ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4

Journal of Coastal Life Medicine

Diplocyclos Palmitus- A Pharmacognostical, in Silico and Invitro Antifungal Evaluation

Received: 16 February 2023, **Revised:** 20 March 2023, **Accepted:** 17 April 2023

Prajitha Biju,
Department of Pharmaceutics, Yenepoya Pharmacy College & Research Centre, Yenepoya (Deemed to be University), Mangaluru-575018

Khathijathul Shahima,
Yenepoya Pharmacy College & Research Centre, Yenepoya (Deemed to be University), Mangaluru-575018.

Salma Ahamed,
Yenepoya Pharmacy College & Research Centre, Yenepoya (Deemed to be University), Mangaluru-575018.

Zuleika Shada Shifana,
Yenepoya Pharmacy College & Research Centre, Yenepoya (Deemed to be University), Mangaluru-575018.

Harsha Ashtekar,
Department of Pharmacology, NGSM Institute of Pharmaceutical Sciences, Nitte (Deemed to be University), Mangaluru-575018.

Muhammed Ameen MP,
Department of Pharmacology, Yenepoya Pharmacy College & Research Centre, Yenepoya (Deemed to be University), Mangaluru-575018

Fathima Majida M,
Department of Pharmacology, Yenepoya Pharmacy College & Research Centre, Yenepoya (Deemed to be University), Mangaluru-575018

Natasha Naval Aggarwal*,
Department of Pharmaceutical Chemistry, Yenepoya Pharmacy College & Research Centre, Yenepoya (Deemed to be University), Mangaluru-575018.

Corresponding author: Natasha Naval Aggarwal
Department of Pharmaceutical Chemistry, Yenepoya Pharmacy College & Research Centre, Yenepoya (Deemed to be University), Mangaluru-575018.
Phone No: 7795578072
Mail id: natashanavalaggarwal@yenepoya.edu.in
ORCID ID: 0000-0002-5464-8625

Keywords
Diplocyclos palmatus, Antifungal, Molecular docking, Luliconazole, Terbinafine

Abstract
Aim: To carry out extraction, phytochemical evaluation, and in vitro & in silico anti-fungal evaluation of the leaves of Diplocyclos palmatus.
Objective: This study is undertaken to carry out extraction of the leaves of Diplocyclos palmatus and to evaluate its antifungal efficacy by In-silico and In-vivo studies.
Methodology: The extraction of the leaves of Diplocyclos palmatus was carried out by the Cold maceration technique, using various solvents such as Chloroform, Methanol, Ethanol, and water. The phytochemical evaluation of each of these extracts was carried out and the extracts were compared for their practical yields.
In-silico analysis was carried out using Pyrex software and the active constituents of Diplocyclos palmatus were docked to the

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

binding site of the Orotidine 5'-phosphate decarboxylase (PDB ID:2CZD). The in-vitro antifungal study was performed by Well Diffusion Assay.
Results: The results depicted that the highest yield was obtained from the ethanolic extract of the leaves. The active constituent, Isoquercetin, depicted the best docking score of -8 Kcal/mol, followed by the standard drugs, Luliconazole and Terbinafine (-7.2 and -7.3 Kcal/mol, respectively). Subsequent In-vitro analysis established that substantial antifungal efficacy upon comparison to the standard was demonstrated by the ethanolic extract of Diplocyclos palmatus compared to chloroform extract (diameter of zone of inhibition: 20±0.43 mm).
Conclusion: The data indicated that the Substantial antifungal efficacy upon comparison to the standard (Luliconazole) and the marketed standard, (Terbinafine), was demonstrated by the ethanolic extracts It was also evident from the results that, as the concentration increased, the antifungal efficacy also increased.

1. Introduction

In recent years, fungal illnesses have grown increasingly relevant. Because few fungi are professional pathogens, fungal pathogenic processes are typically complicated, resulting in large part from adaptations of nonparasitic lifestyle traits of the organisms[1]. Invasive fungal infection, which can be fatal, now accounts for almost 3 million cases of serious illness each year, owing to advances in modern medicine and the HIV epidemic, which left a large number of people with weakened immune systems [2,3].

