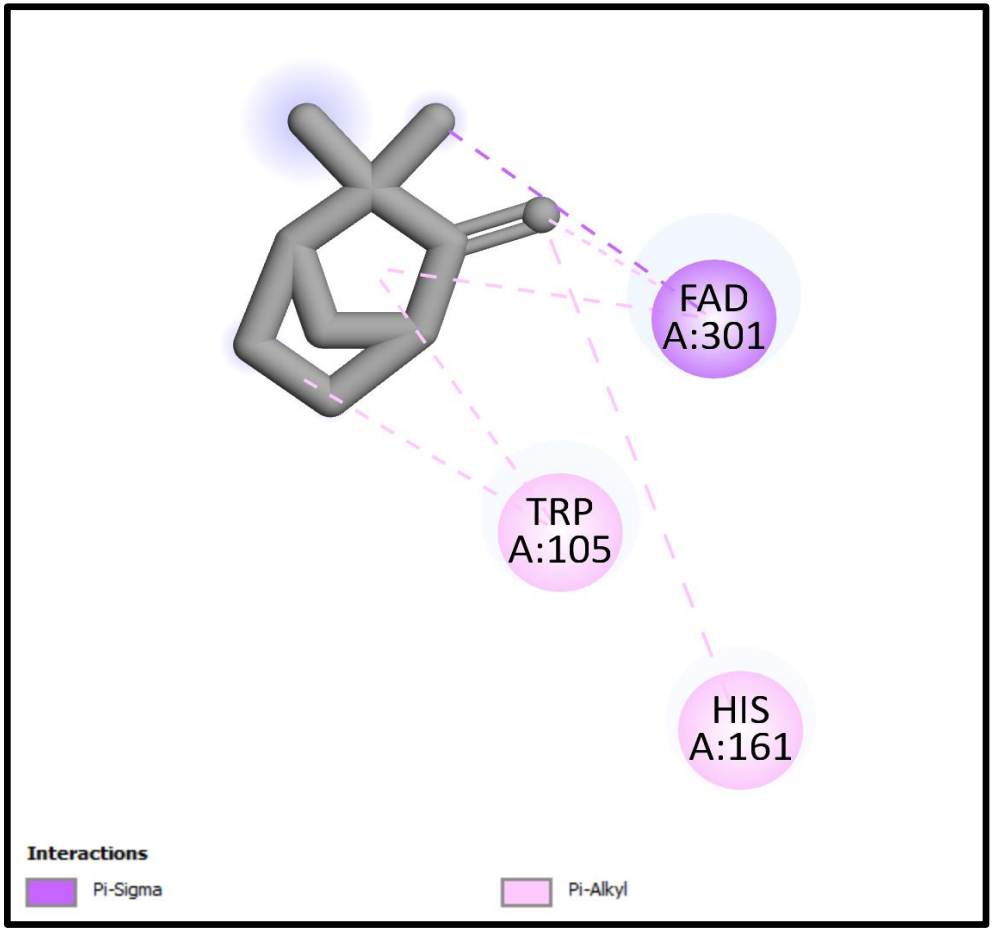


Figura 11 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Borneol(+)



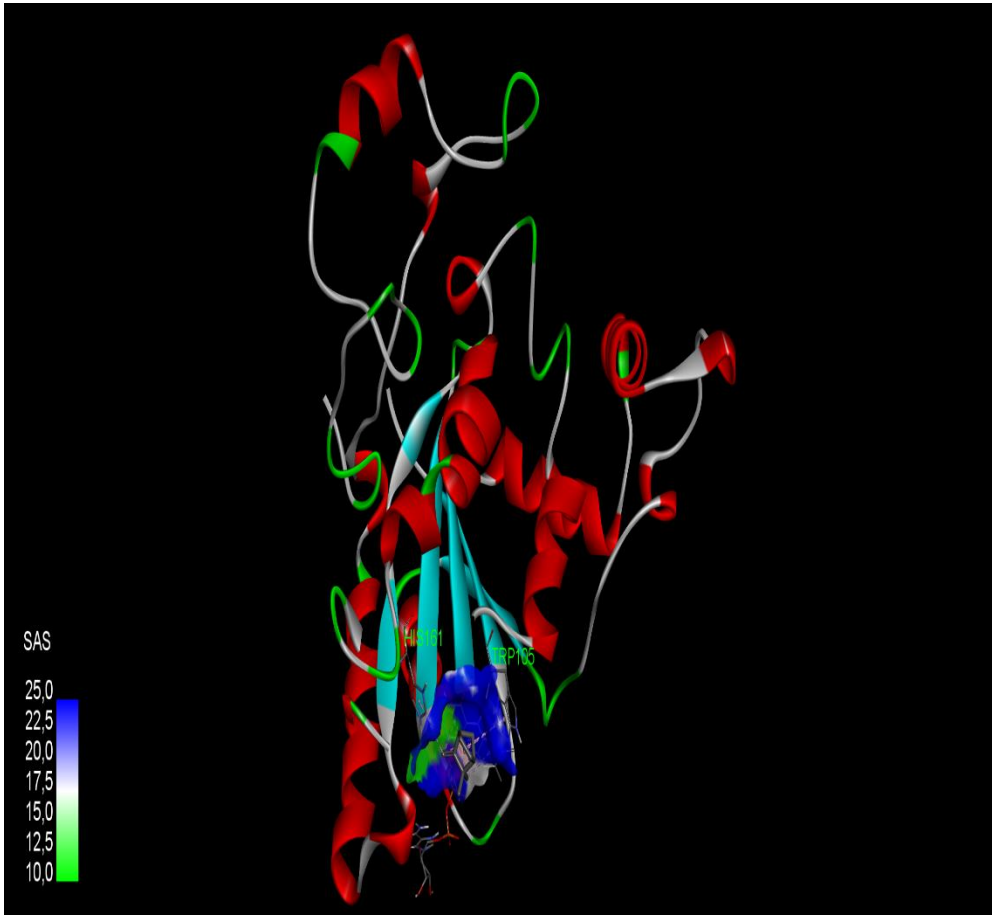
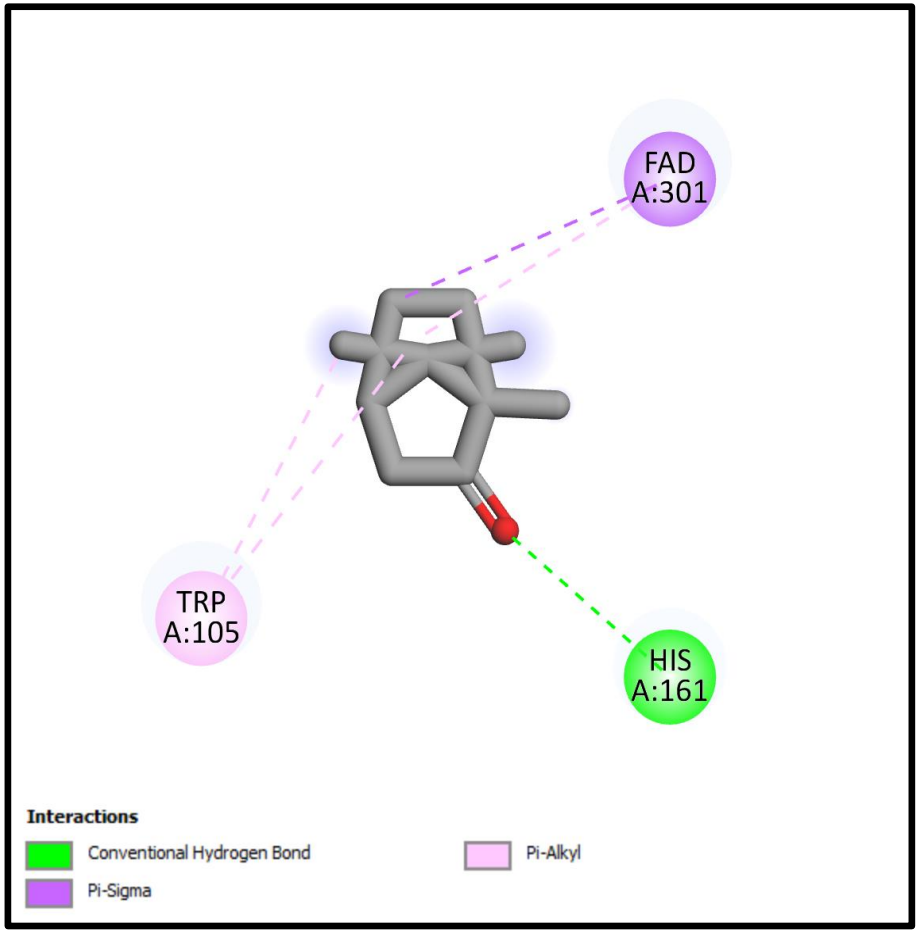


Figura 12 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Canfeno



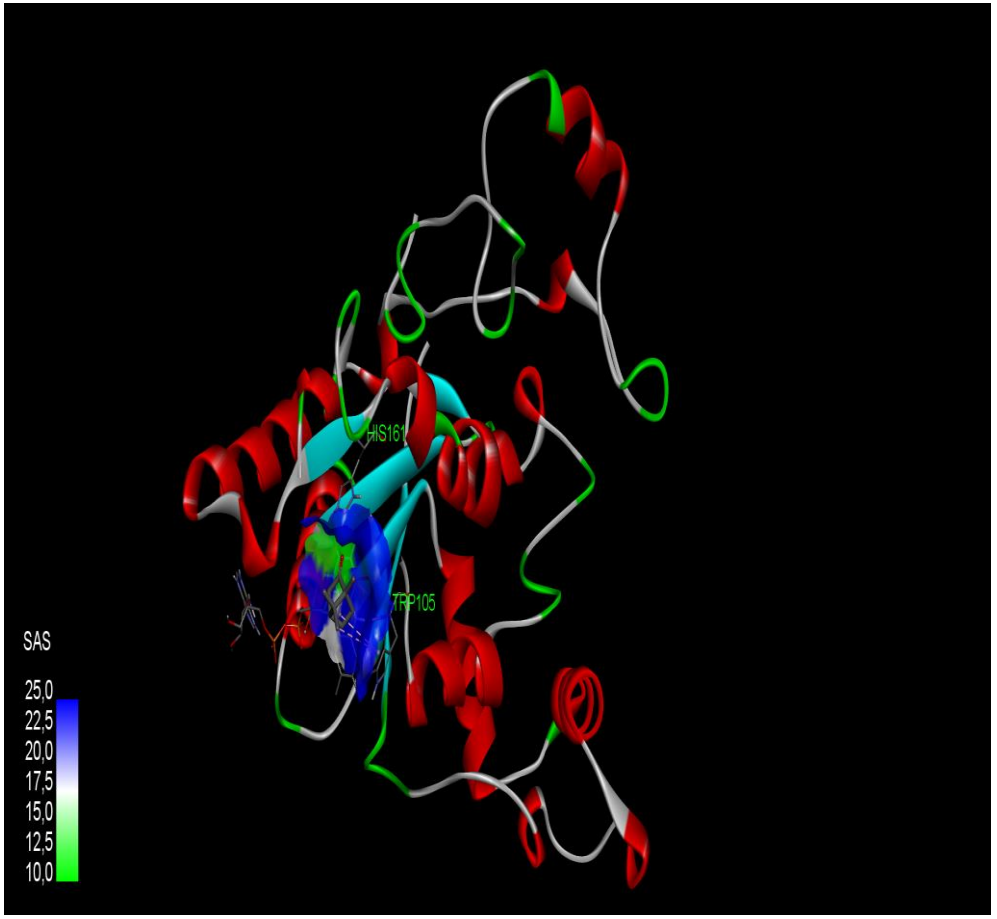
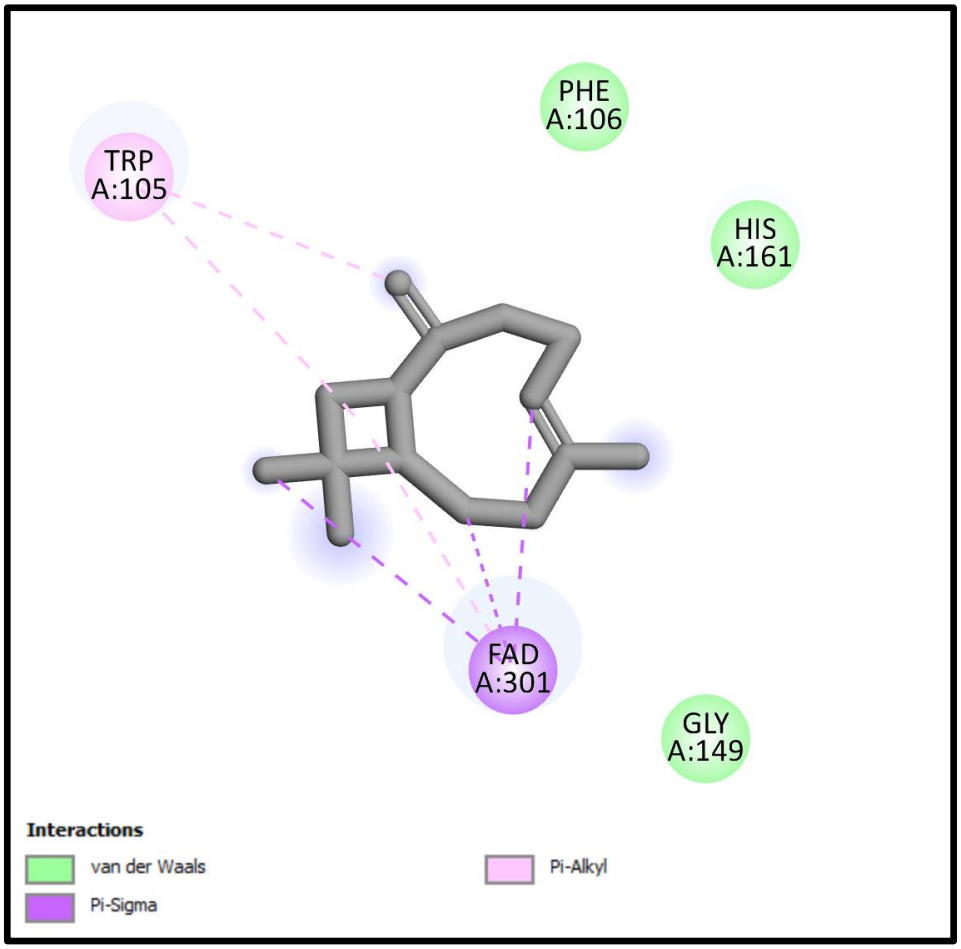


Figura 13 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Canfora



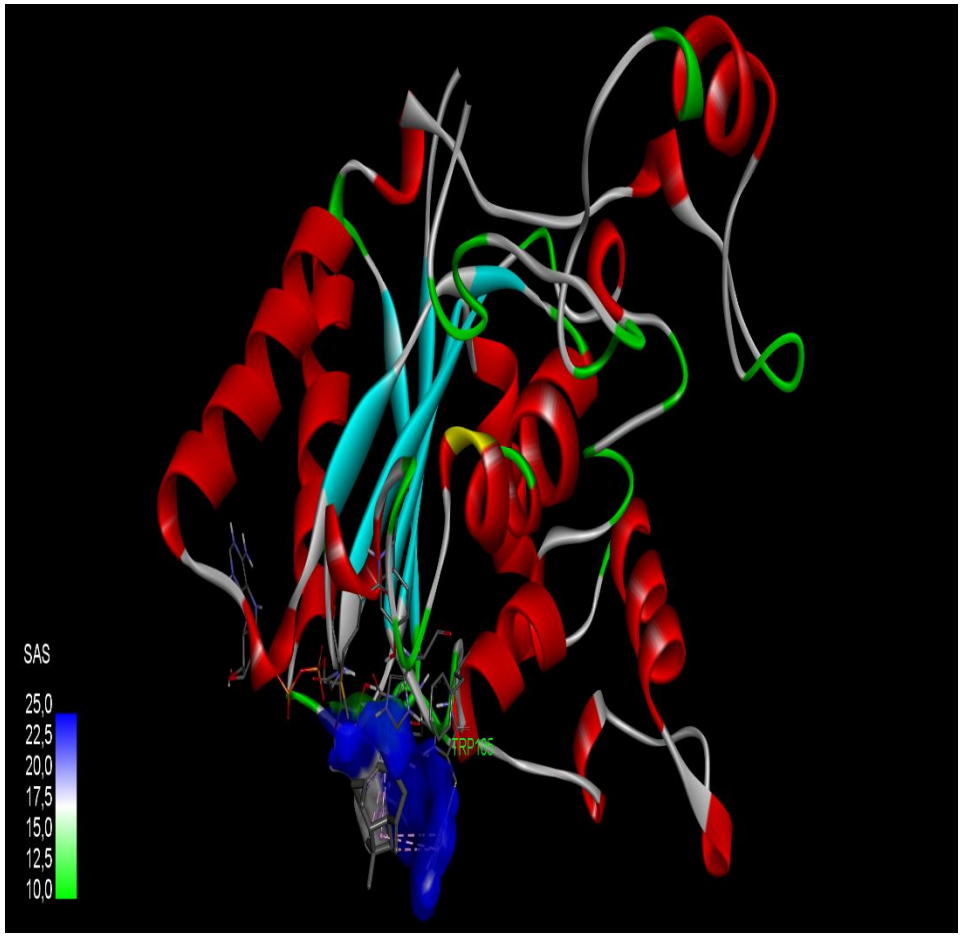
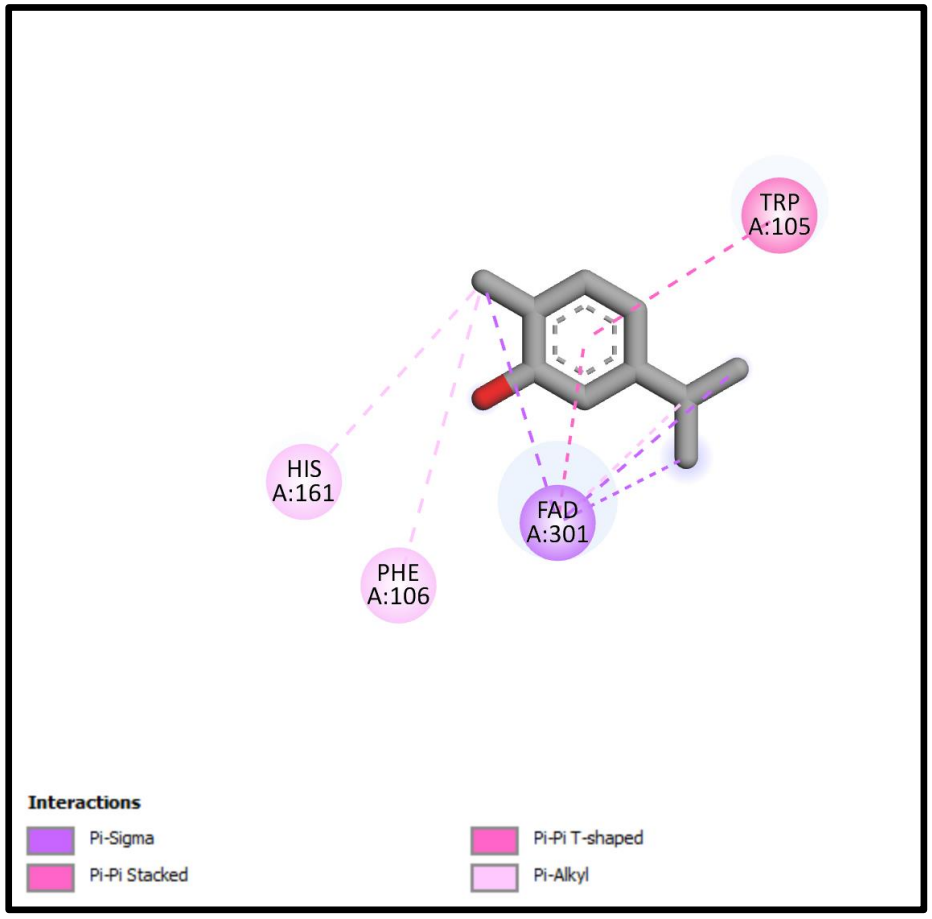