Of late, there has been a new breakout, particularly the black fungus, since the appearance of the Covid-19 pandemic, in an attempt to counteract Covid-19 when the vaccine was created. This fungus affects the sinuses, brain, and lungs, as well as being a very harmful factor that can lead to death if the death rate from fungus infection reaches over 50%, especially in people with diabetes and immunodeficiency illnesses [4]. All indicators point to a rise in the importance of fungal infections in the twenty-first century, and better human readiness for this scourge will necessitate more research funding in this group of infectious diseases.

80 percent of people in underdeveloped countries use traditional medicine, according to the World Health Organization (WHO). Throughout recent decades, the use of complementary and alternative medicine (CAM), particularly herbal treatments, has increased in the industrialized world. Medical systems and folk medicine contain a wide range of herbal concoctions and therapeutic plants. The foundation for a vast array of modern medicines is found in the plants used in Ayurveda and other traditional medical systems [5,6]. Ayurveda and other conventional herbal treatments can support future medication development and help bridge some of the medical gaps. The usage of herbal medicines has made a huge resurgence recently as a result of the side effects of modern drugs, the failure of existing therapy for chronic conditions, and microbial resistance [7-8].

The plant Diplocyclos palmatus (L.) Jeffrey, well known as Shivalinga, is a seasonal climber plant with a myriad of medicinal properties. It can be found in India, Africa, Indo-China, and parts of Australia8. In traditional medicine, several parts of the Diplocyclos palmatus plant, including the leaves, stem, flower, seeds, and possibly the entire plant, have been used to cure a range of illnesses. It has a long history of use as a supplement for a variety of illnesses, such as fever, diarrhoea, inflammation, asthma, and constipation [9-13].

Fig.1 Diplocyclos palmatus

Various reports on phytochemical screening of Diplocyclos palmatus indicate the presence of Triterpenoids, Alkaloids, Saponins, Flavonoids, steroids as well as proteins. In addition, they are also reported to contain starch, various sugars, resins, lipids, Punicic acid, Goniothalamin, Glucamannon, Isoquercitin, Gallic acid, Bryonin, etc.

Of these, Alkaloids are reported to exhibit antifungal activity by altering the permeability of the fungal

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

membranes and impairing the mitochondrial function [14]. The antifungal activity of Triterpenoids and Saponins is also well-reported [15]. These findings, therefore, bolstered us to screen the anti-fungal potential of the constituents of Diplocyclos palmatus with the help of molecular docking, molecular dynamics, and pharmacophore modeling techniques, in addition to in vitro assay.

In this study, we have carried out the extraction of the leaves of Diplocyclos palmatus using solvents of varying polarity followed by preliminary phytochemical screening. Furthermore, the anti-fungal potential of the leaves was screened through in silico studies against Orotidine 5'-phosphate decarboxylase as well as through in vitro analysis against Candida albicans.

2. Methodology

2.1. Reagents and chemicals

All the required chemicals, solvents were of Analytical (AR) grade and obtained from standard sources. Luliconazole was obtained as a gift sample from Optrix, Mumbai,India. Instruments like UV/Vis Spectrophotometer (Shimadzu, UV 6000+), Micro centrifuge (Remi, RM-12 C) were used for the study. Rotary vacuum evaporator (Remi, India) was used for recovery of the solvent under reduced pressure.

2.2. Plant material used

Fresh leaves of Diplocyclos palmatus were collected from the local areas of Mangalore, Karnataka, India. The plant was authenticated by the botanist.

2.3. Preparation of extracts

The leaves of Diplocyclos palmatus were cleaned and shade dried for about 7 days. The shade dried leaves were ground into coarse powder using a mechanical grinder. The coarse powder of leaves of Diplocyclos palmatus was subjected to cold maceration extraction using various solvents viz., Chloroform, Methanol, Ethanol and water [16].

2.4. Test microorganisms

The fungal strain used in this study is Candida albicans which was collected from the department of Yenepoya Medical College, Yenepoya University, Mangalore, Karnataka, India

2.5. Culture media

Sabourad dextrose agar (SDA) medium was used for the culturing and growth of Candida albicans used in this study.

2.6. Phytochemical evaluation

15 mg of extract was agitated for 5 minutes on a water bath with 6 ml of 1 percent HCl and filtered. The filtrate was tested for the presence of Alkaloids, Terpenoids, Steroids, Tannins, Saponins, Glycosides, Proteins, Flavonoids, Phenolic compounds according to the standard procedures [20,21].