Figura 14 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Cariofileno (beta)



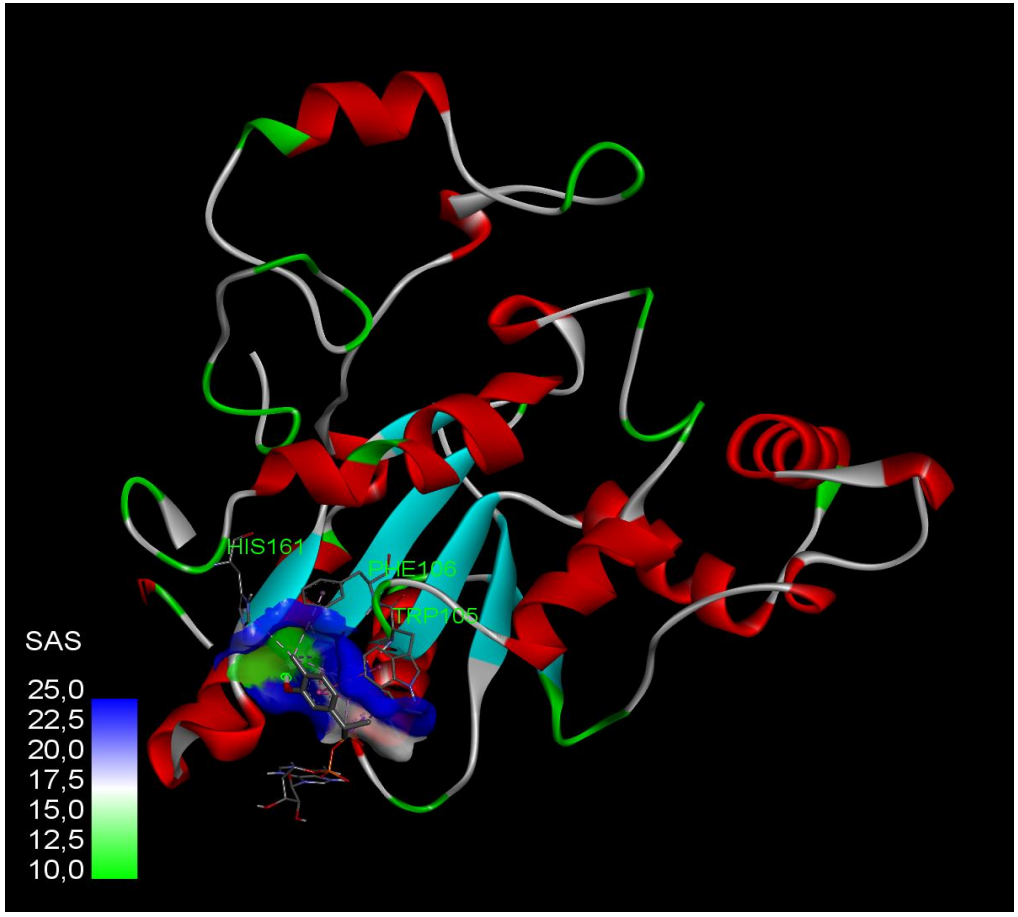
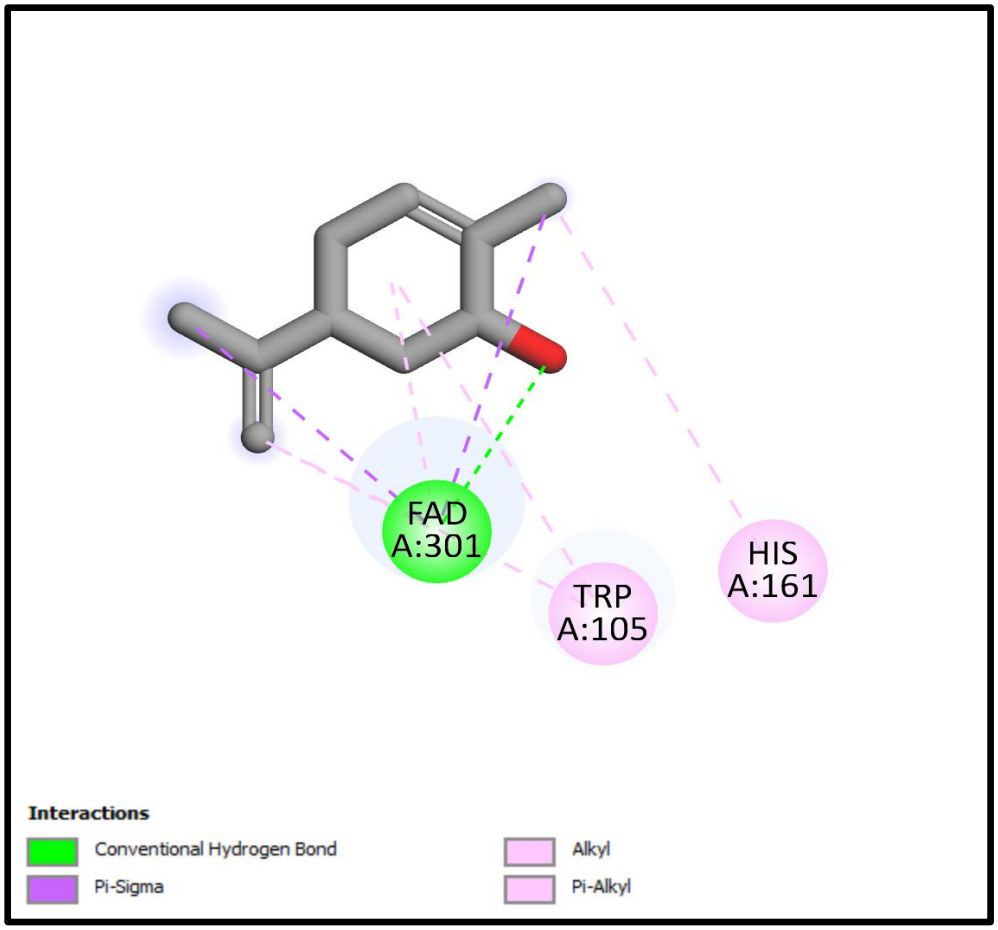


Figura 15 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Carvacrol



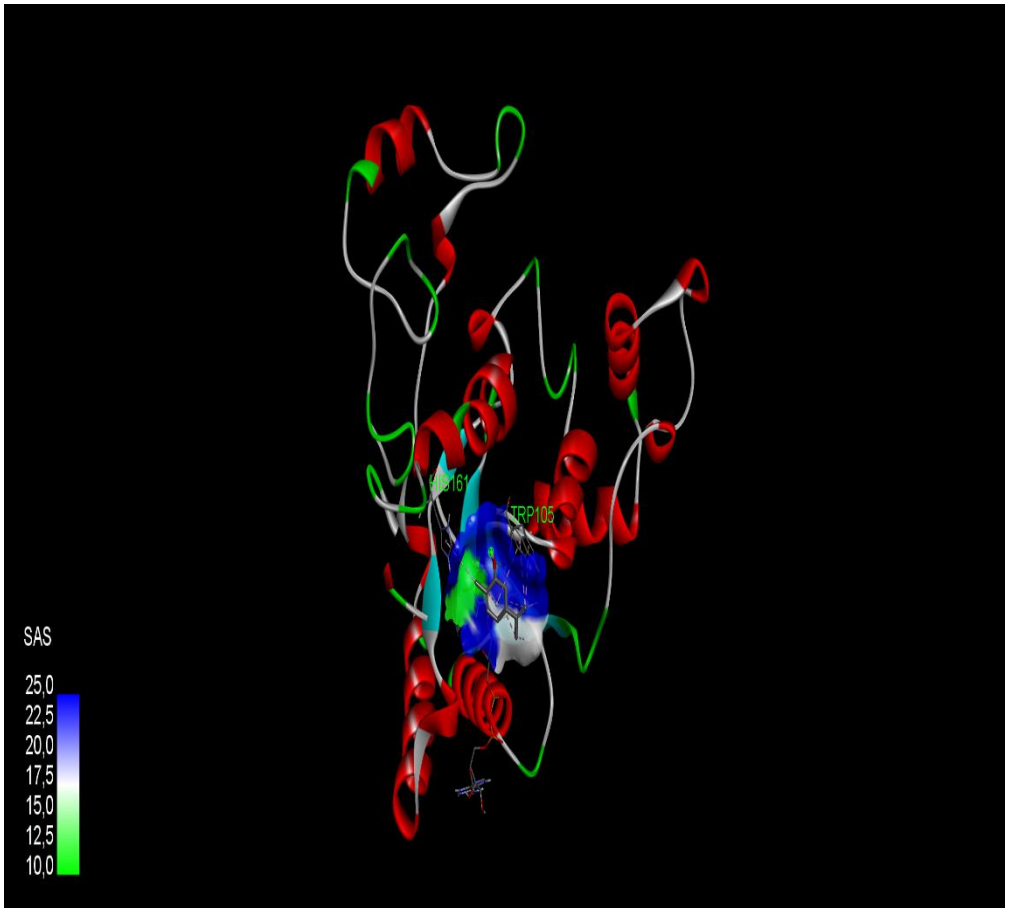
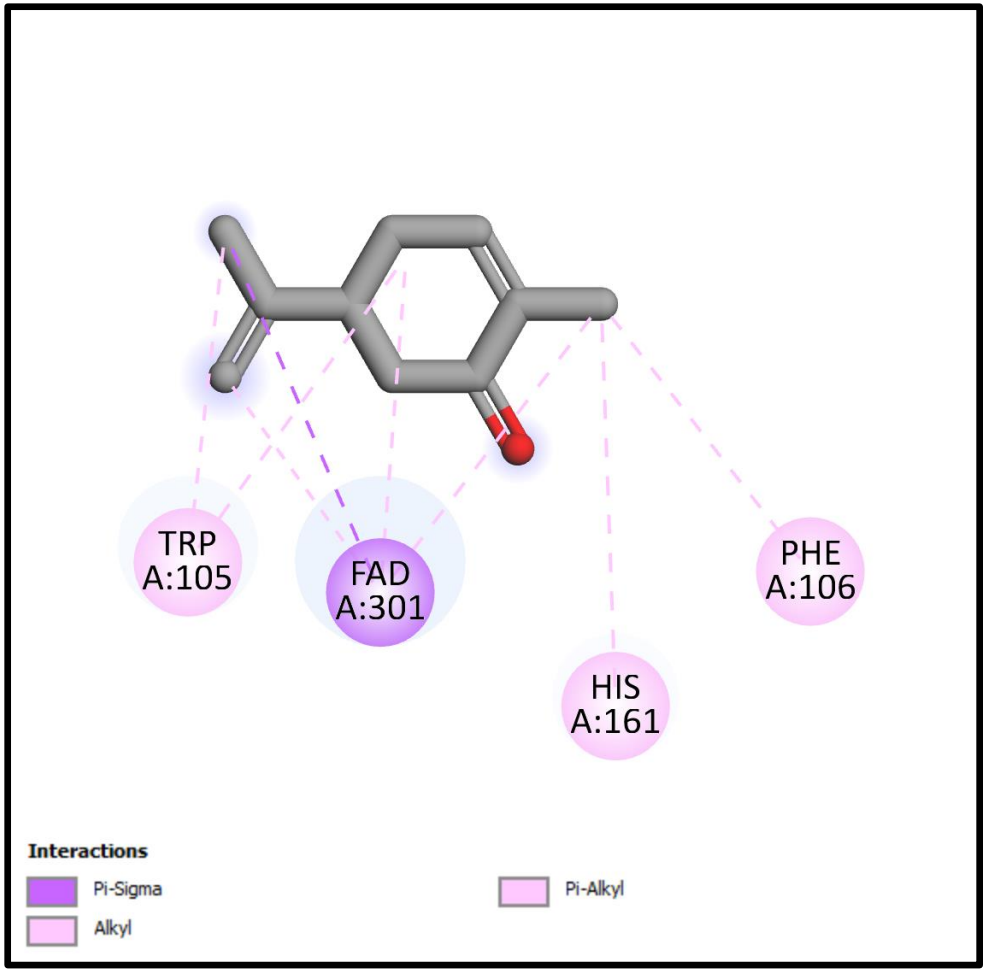


Figura 16 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Carveol (L)



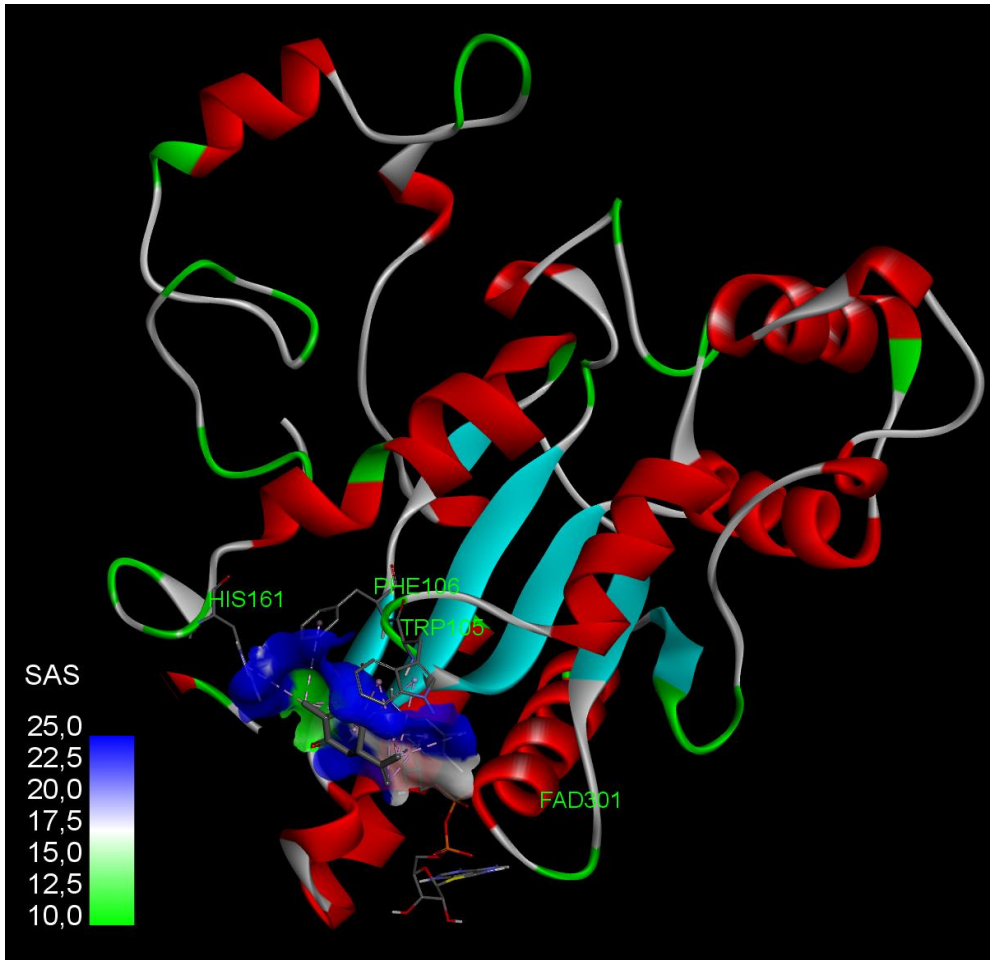


Figura 17 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Carvona(+)