2.7. In silico Anti-fungal analysis

2.7.1. Ligand preparation:

The 2D structures of the active constituents of Diplocyclos palmatus were prepared by employing Chemsketch freeware (https://www.acdlabs.com/resources/freeware/chemsketch/index.php). Thereafter, energy optimization of the 3D ligand structures was carried out using the Ligprep module in Schrodinger 2018-3 suite device Maestro 11.7.012. All the structures were optimized to a globalminima by the addition of hydrogens and removal of counter ions. In addition, distinct stereoisomers and ionization states tautomers were generated [17].

2.7.2. Protein preparation:

The structure of Aminotransferase isoenzyme derived from X-ray crystallography (PDB ID: 2CZD) was obtained from the protein data bank(www.rcsb.org).

PDB ID: 2CZD is the crystal structure of Orotidine 5'-phosphate decarboxylase. This enzyme is involved in the biosynthesis of pyrimidines. In order to generate uridine monophosphate (UMP), it catalyzes the decarboxylation of orotidine monophosphate (OMP). The de novo synthesis of the pyrimidine nucleotides, uridine triphosphate, cytidine triphosphate, and thymidine triphosphate depend on the activity of this enzyme.21 The protein was prepared and preprocessed using the Protein preparation module of Schrodinger 2018-3 suite device Maestro 11.7.012. The energy of the proteins was then minimized using the OPLS3 force field. The active sites within a radius of 10 Å were identified, and a receptor grid box was generated at the

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

center of the enzyme's active site. The ligands were then docked into the active site of the enzyme using Grid-based ligand docking using Glide extra precision (XP) scoring functions. Positive interactions such as lipophilia, hydrogen bonding, and metal-linking are rewarded, while steric conflicts are punished. Finally, re-scoring of a few positions was done via glide score's ability to score. The docking results were analysed using the Glide module's XP visualizer [17].

2.7.3. ADME prediction:

Screening of the docked compounds was carried out based on their absorption, distribution, metabolism, and excretion with the help of the Qikprop module in Schrodinger software. This module yielded several properties such as the molecular weight, volume, solubility, number of rotatable bonds, partition coefficient, number of hydrogen bond donors and acceptors, etc. The total polarizable surface area of all the compounds was predicted using Molinspiration freeware(https://www.molinspiration.com/cgi-bin/properties) [17].

2.8. In vitro Anti-fungal activity

2.8.1. Well-diffusion method

A sterile swab was used to spread the fungal culture evenly over the medium. A well of 10mm diameter was made using a well borer. The standard drug was prepared by dissolving 10mg of Luliconazole in 10 ml of methanol. The standard well of 10 mm diameter was injected with Luliconazole (1mg/ml) and two control wells were maintained containing ethanol and chloroform (10 µl). The plant extract was injected into the wells in the increasing concentration of 10µl/ml, 30µl/ml, and 50µl /ml. A marketed standard drug (Terbinafine,1mg/ml) was also injected in Antifungal assay plates were incubated at 37 ± 2◦C for 24-48 hours. The diameter of the zone of inhibition was measured and tabulated [18,19].

2.8.2. Statistical Analysis

The results obtained from the pharmacological studies are presented as Mean ± Standard deviation (S.D.) for three triplicates (n=3). IC50 value was calculated by using the Linear regression analysis method.

3. Result:

3.1. Extraction of Diplocyclos palmatus:

The following percentage yields were found upon extraction of the leaves using various solvents:

Table 1: % yield of the different extracts of Diplocyclos palmatus

Extract	% Yield
Chloroform	45
Ethanolic	66
Methanolic	50
Aqueous	61

3.2. Phytochemical evaluation:

The phytochemical screening of the various extracts was conducted and the results are depicted in Table-2.