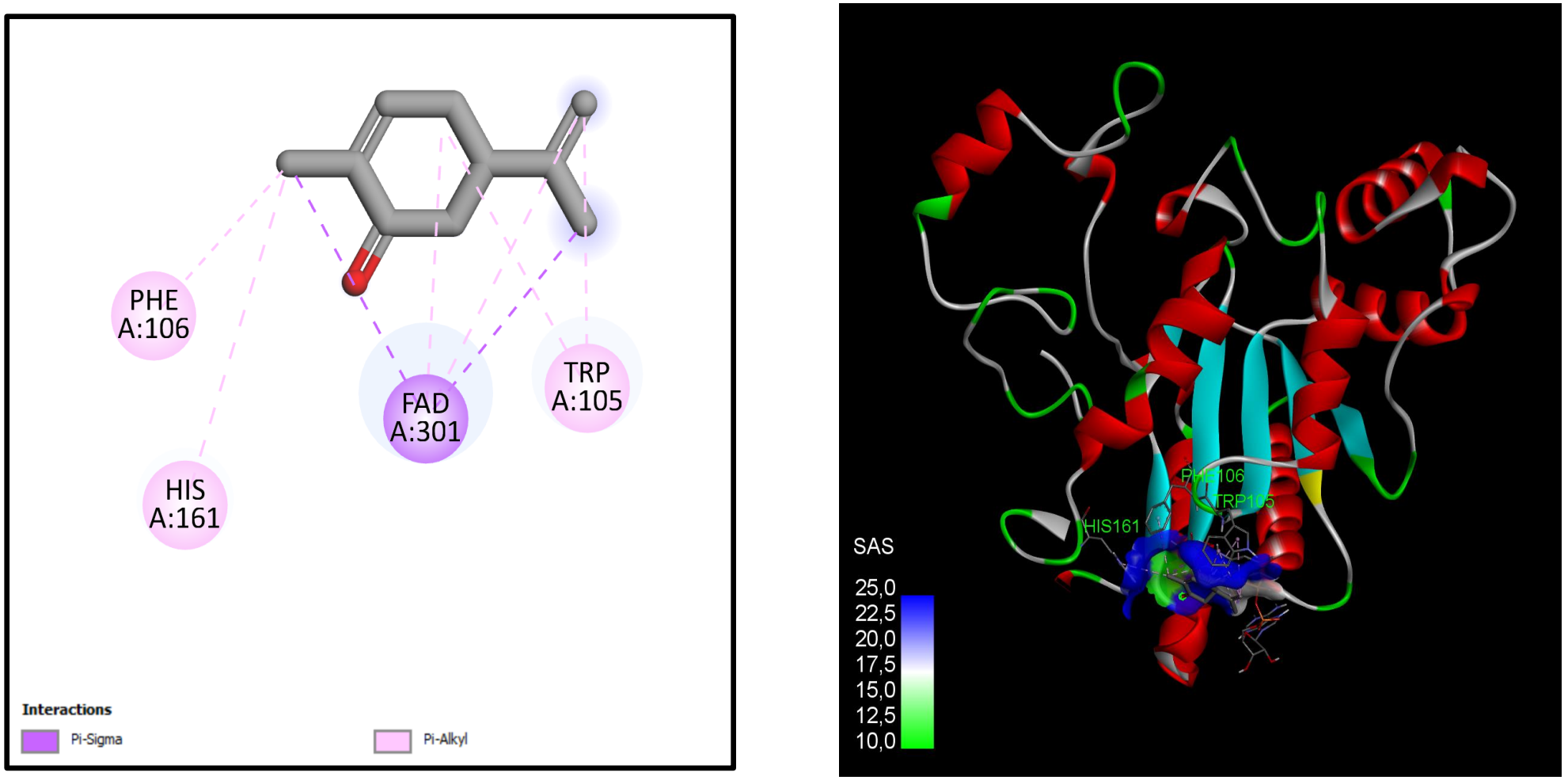


Figura 18 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Carvona(L)

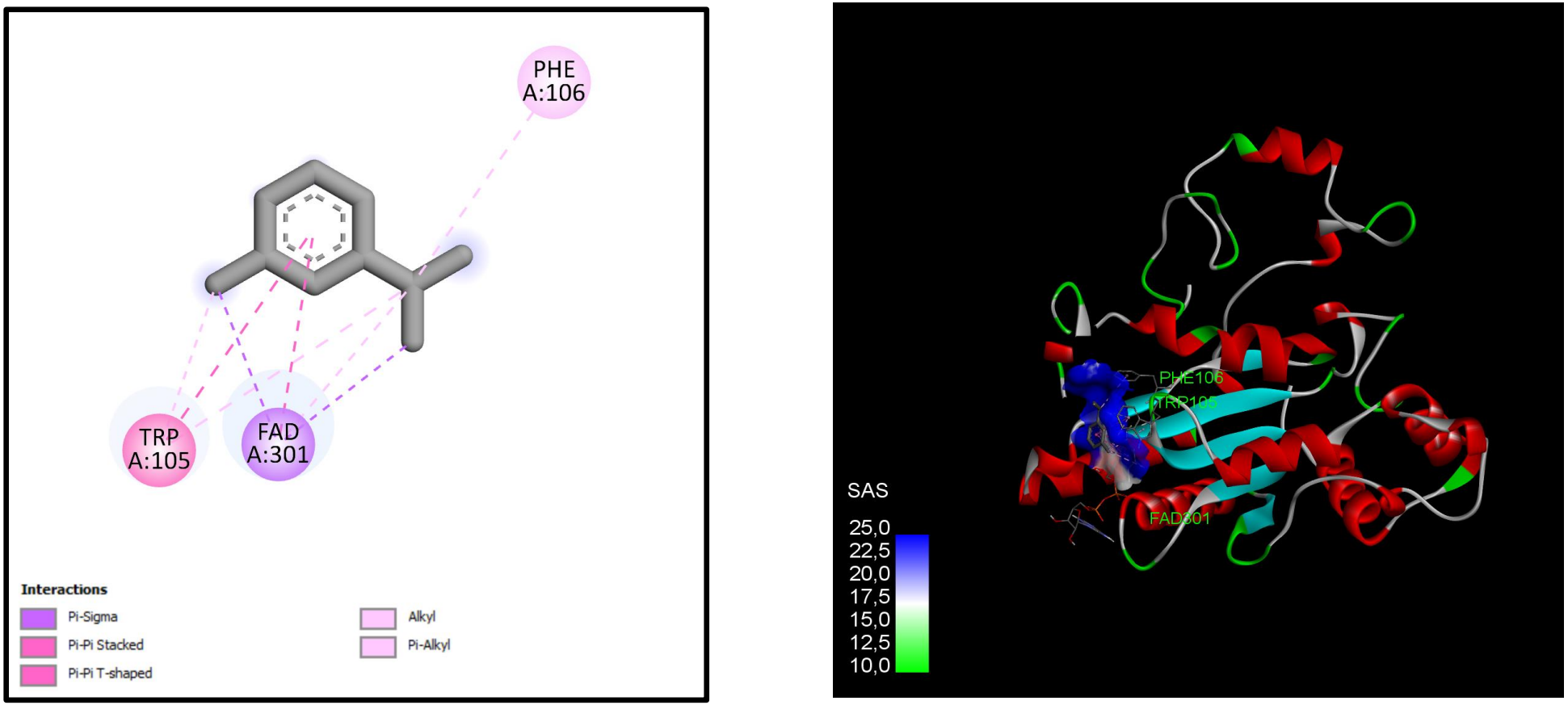
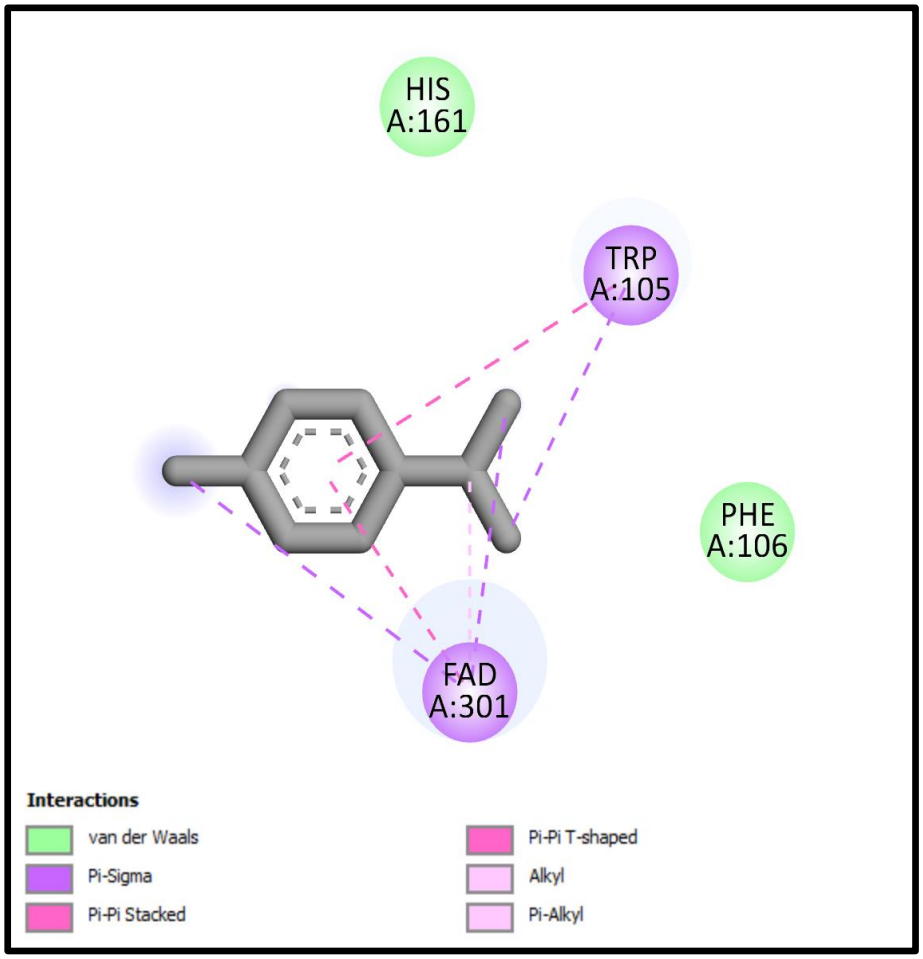


Figura 19 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Cimeno (m)



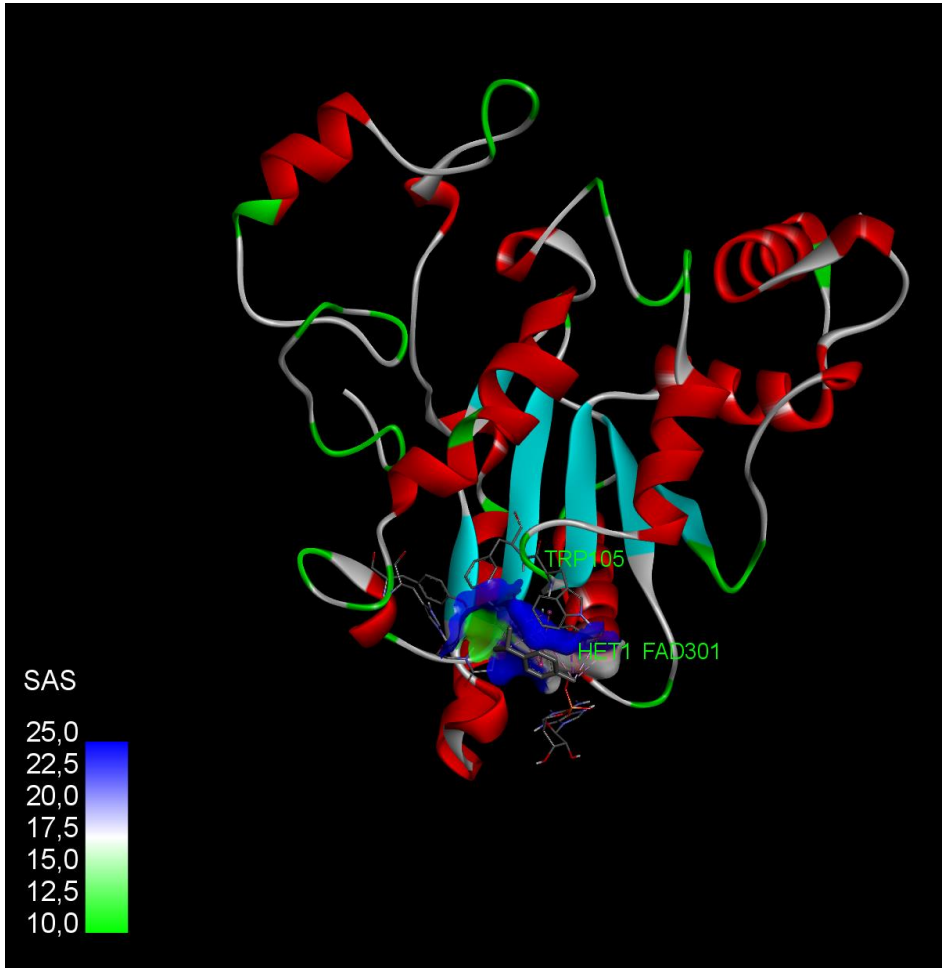
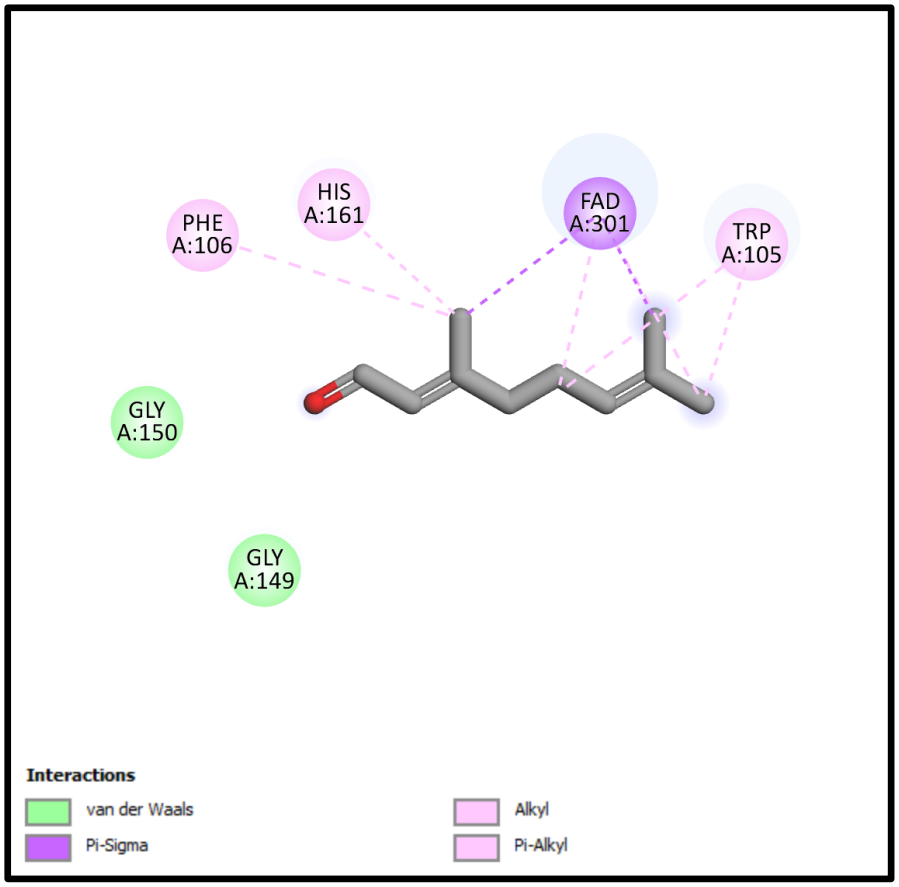


Figura 20 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Cimeno (p)



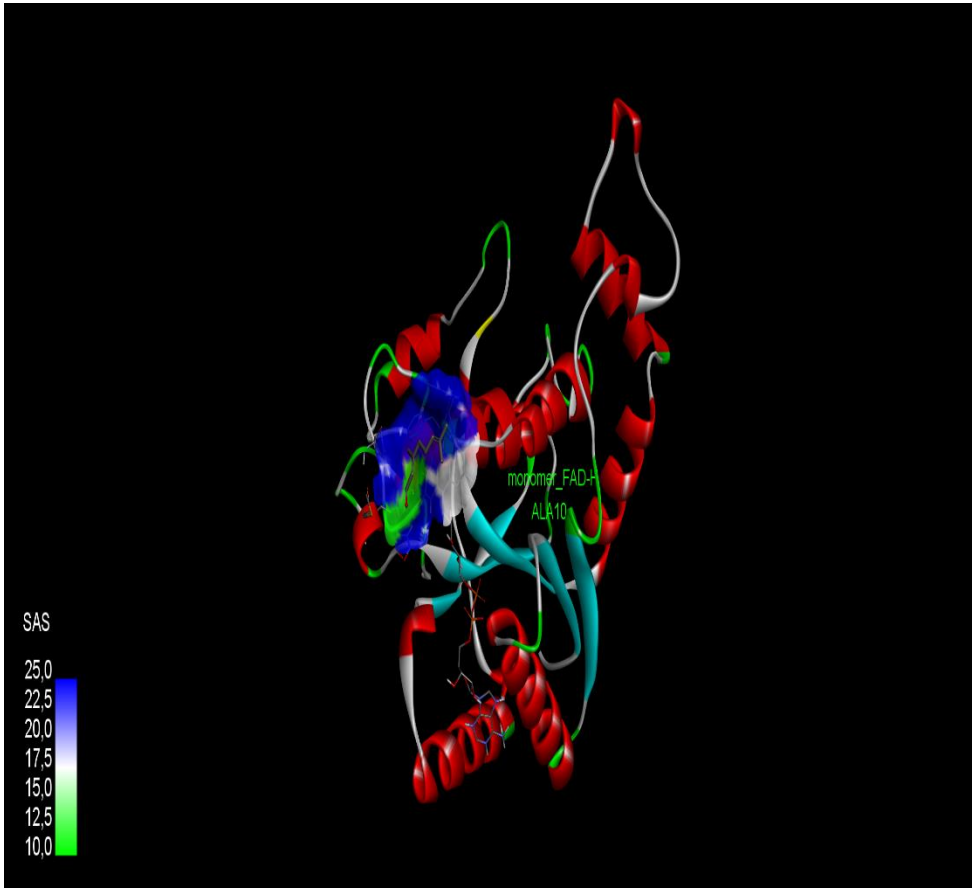
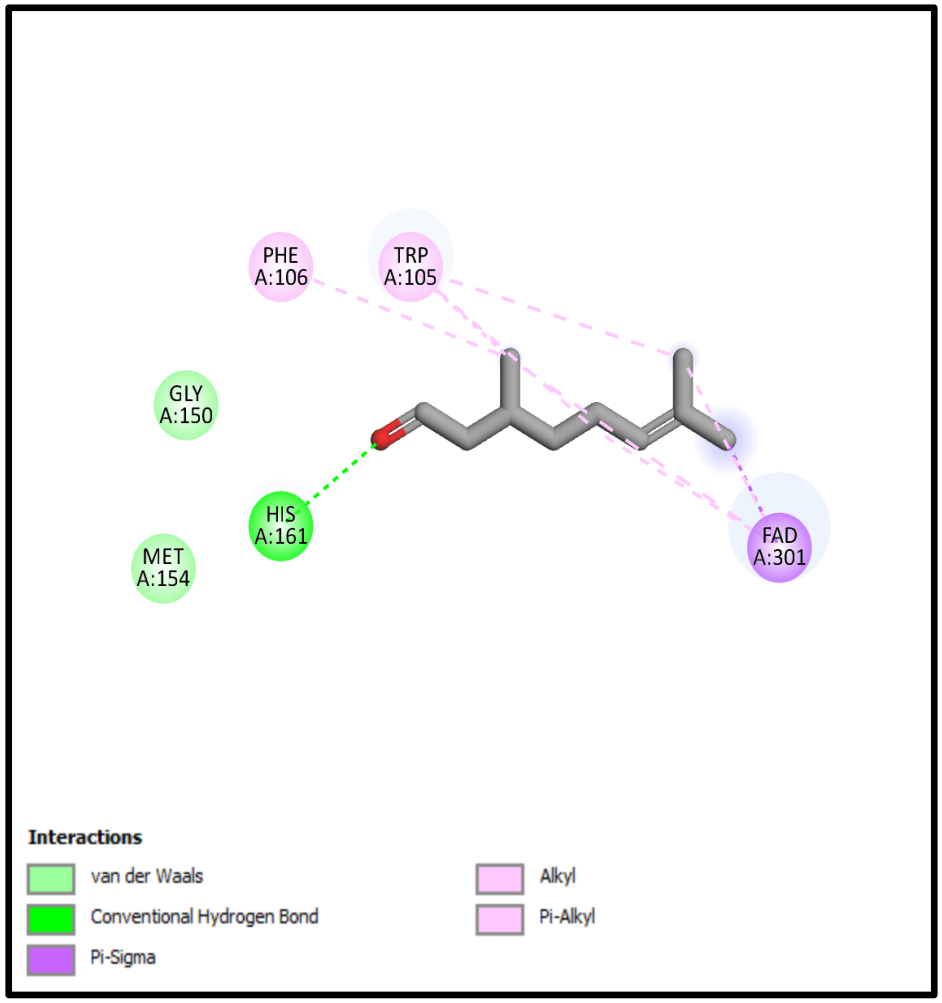


Figura 21 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Citral



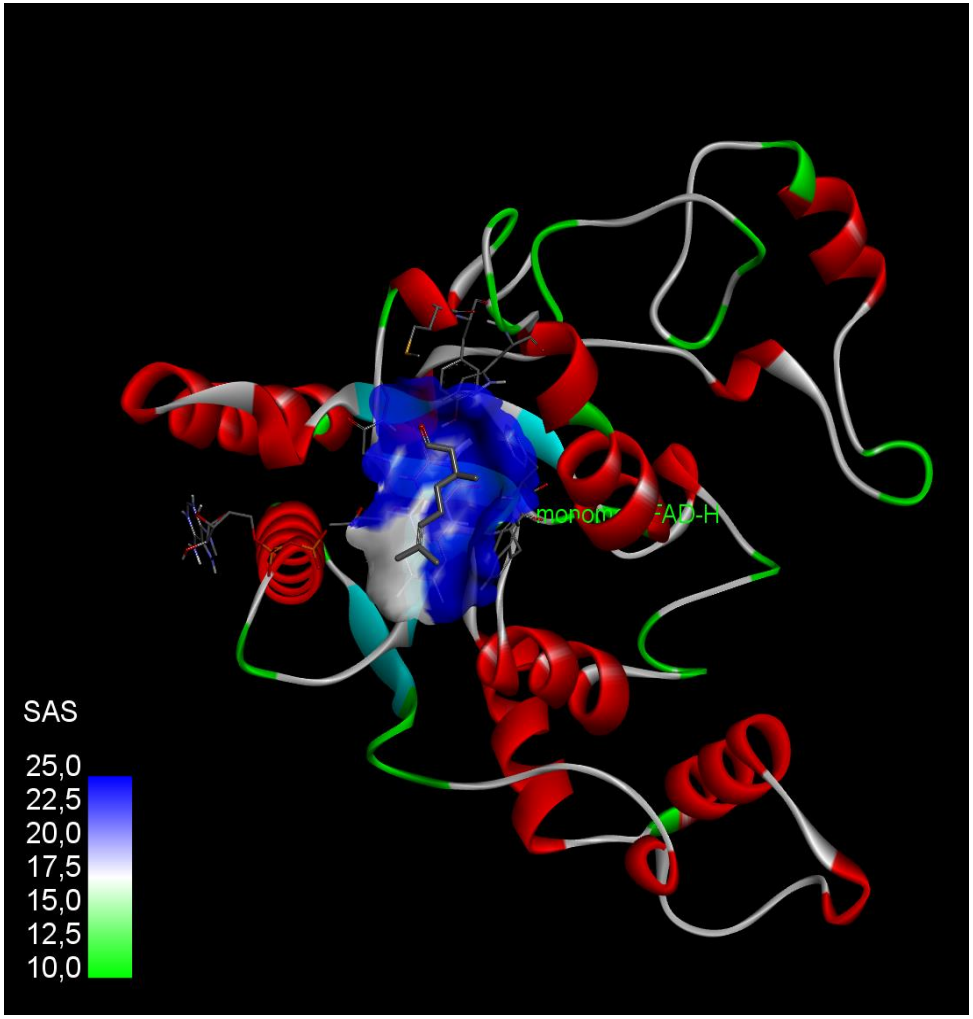
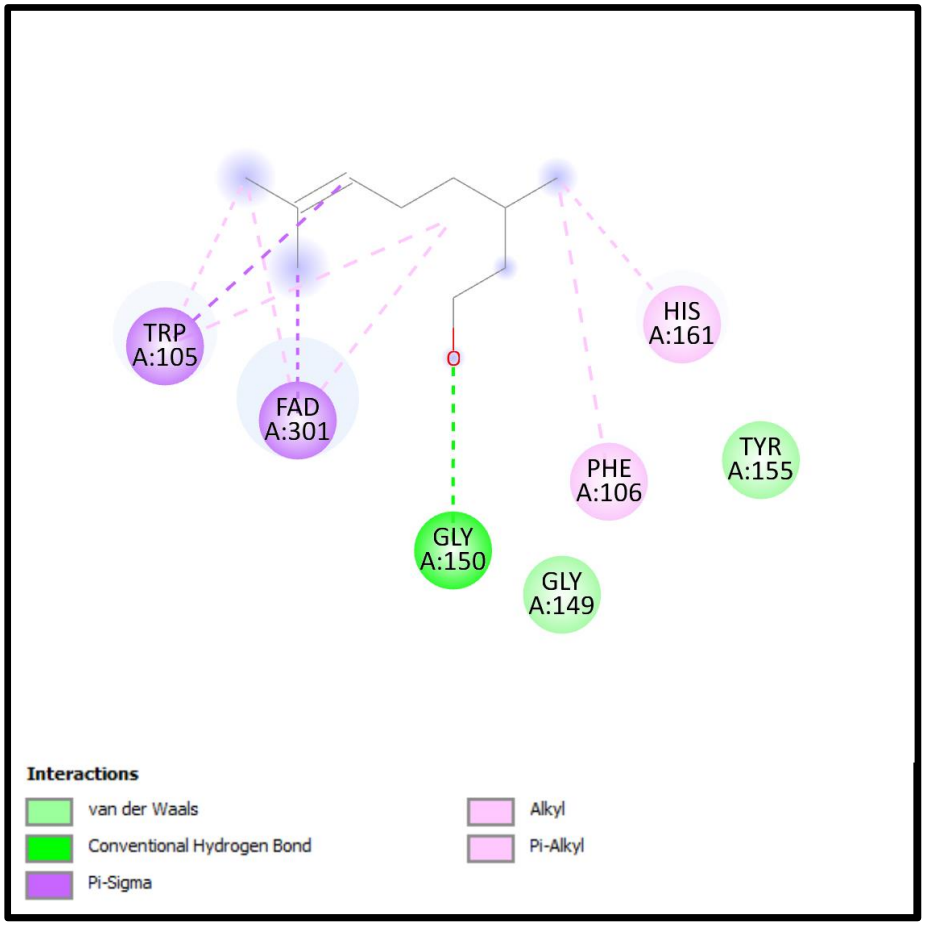


Figura 22 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Citronelal(±)



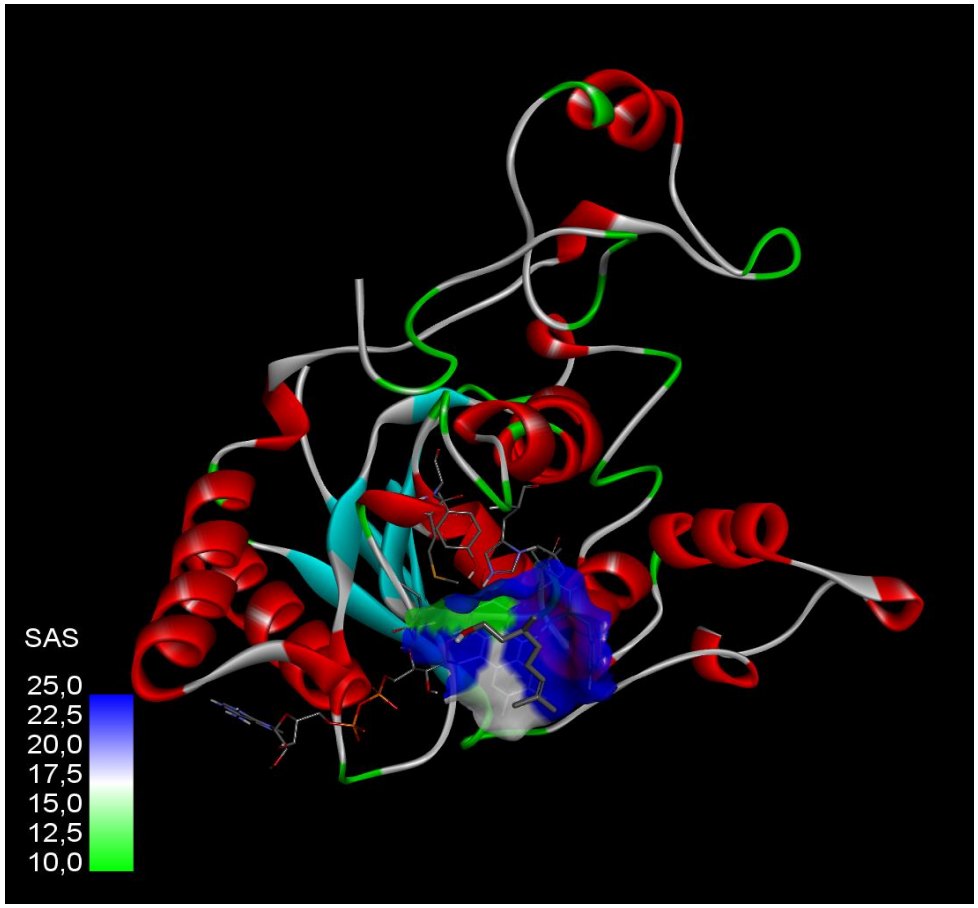
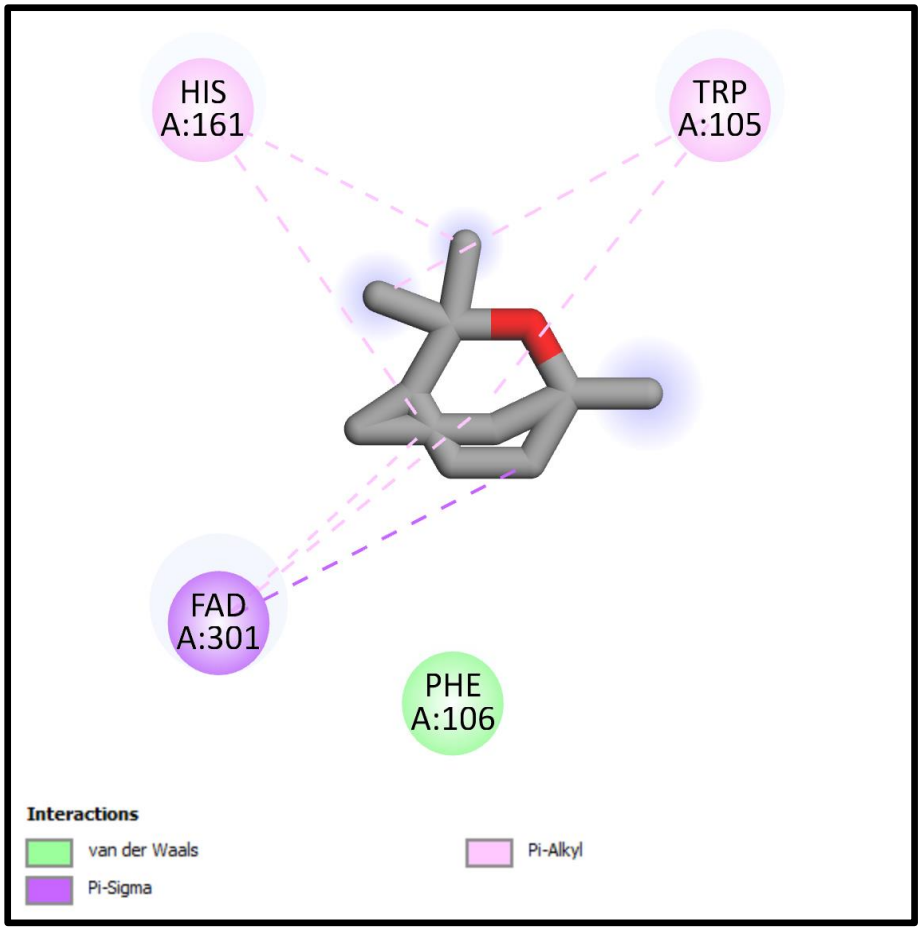


Figura 23 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Citronelol (beta)