Table 2: Phytochemical screening of plant using different extracts

Chemical tests	Different extracts of plant			
	Chloroform	Ethanol	Methanol	Aqueous
ALKALOIDS				
Hager's test	+ve	+ve	+ve	+ve
Wagnor's test	+ve	+ve	+ve	+ve
Mayer's test	-	-	-	-
Dragendroff's test	-	-	-	-
FLAVONOIDS				
Lead acetate test	+ve	+ve	+ve	+ve

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

Alkaline reagent test	+ve	+ve	-	+ve
TANNINS				
Gelatin test	+ve	-	-	-
DITERPENES AND TRITERPENES				
Copper acetate test	+ve	+ve	-	-
SAPONIN				
Froth test	+ve	+ve	-	-
Foam test	+ve	+ve	-	-
GLYCOSIDE				
Borntragers test	+ve	+ve	-	-
Legals test	+ve	+ve	-	-
PROTEINS AND AMINO ACIDS				
Xanthoproteic test	-	-	-	-
Biuret test	-	-	-	-
Ninhydrin test	-	-	-	-
CARBOHYDRATES				
Molisch test	+ve	+ve	-	-
Benedict's test	+ve	-	-	-
Barfoed's test	-	-	-	-
Fehling test	+ve	+ve	+ve	+ve
PHENOLIC COMPOUNDS				
Ferric chloride test	-	-	-	+ve
Lead acetate test	-	+ve	-	-
PHYTOSTEROLS				
Salkowski test	+ve	-	-	-
Liberm ann test	-	-	-	-

3.1.3. In- silico Antimicrobial activity:

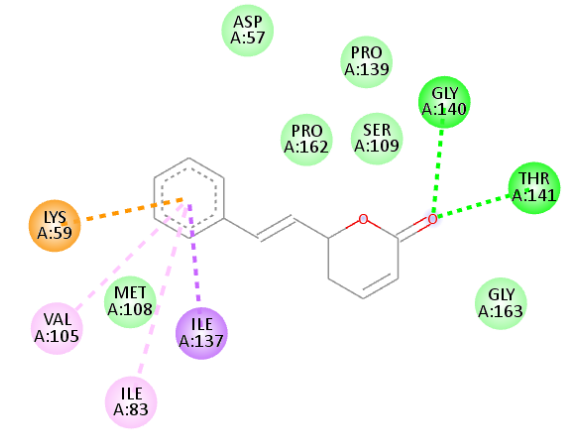
The molecular weight, Log P value, and number of hydrogen bond acceptors (Acceptor HB) and donors (Donor HB) were calculated in order to determine whether the compounds obey Lipinski's rule of 5. In addition, the docking scores of the compounds at the active site of 2CZD were also calculated and are reported in Table-3. The 2D and 3D docking interactions of the same are depicted in Figures 2-10.

Table 3: Lipinski's RO5 and Dock scores at the active site of 2CZD of compounds

Comp	**Molecular weight (Daltons)**	**Log P**	**Donor HB**	**Acceptor HB**	**Number of violations**	**Dock score (Kcal/mol)**
Acceptable Range	**≤ 500**	**5**	**≤ 5**	**≤ 10**	**< 5**	
Standard (Luliconazole)	354.28	2.82	0	2	0	-7.2
Marketed Standard (Terbinafine)	291.43	4.15	0	1	1	-7.3
Goniothalamin	200.23	2.33	0	2	0	-7.1

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

Isoquercetin	464.38	2.11	8	12	2	-8
Glucomannan	666.58	0.53	14	21	3	-
Gallic acid	170.12	0.21	4	5	0	-5.6
Chlorogenic acid	510.4	0.88	10	14	3	
Protocatechuic acid	154.12	0.66	3	4	0	-5.5
Punicic acid	278.43	0.66	1	2	0	-5.7



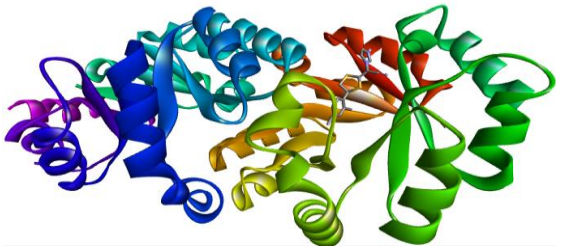
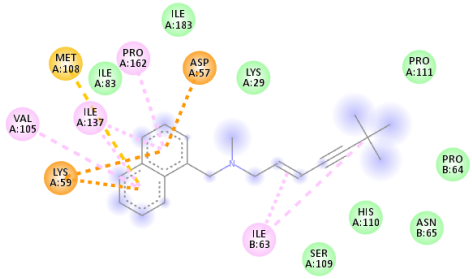


Fig. 2a and 2b: 2D & 3D Docking interaction of Luliconazole with 2CZD



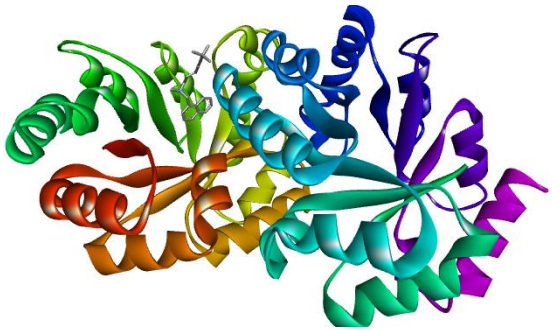
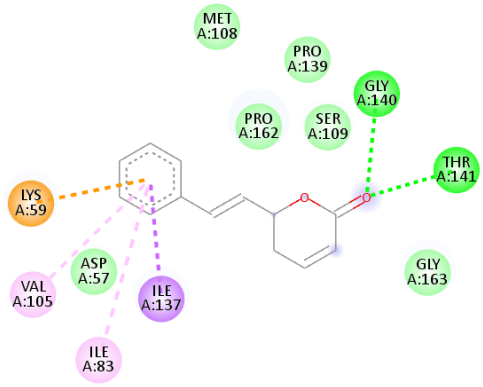


Fig. 3a and 3b: 2D & 3D Docking interaction of Terbinafine with 2CZD



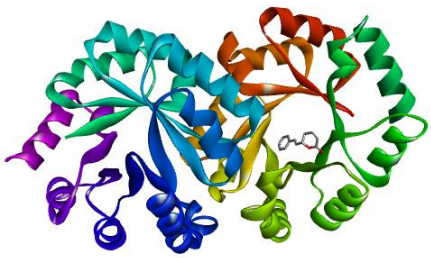
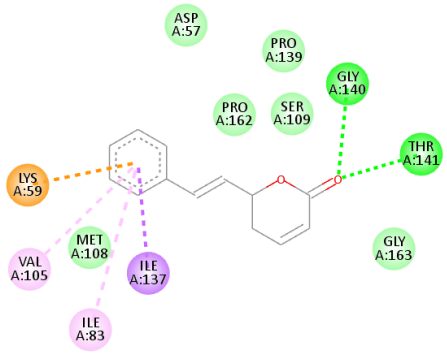


Fig. 4a and 4b: 2D & 3D Docking interaction of Goniothalamin with 2CZD

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine



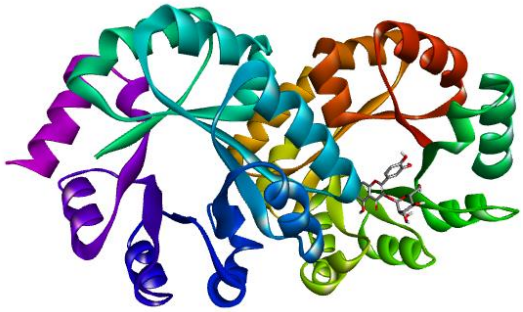
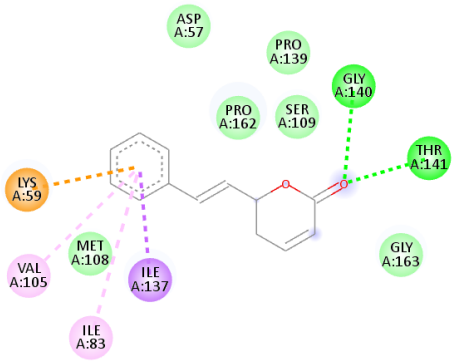


Fig. 5a and 5b: 2D & 3D Docking interaction of Isoquercitrin with 2CZD



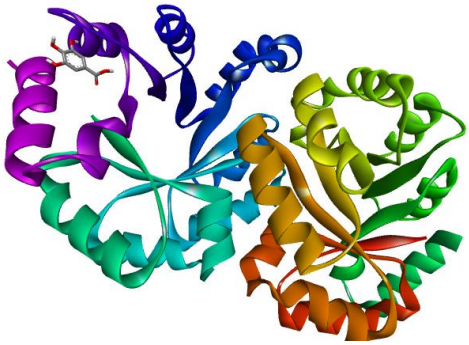
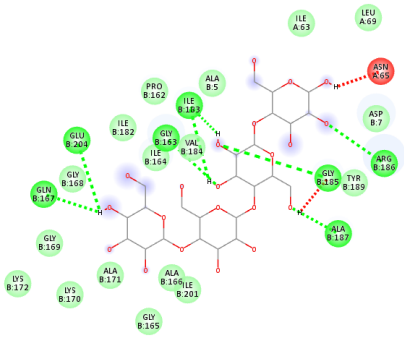