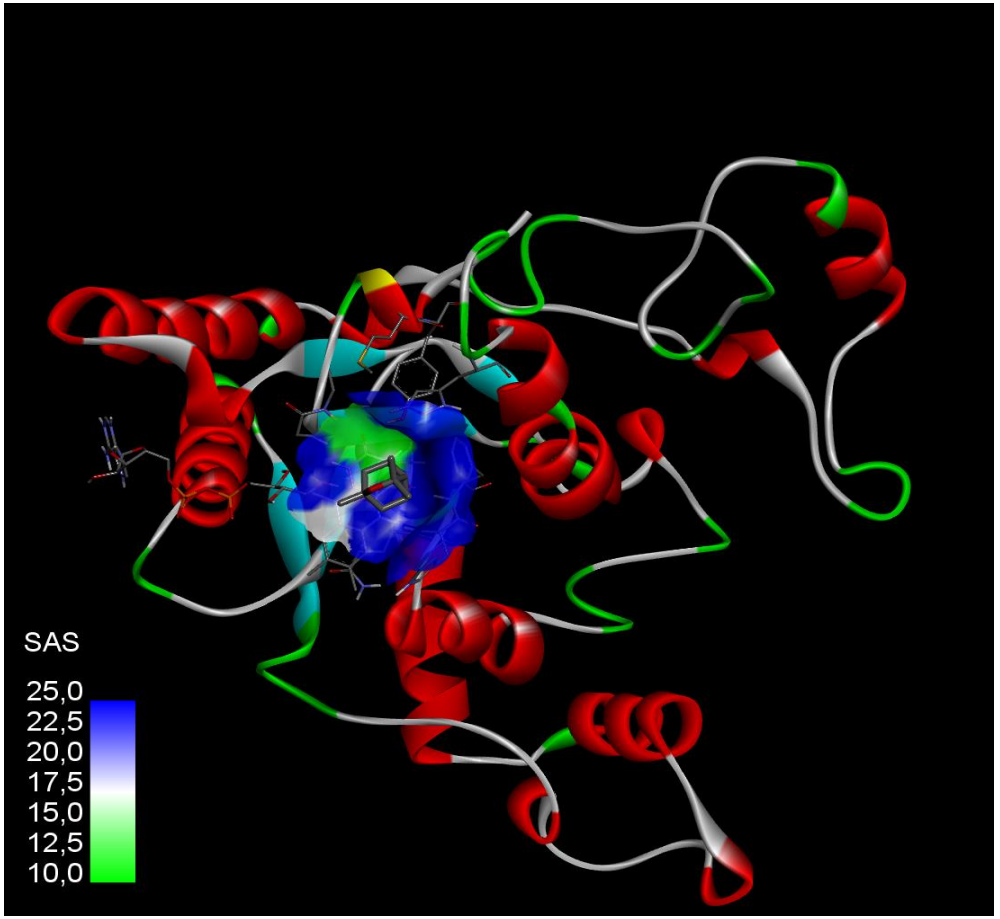


Figura 24 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Eucaliptol

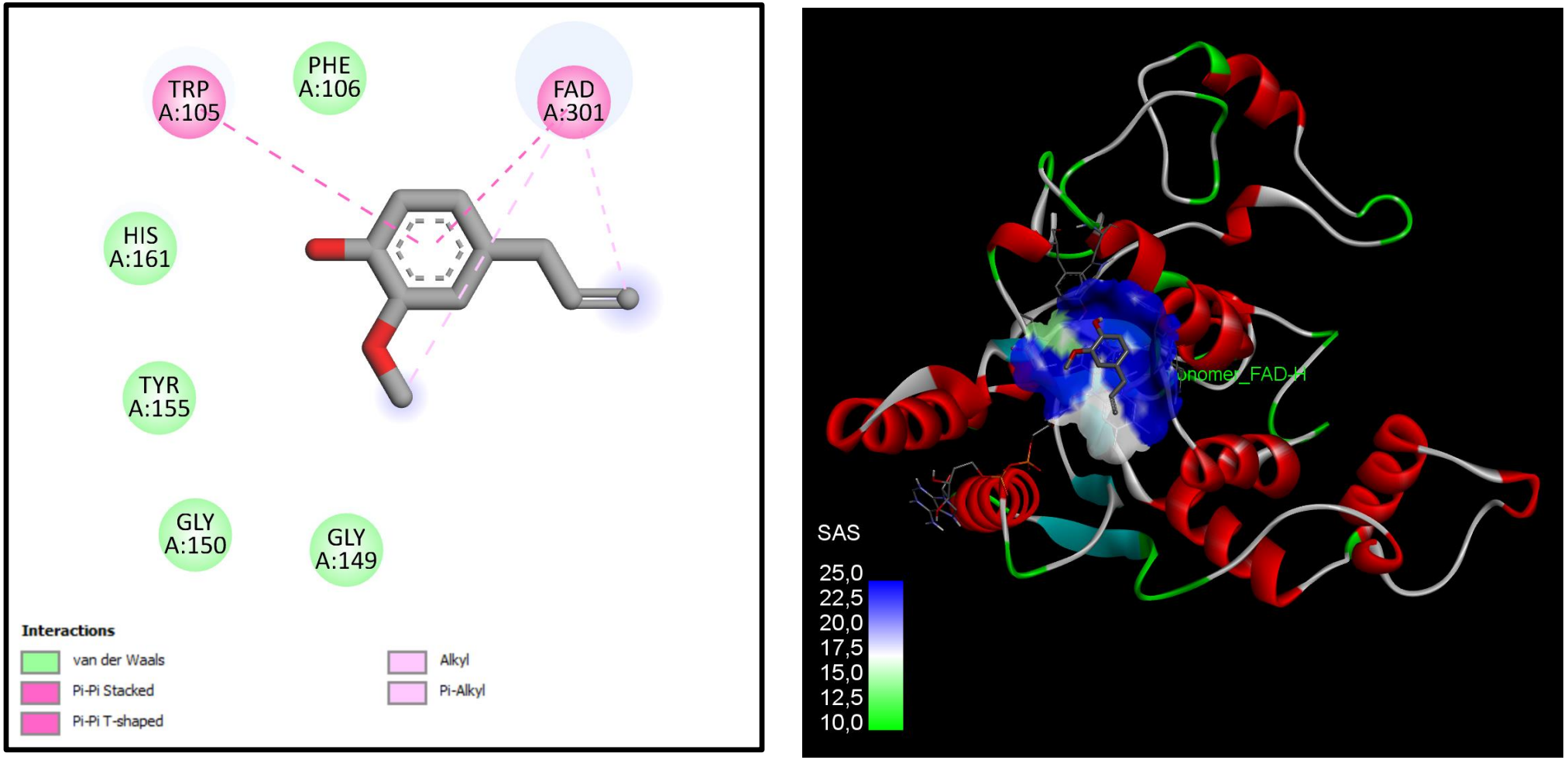
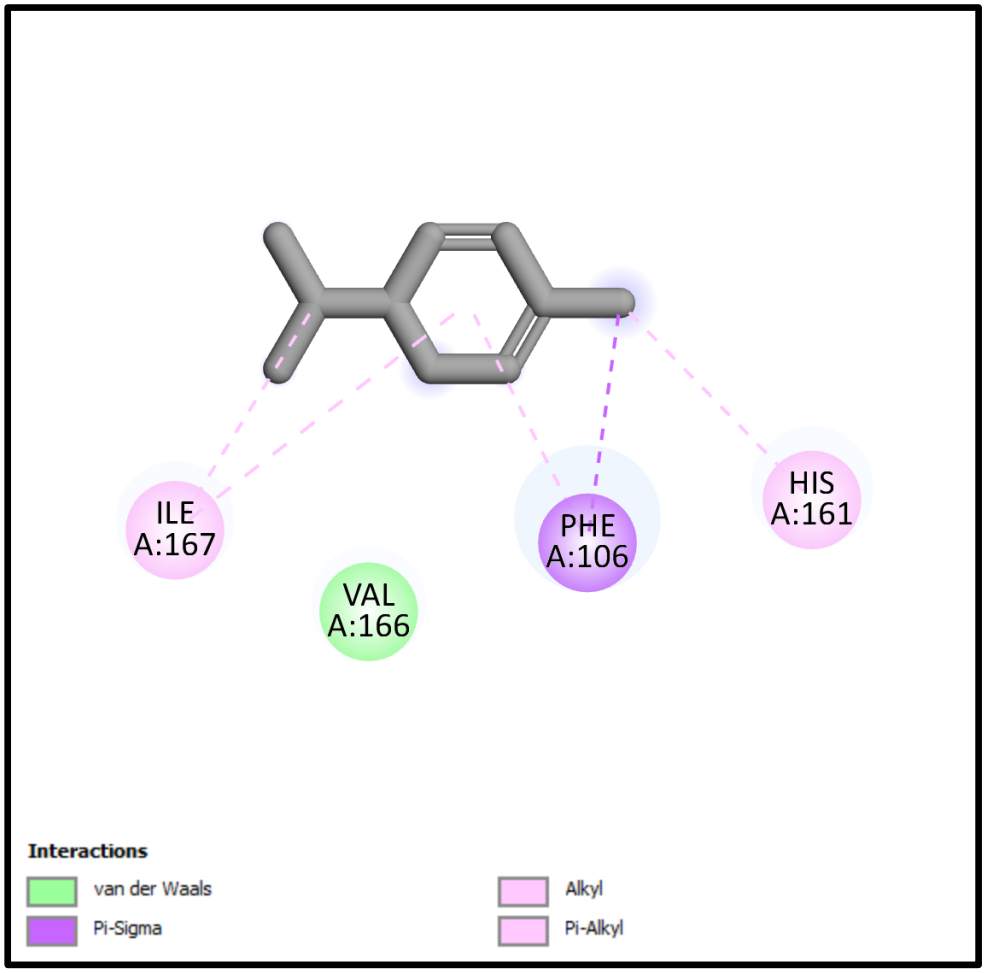


Figura 25 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Eugenol



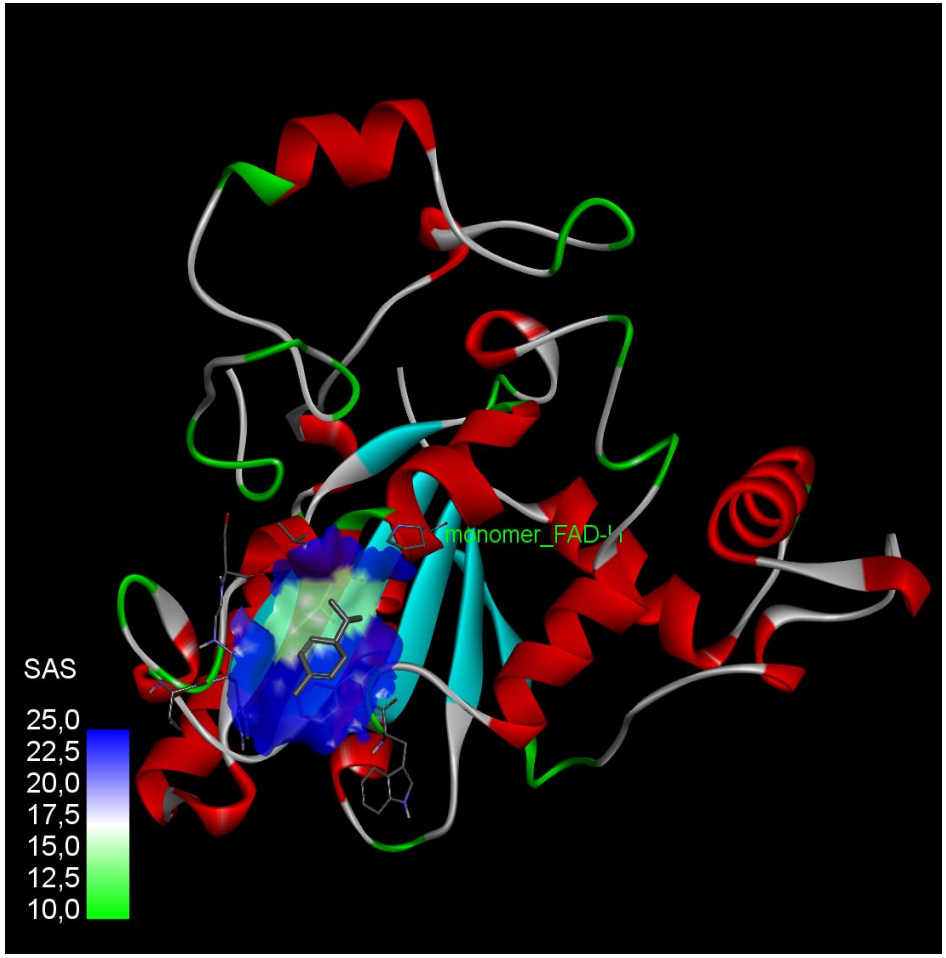
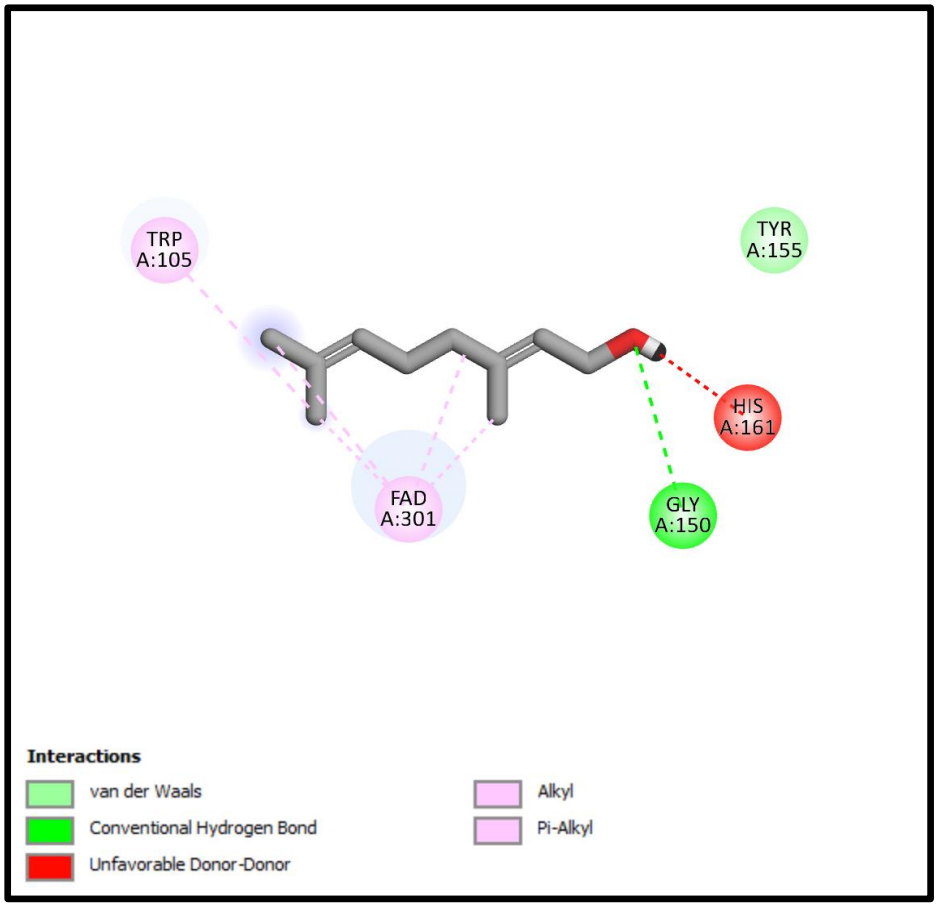


Figura 26 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Felandreno (alfa)



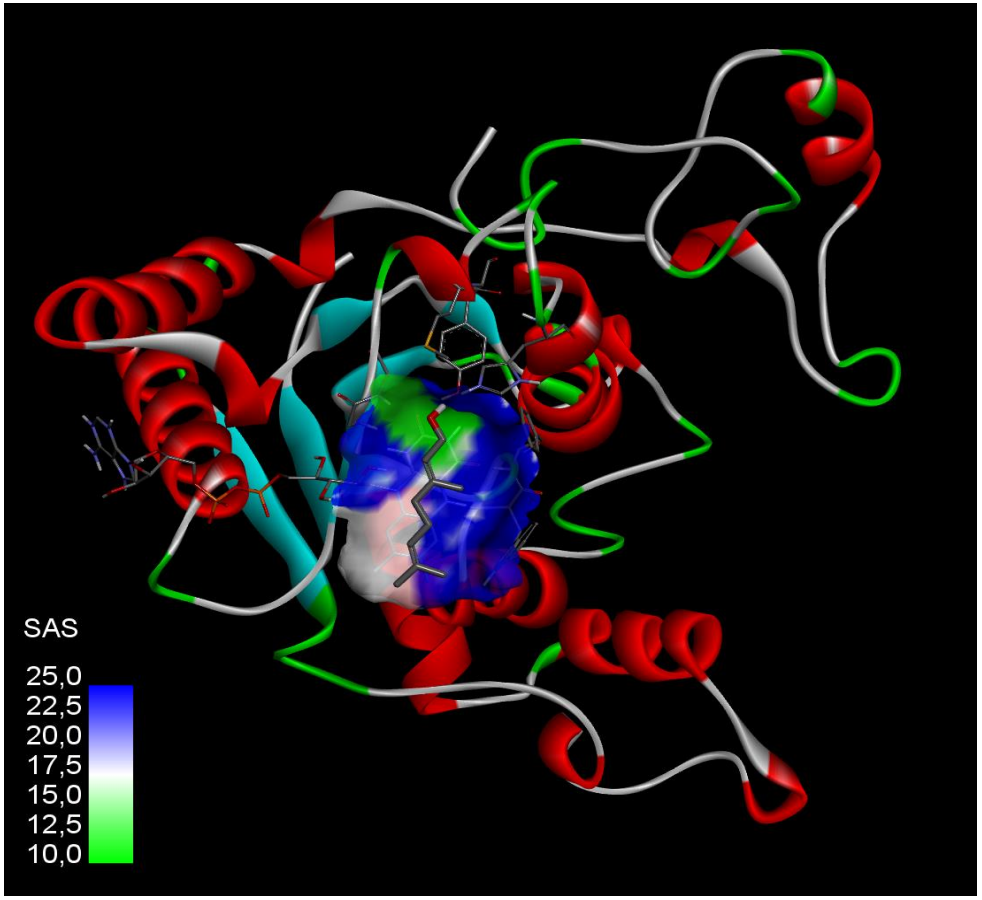
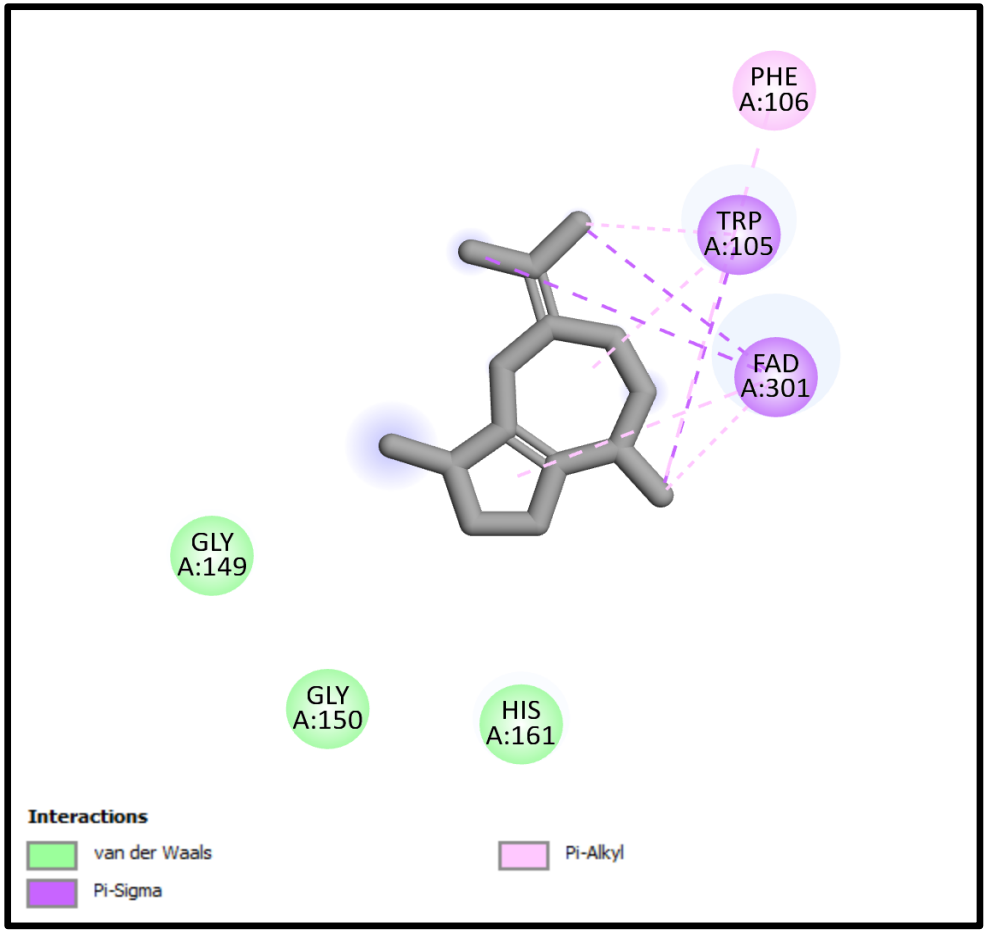