Fig. 7a and 7b: 2D & 3D Docking interaction of Gallic acid with 2CZD



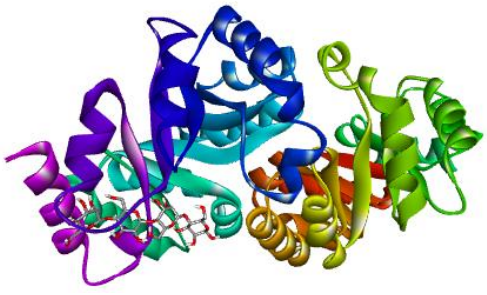
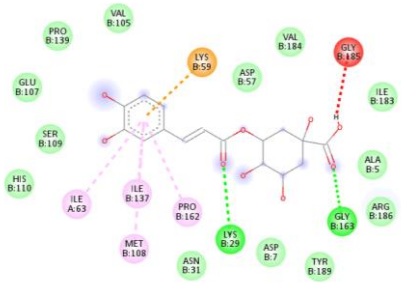


Fig. 6a and 6b: 2D & 3D Docking interaction of Glucomannan with 2CZD



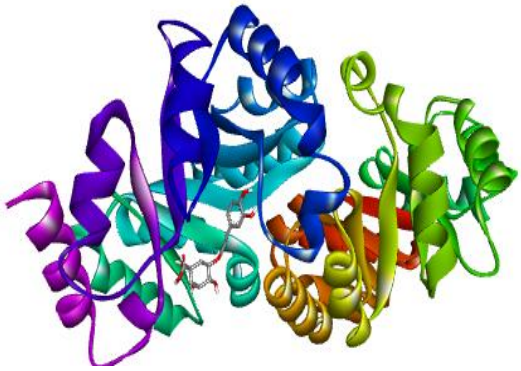


Fig. 8a and 8b: 2D & 3D Docking interaction of Chlorogenic acid with 2CZD

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

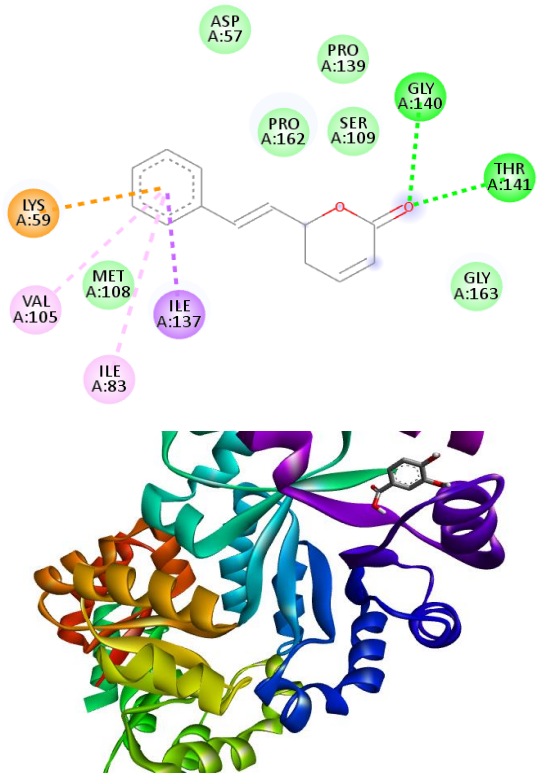


Fig. 9a and 9b: 2D & 3D Docking interaction of Procatechunic acid with 2CZD

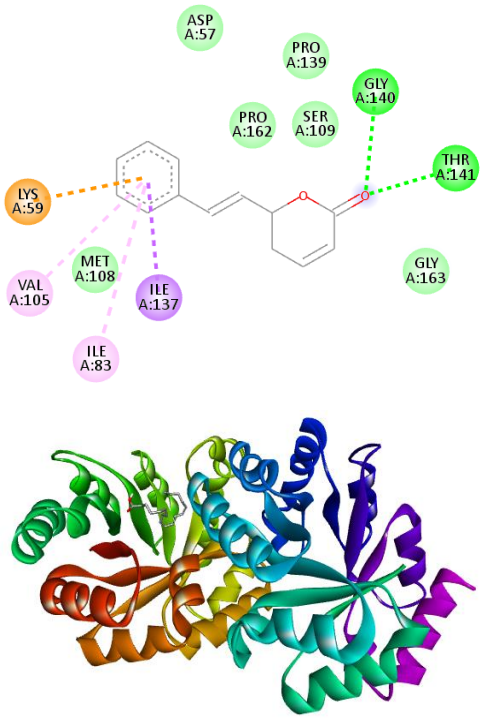


Fig. 10a and 10b: 2D & 3D Docking interaction of Punicic acid with 2CZD

The ADME properties such as Molar refractivity (MR), tPSA (total polarizable surface area), Number of rotatable bonds (Nrobs), Blood brain barrier penetration capability (BBB penetration), Skin permeability (logKp) as well as the bioavailability score and absorption through the GI tract was predicted and the results are tabulated in Table-4.

Comp	MR	tPSA	Nrobs	BBB Penetration	logKp	Bioavailibility score	GI absorption
Standard (Luliconazole)	90.36	92.21	2	No	-5.64	0.55	High
Marketed Standard (Terbinafine)	97.31	3.24	4	No	-4.11	0.55	High
Gonothalamin	59.27	26.3	2	Yes	-5.5	0.55	High
Isoquercetin	110.16	210.51	4	No	-8.88	0.17	Low
Glucomannan	132.89	347.83	10	No	-16.77	0.17	Low
Gallic acid	39.47	97.99	1	No	-6.84	0.56	High
Chlorogenic acid	118.16	262.74	6	No	-9.31	0.11	Low

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

Protocatechuic acid	37.45	77.76	1	No	-6.42	0.56	High
Punicic acid	88.99	37.3	13	No	-6.42	0.56	High

3.1.4. In- vitro Antimicrobial activity:

The following table (Table-5) depicts the results of in vitro antimicrobial activity of chloroform and ethanolic extract against Candida albicans.

Table 5: In vitro antimicrobial activity of chloroform and ethanolic extract against Candida albicans

Comp	Diameter of zone of inhibition(mm)	
	Chloroform	***Ethanolic***
10 µg/ml	9.48±0.112	15±0.043
30 µg/ml	11.21±0.013	16±0.58
50 µg/ml	13.31±0.26	20±0.030
Standard drug – 10 µg/ml (Luliconazole)	12±0.007	
Standard drug - 30 µg/ml (Luliconazole)	15.23±097	
Standard drug – 50 µg/ml (Luliconazole)	16.4±0.062	
Standard marketed drug – 50 µg/ml	20.53±0.085	
Control	--	

The values are represented as mean±SD with n=3

Fig 11: Antimicrobial activity by well-diffusion method

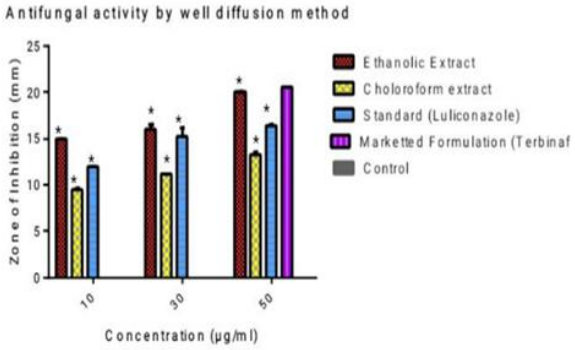


Fig 12: Graphical representation of zone of inhibition (mm) v/s concentration using different extracts at various concentrations

The above data is presented as mean ± SEM, n=6. Statistical analysis was performed using One way analysis of variance (ANOVA), followed by Dunnet's multiple comparison test. Levels of significance: *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 compared to control group.

4. Discussion

Phytochemical Screening:

The leaf extracts were found to have the following constituents after the phytochemical evaluation:

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

Alkaloids, flavonoids, tannins, carbohydrates, phenolic compounds and phytosterols.

The chloroform extract of the leaves of Diplocyclos palmitus showed the presence of alkaloids, flavonoids, tannins, Diterpenes & Triterpenes, saponins, glycosides, carbohydrates and phytosterols.