Figura 27 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Geraniol



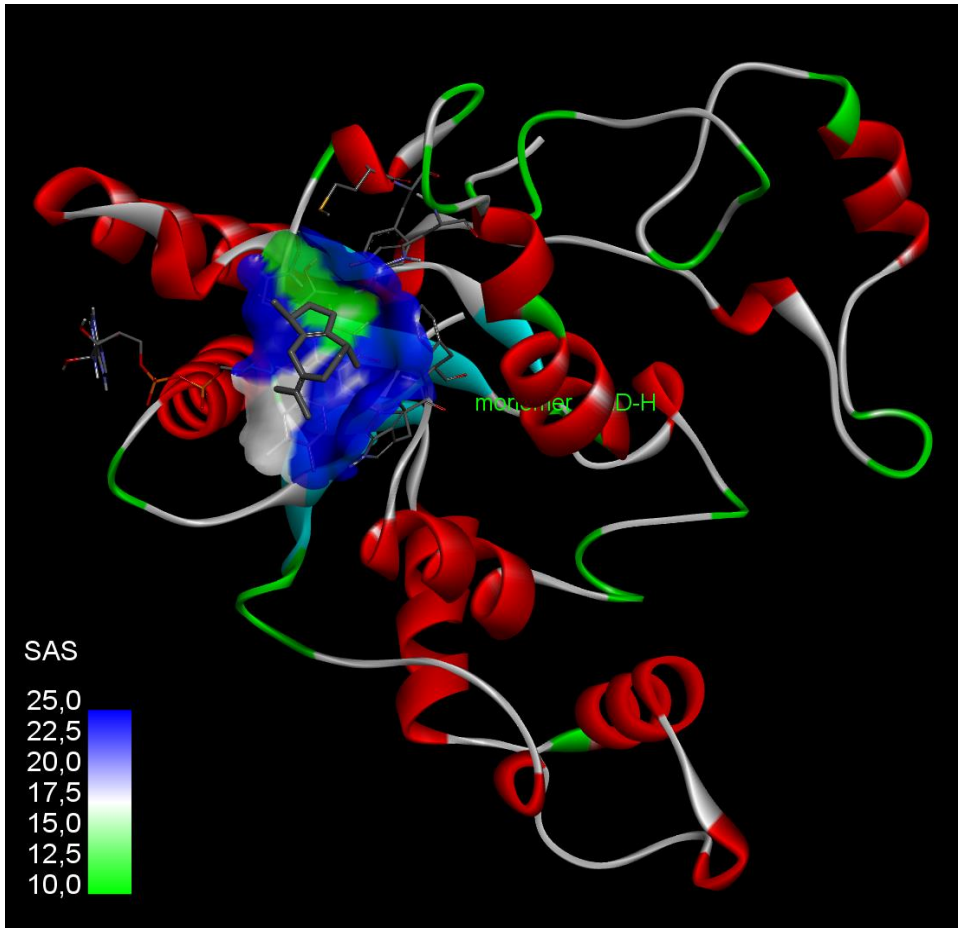


Figura 28 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Guaieno

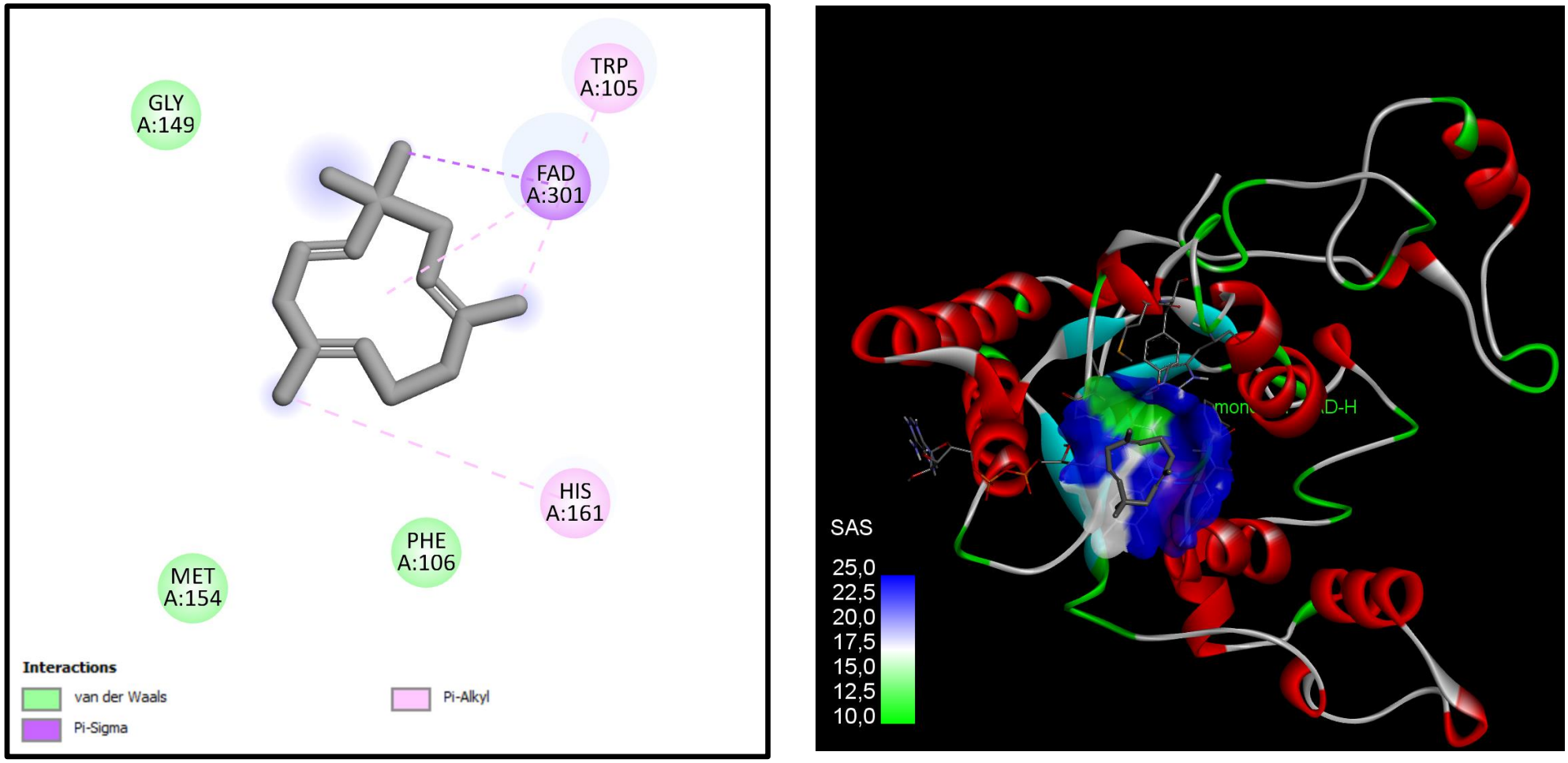


Figura 29 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Humuleno (alfa)

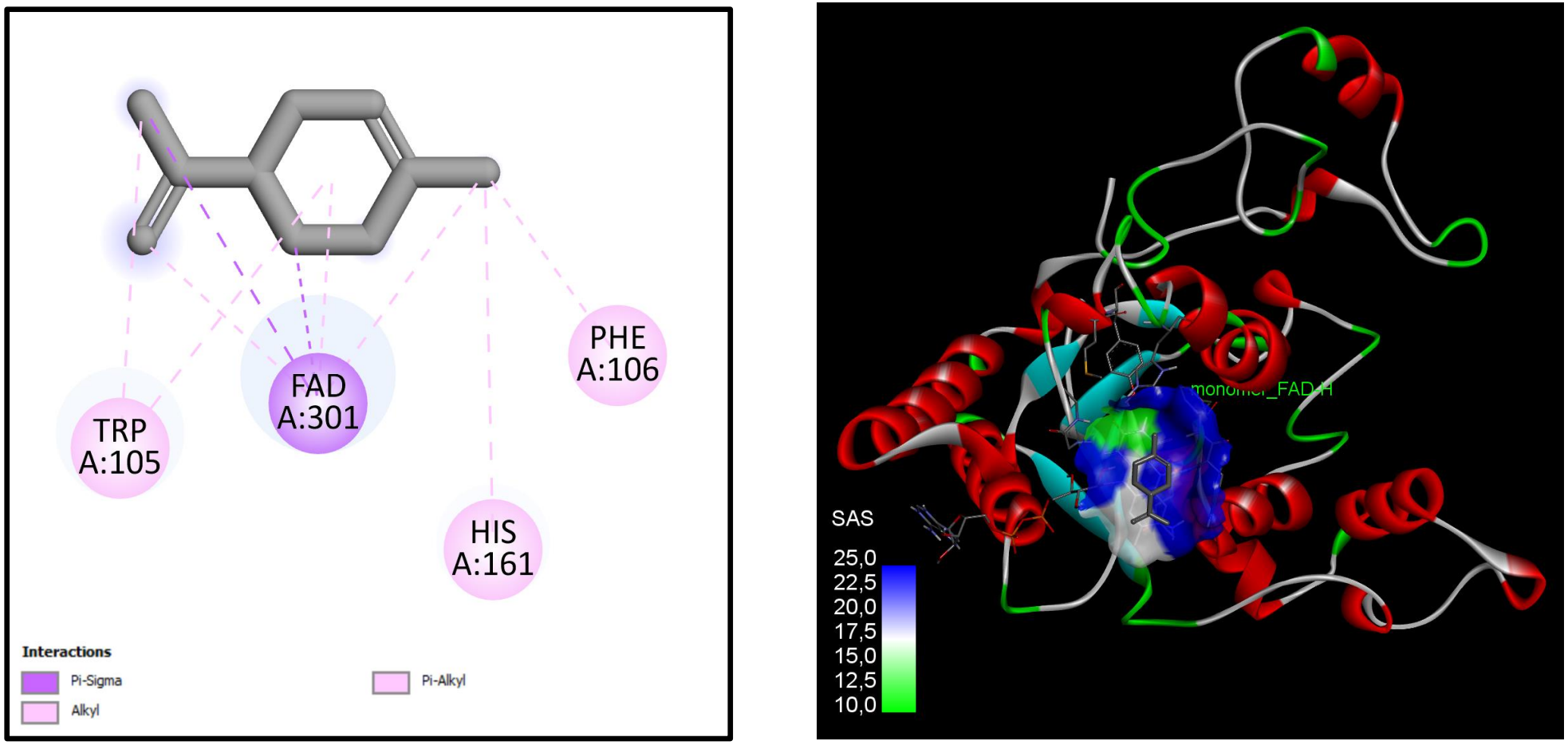


Figura 30 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Limoneno (R)

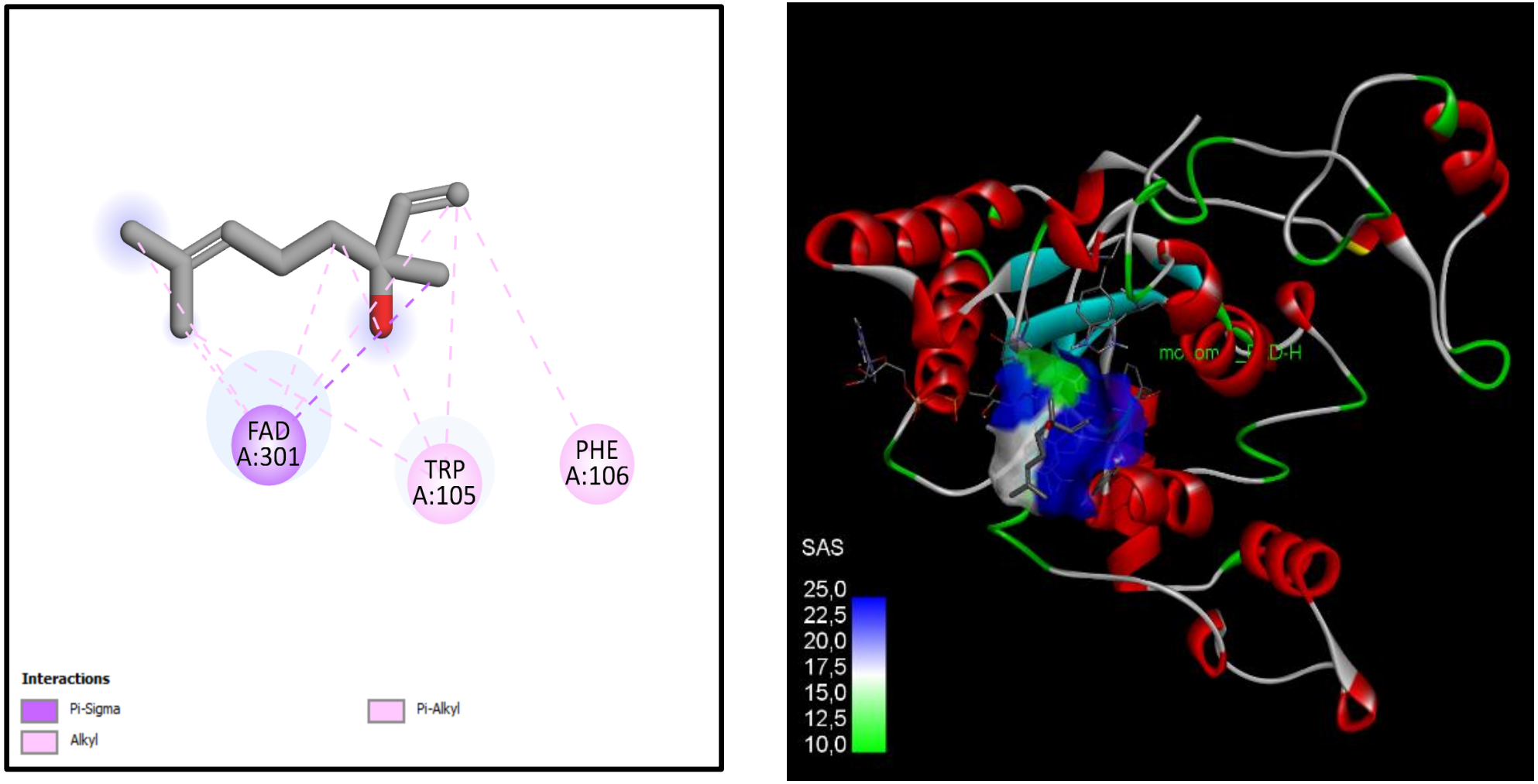
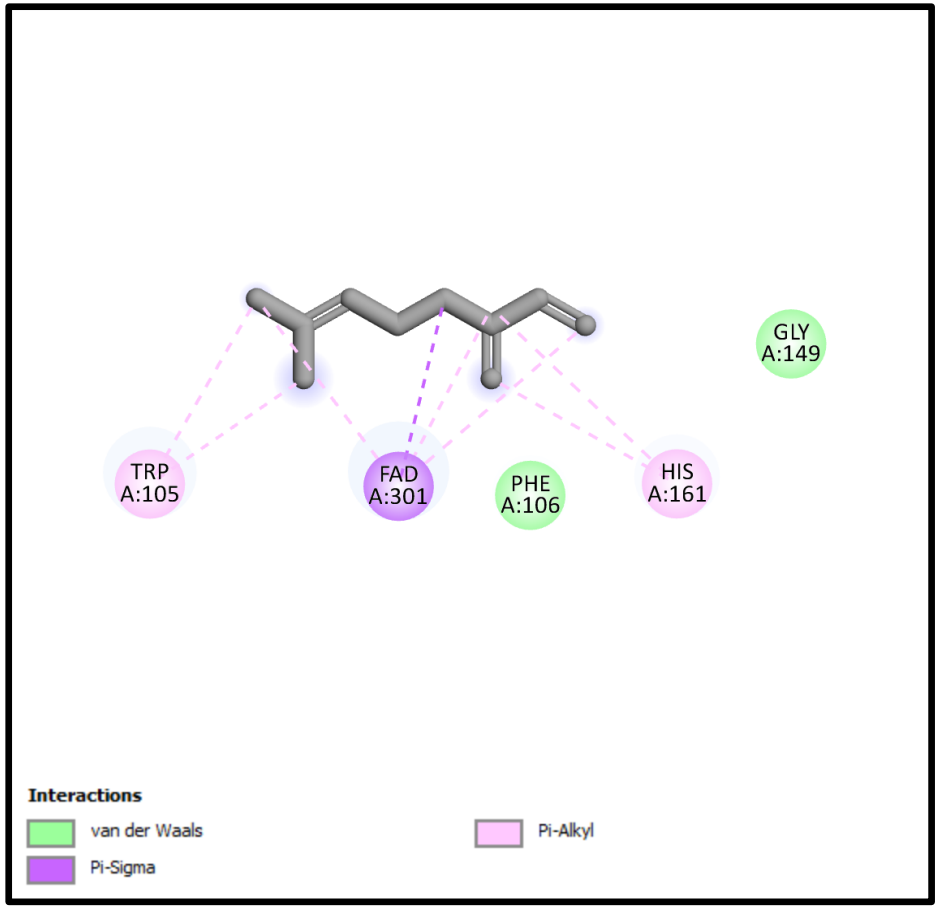


Figura 31 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Linalol



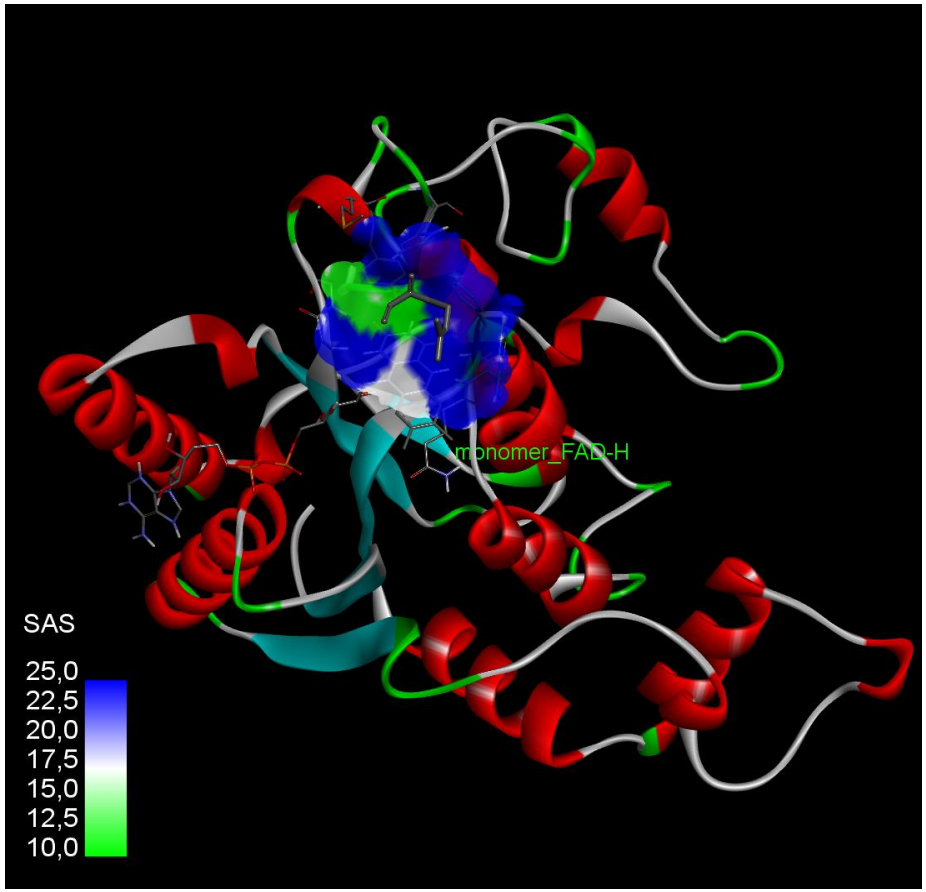


Figura 32 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Mirceno

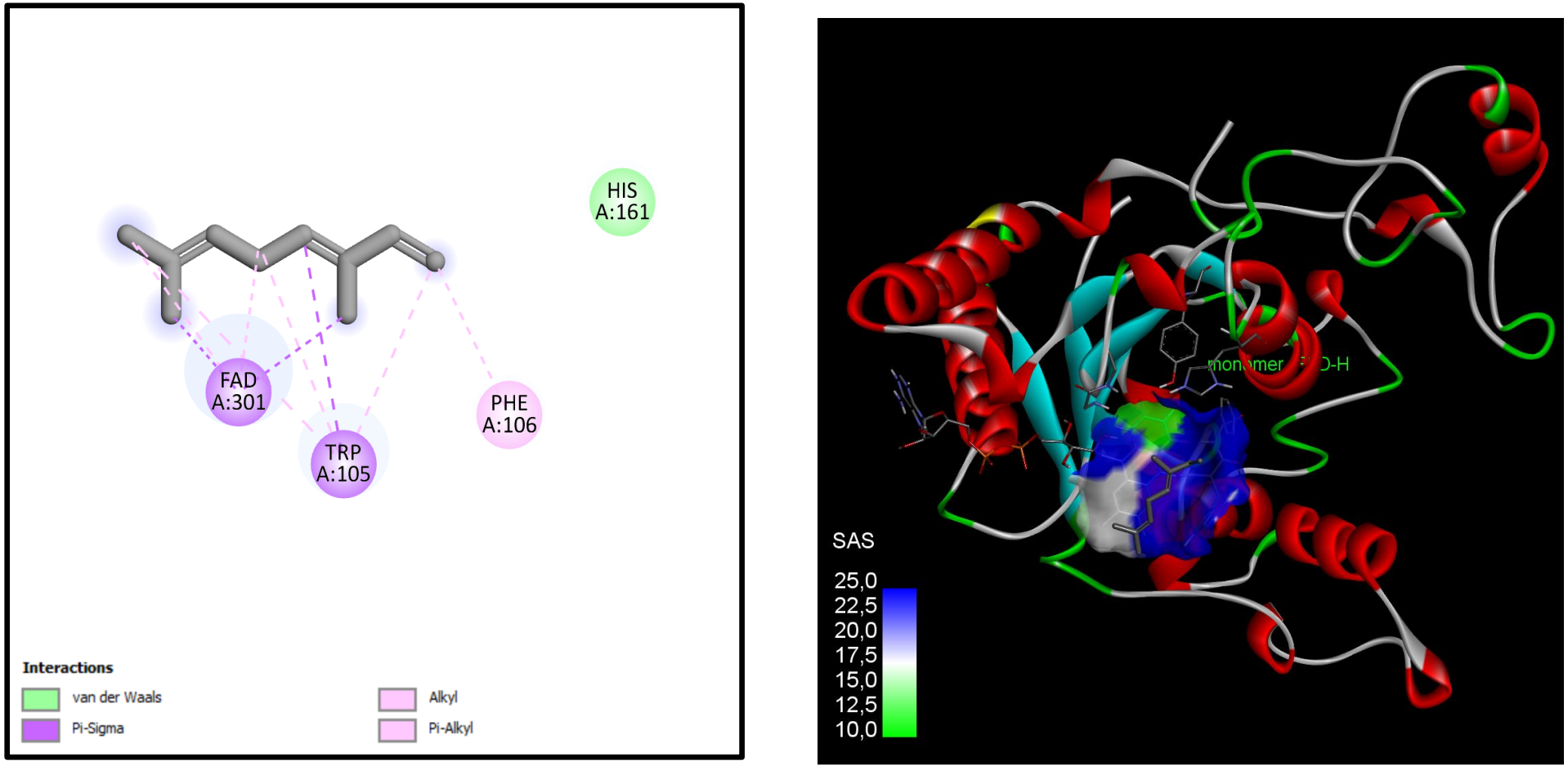


Figura 33 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Ocimeno

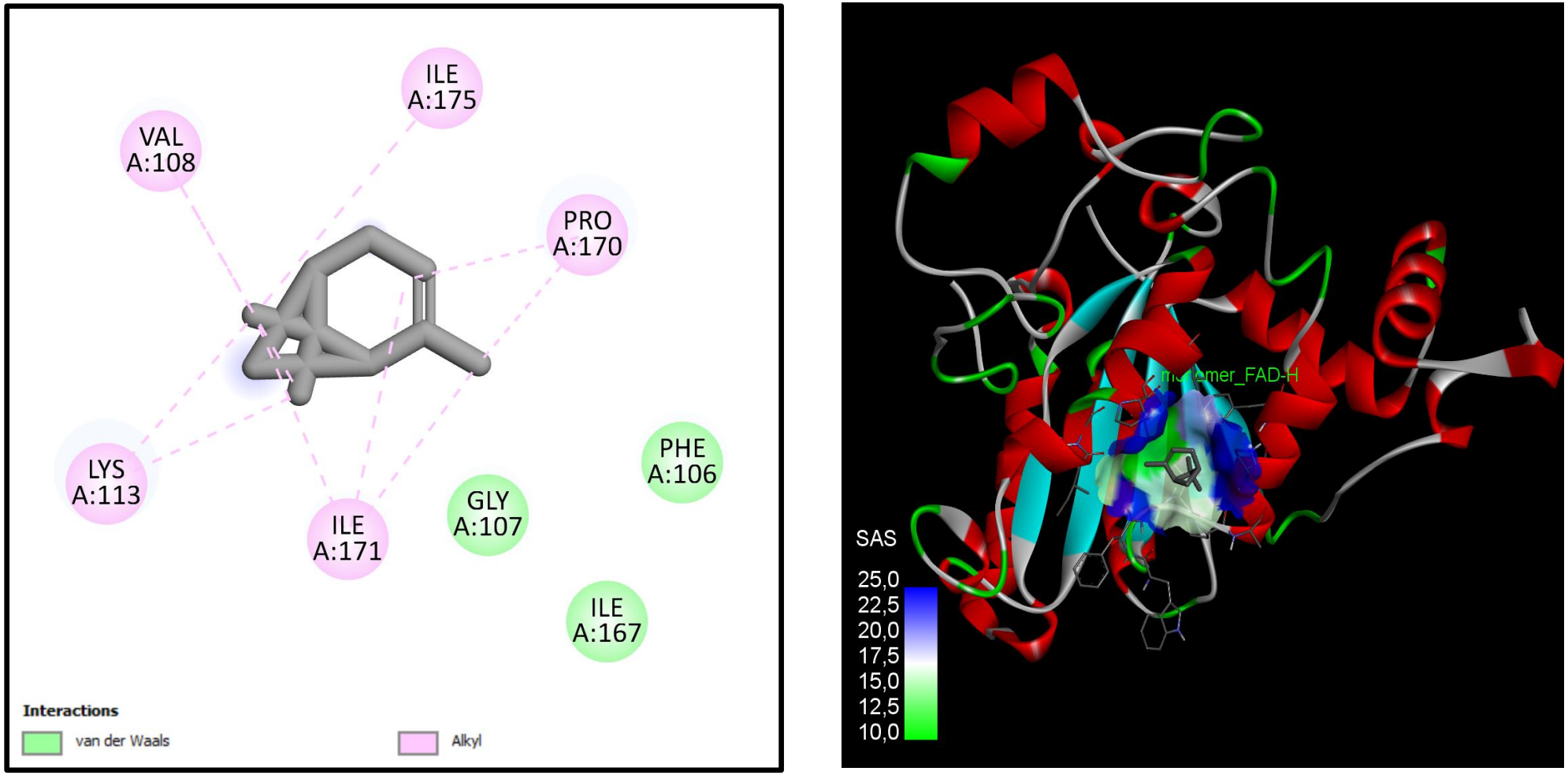


Figura 34 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Pineno (alfa)(+)

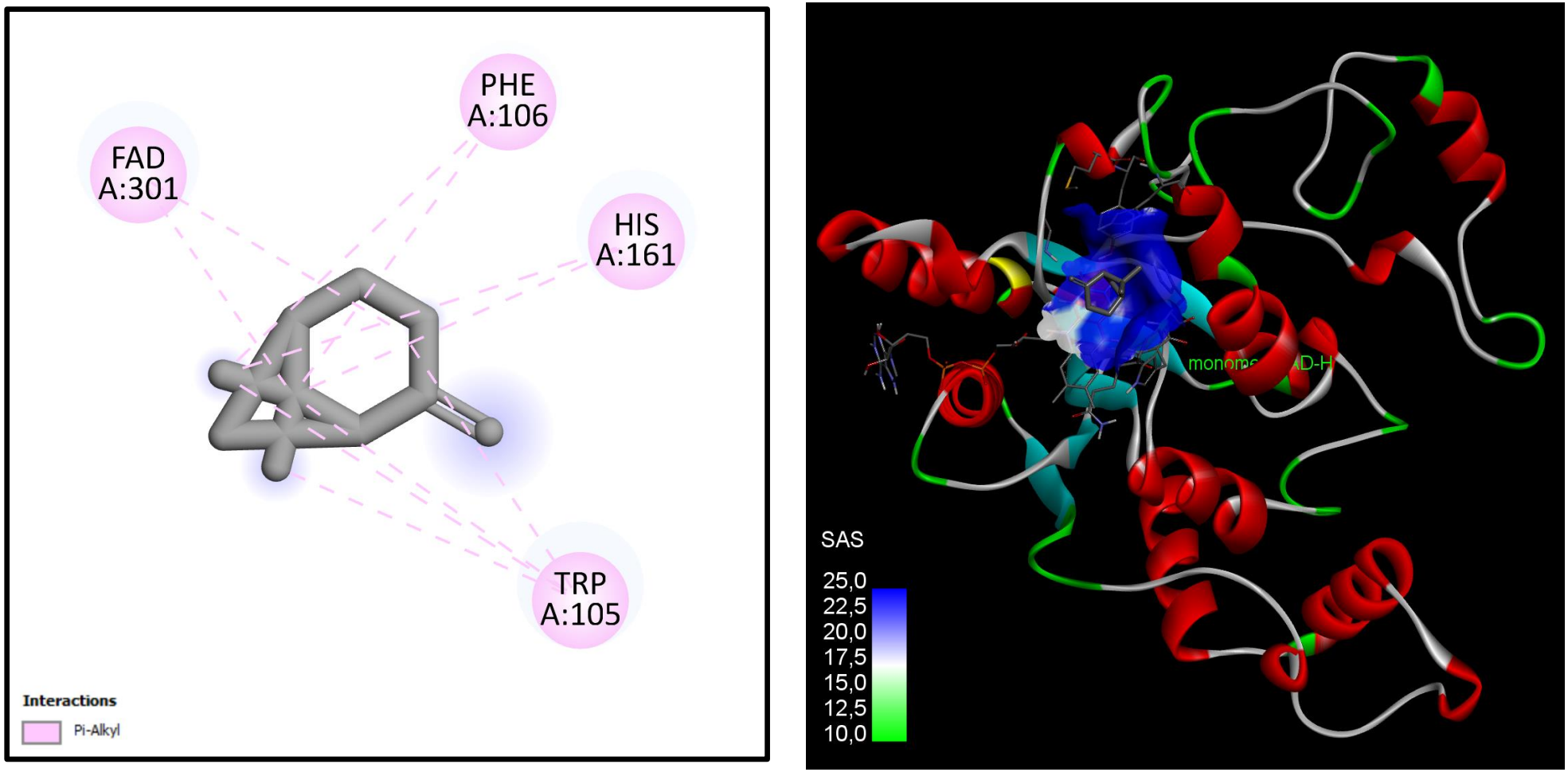
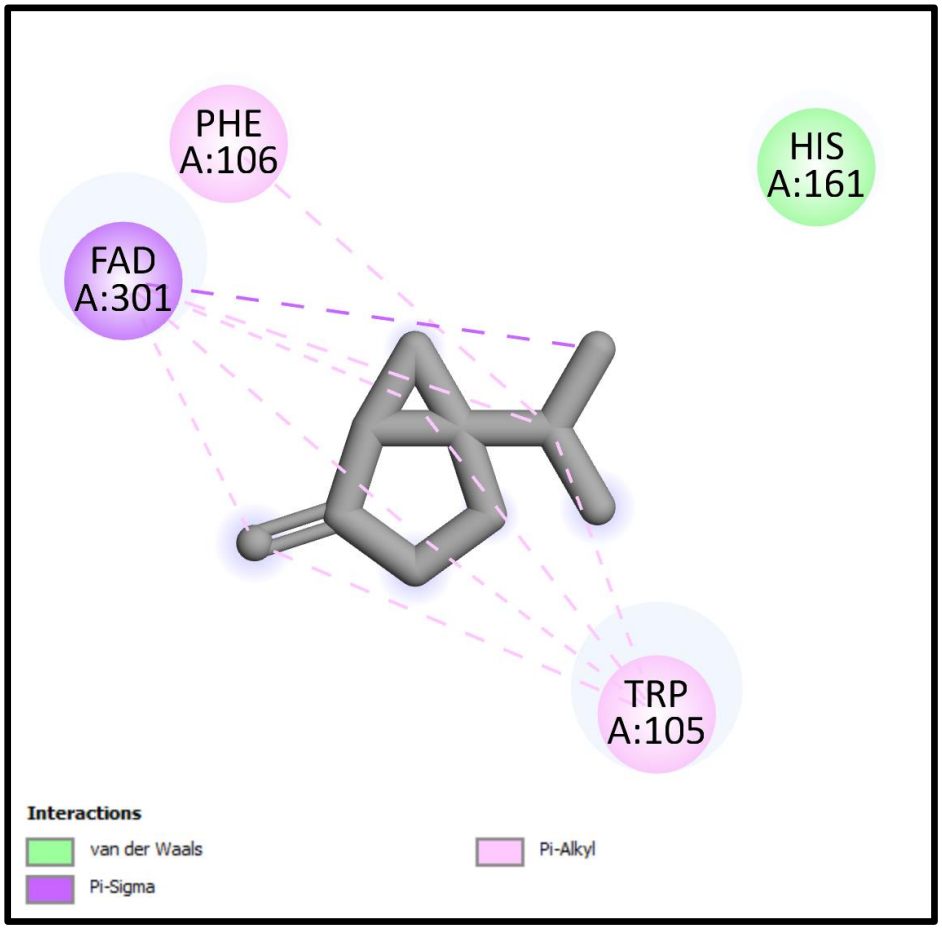


Figura 35 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Pineno (beta)(+)



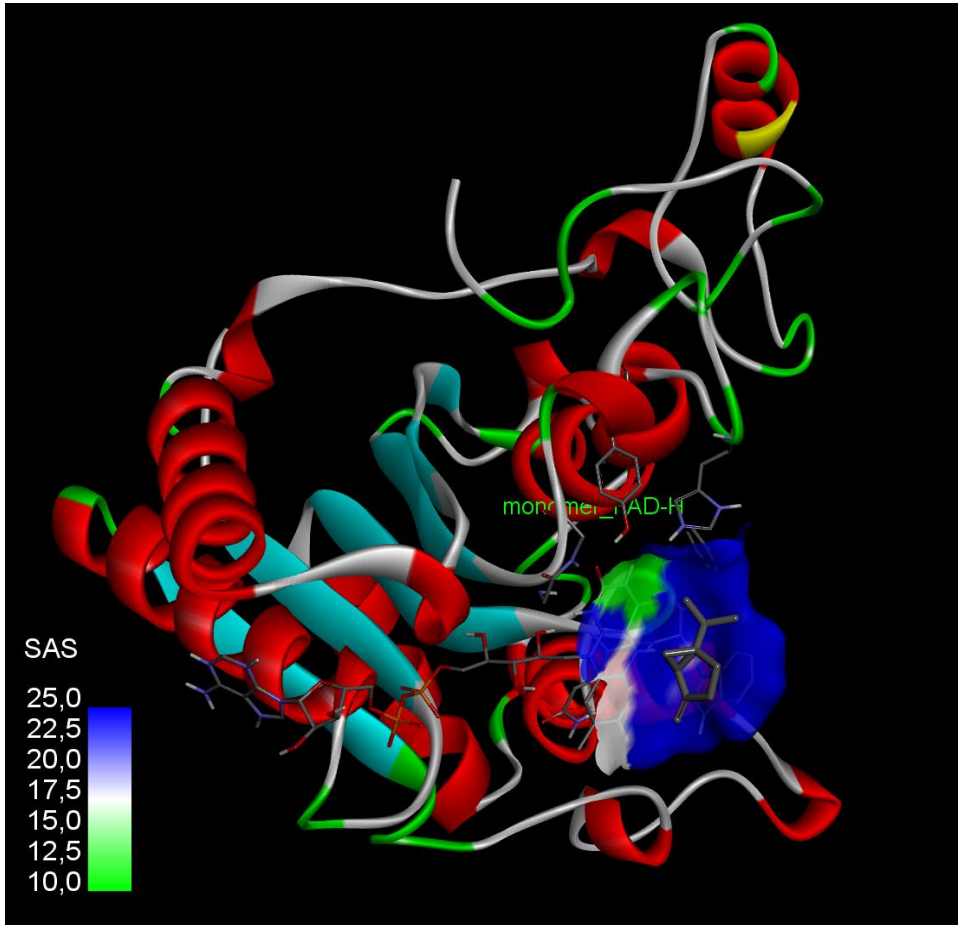


Figura 36 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Sabineno

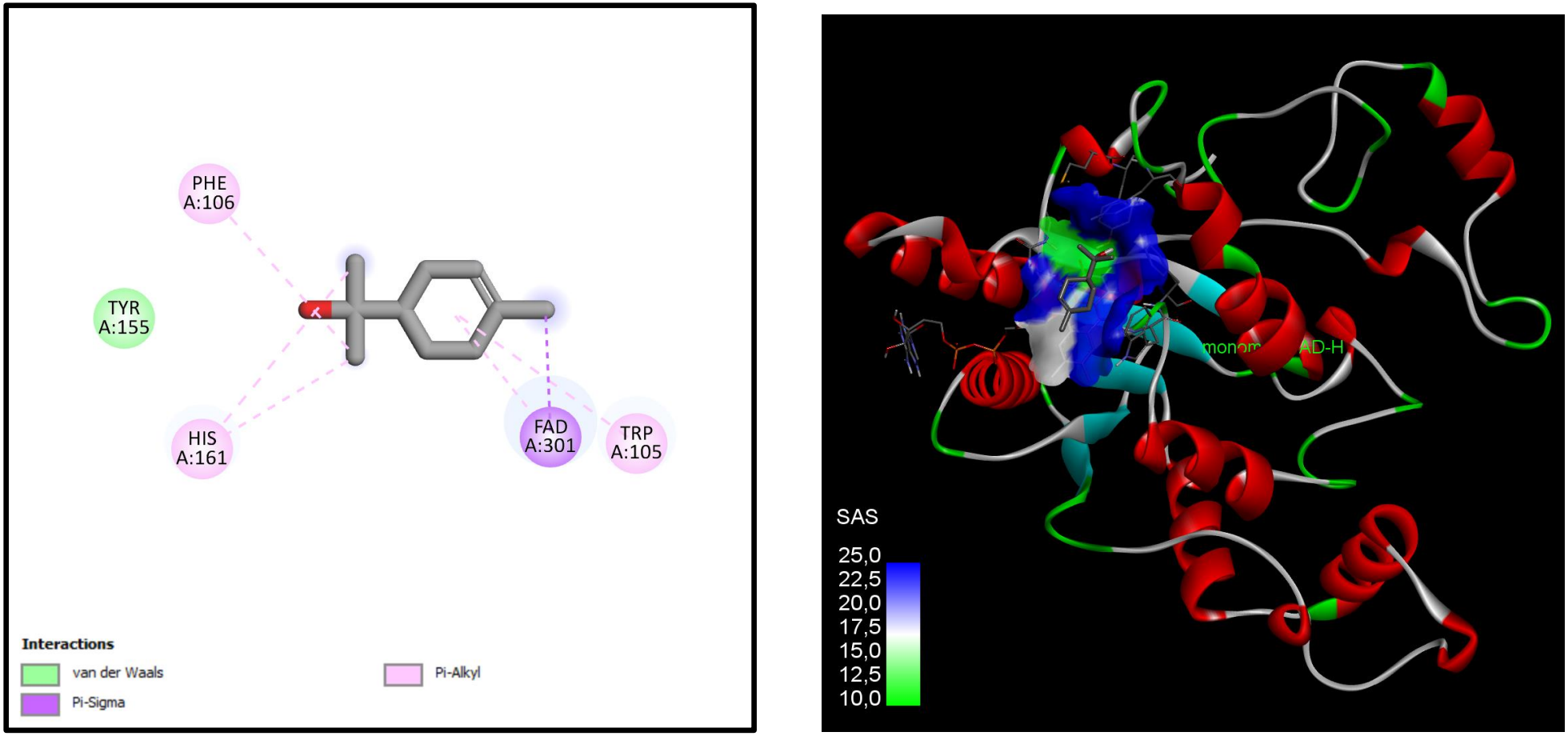


Figura 37 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Terpineol

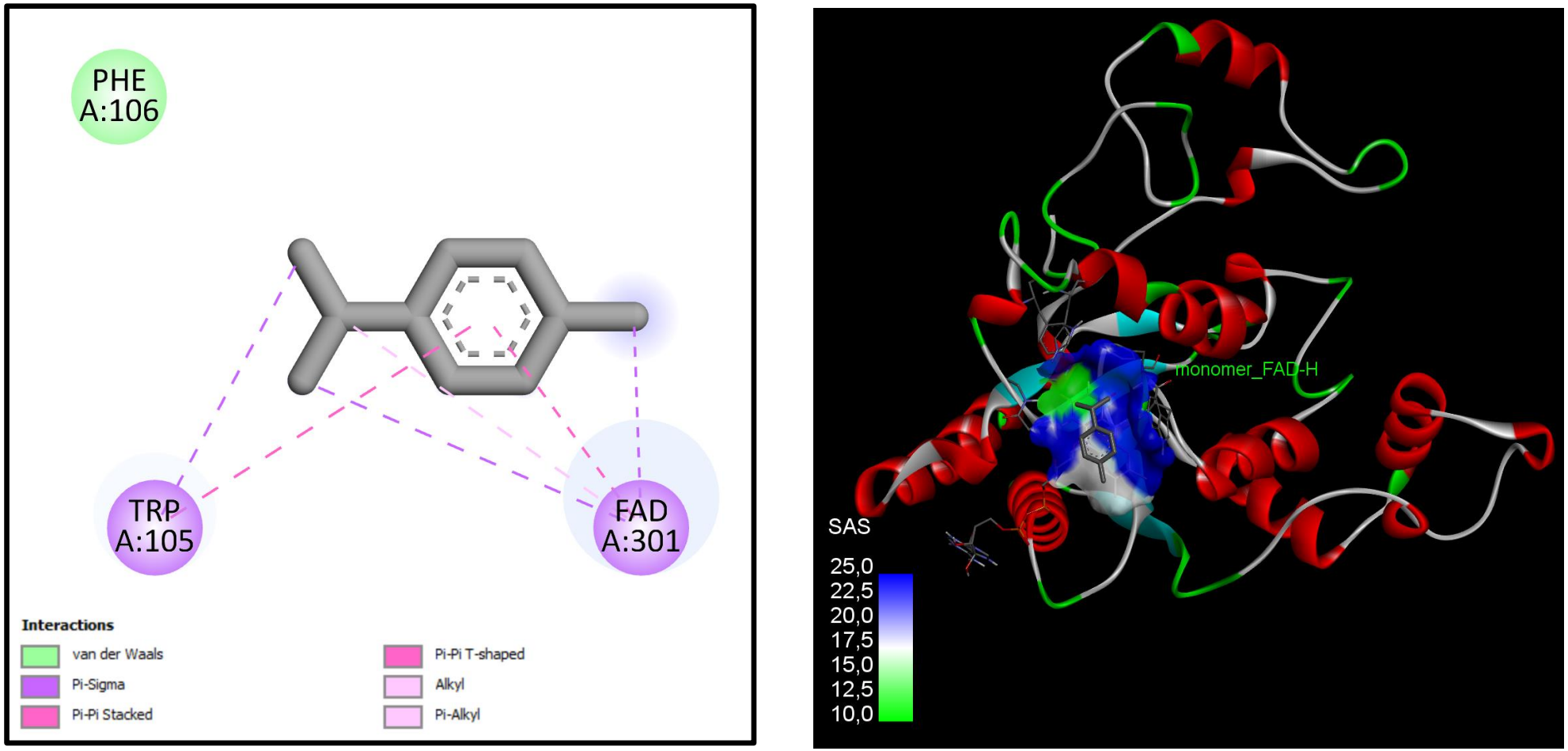


Figura 38 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Terpineno (gama)

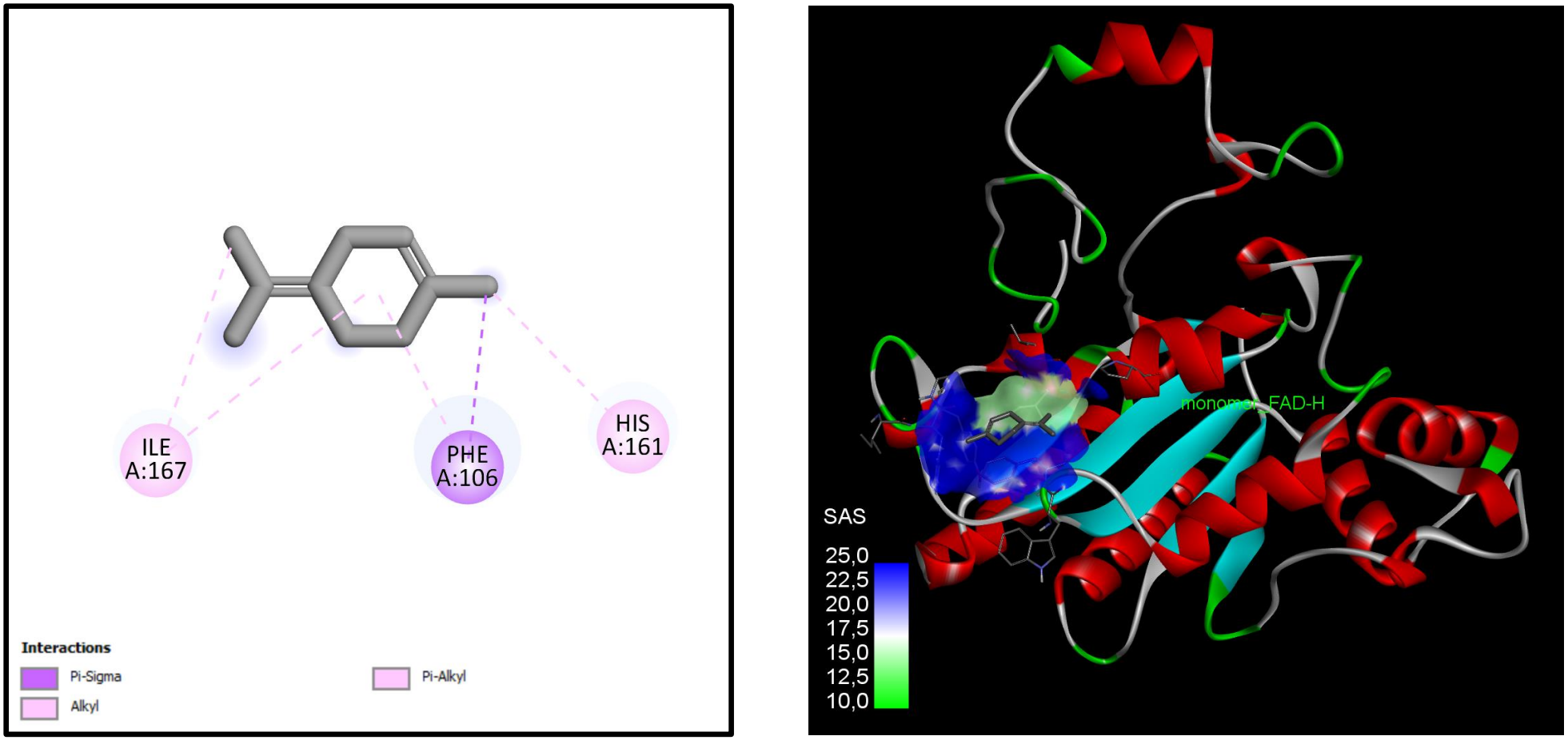


Figura 39 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Terpinoleno

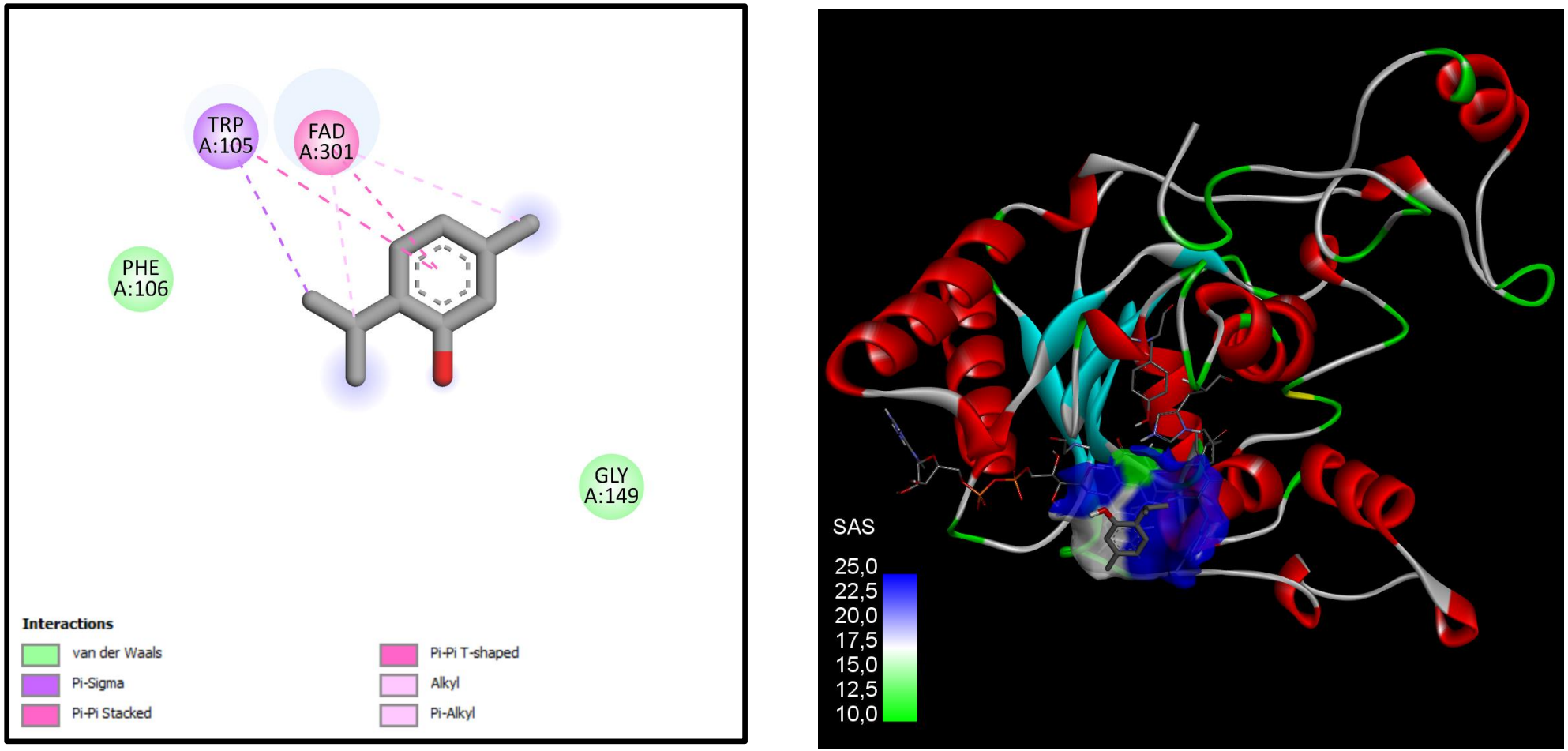


Figura 40 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Timol

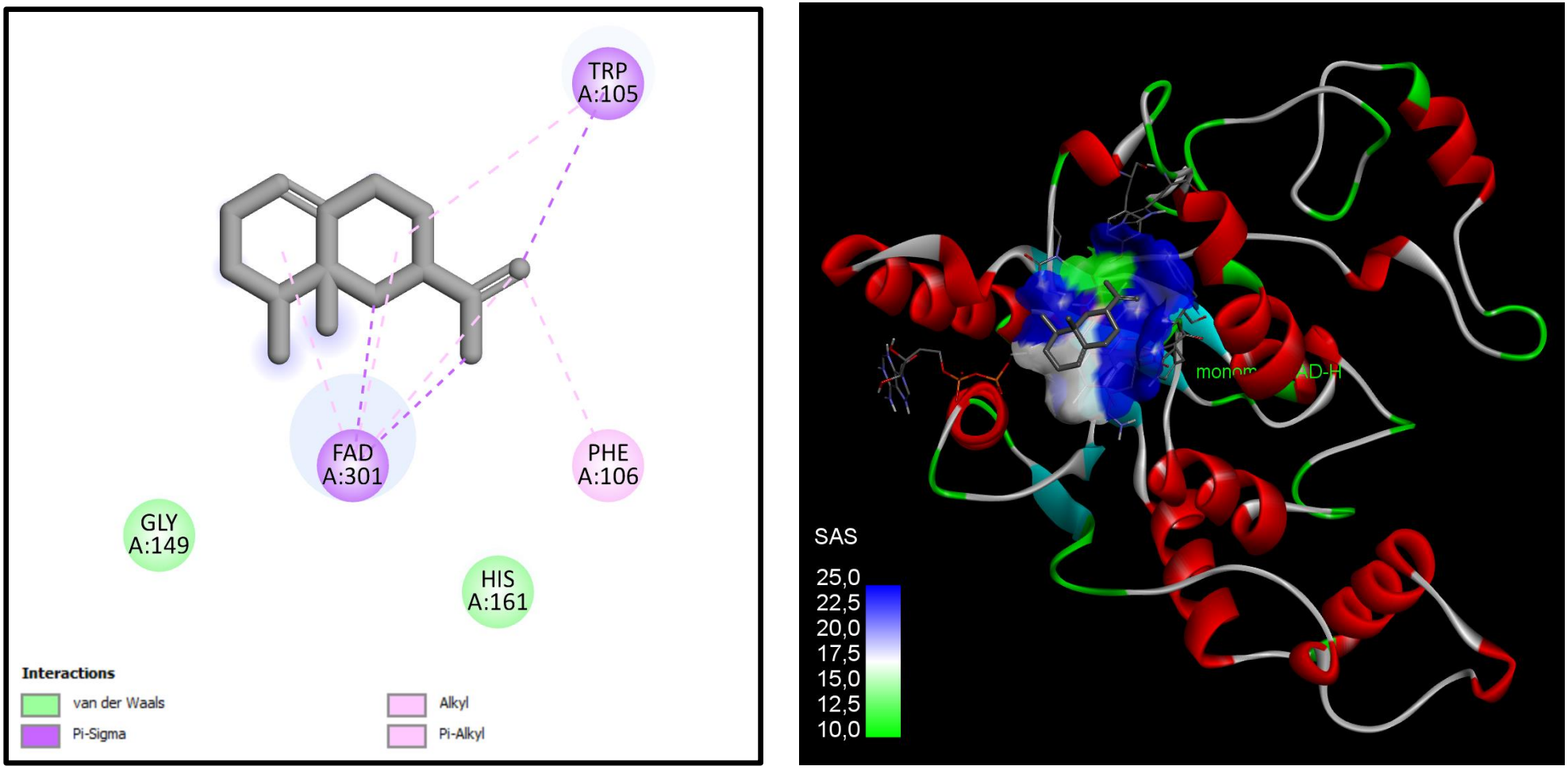


Figura 41 Estrutura Bidimensional e Tridimensional do Complexo Quinina redutase, FAD e Valenceno