The ethanolic extract of the leaves of Diplocyclos palmitus contained alkaloids, flavonoids, Diterpenes & Triterpenes, saponins, glycosides, carbohydrates and phenolic compounds.

Alkaloids, flavonoids, saponins, glycosides, and carbohydrates were found to be present in the methanolic extract of the leaves of Diplocyclos palmitus.

The aqueous extract of the leaves of Diplocyclos palmitus showed the presence of Alkaloids, flavonoids, carbohydrates and phenolic compound.

In silico analysis

3.1. Molecular docking

The active residues in the A chain of 2CZD are LYS:29, ASP 57, LYS:59, ILE:83, VAL:105, MET:108, SER:109, HIS:110, PRO 111, ILE:137, PRO 139, GLY 140, THR 141, PRO 162, GLY 163 and that of the B chain are PRO:64, ASN:65 and, ILE:63. The active constituents of Diplocyclos palmitus fit into the binding cleft of the 2CZD receptor displaying excellent glide energy scores, docking scores and hydrogen bond interaction with 2CZD. The affinity of the compounds with receptor 2CZD is given in Table-2 in terms of dock score. The constituents possessed binding free energy in the range of -5.6 to -8.0 kcal/mol. The highest affinity as well as the binding energy of -8.0 kcal/mol is displayed by Isoquercetin in comparison to the other constituents followed by Goniothalamine with a docking score of -7.1 kcal/mol.

Luliconazole underwent pi-pi stacking interaction with LYS:59 and Pi-Allyl bond interaction with VAL:105 and ILE:83. It also formed Hydrogen bonds with GLY:140 and THR:141. The 2D and 3D docking conformations are depicted in Fig.2a and 2b respectively.

Terbinafine formed pi-pi stacking interaction with ASP:57, LYS:59 and MET:108. It was also observed that Pi-Allyl bonds were formed between Terbinafine and PRO:162, ILE:137 and VAL:105 of the A chain and with ILE:63 of the B chain. The 2D and 3D docking conformations are depicted in Fig.3a and 3b respectively.

Goniothalamin displayed with Pi anion bond interaction with LYS:59, and Pi-Pi stacking interaction with VAL:105 and ILE:83. It also formed hydrogen bonds with GLY:140 and THR:141. The 2D and 3D docking conformations are depicted in Fig.4a and 4b respectively.

Isoquercetin underwent Pi anion bond interaction with LYS:59 and Pi-Pi stacking interaction with VAL:105 and ILE:83. It also formed bonds with GLY:140 and THR:141. The 2D and 3D docking conformations are depicted in Fig.5a and 5b respectively.

Glucomannan undergoes Hydrogen bonding interaction with GLU:204, GLN:167, ILE:183, GLY:163, GLY:185,ALA:187, and ARG:186 of the B chain of 2CZD. It also undergoes Donor-Donor interaction with ASN:65 of the A chain of 2CZD. The 2D and 3D docking conformations are depicted in Fig.6a and 6b respectively.

Gallic acid underwent Pi anion bond interaction with LYS:59 and Pi-Pi stacking interaction with VAL:105 and ILE:83. It also formed bonds with GLY:140 and THR:141. The 2D and 3D docking conformations are depicted in Fig.7a and 7b respectively.

Chlorogenic acid displayed Pi-alkyl interaction with ILE:63 of the A chain and ILE:137, MET:108, and PRO:162 of the B chain of 2CZD. It formed Hydrogen bond interactions with LYS:29, GLY:163, and GLY:185 of the B chain of 2CZD. It also undergoes Donor-Donor interaction with GLY:185 of the B chain of 2CZD. The 2D and 3D docking conformations are depicted in Fig.8a and 8b respectively.

Procatechunic acid underwent Pi anion bond interaction with LYS:59 and Pi-Pi stacking interaction with VAL:105 and ILE:83. It also formed bonds with GLY:140 and THR:141. The 2D and 3D docking conformations are depicted in Fig.9a and 9b respectively.

Punicic acid underwent Pi anion bond interaction with LYS:59 and Pi-Pi stacking interaction with VAL:105 and ILE:83. It also formed bonds with GLY:140 and

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

THR:141. The 2D and 3D docking conformations are depicted in Fig.10a and 10b respectively.

3.2. Physicochemical properties

All the active constituents of Diplocyclos palmitus obeyed Lipinski's rule of five except, Isoquercetin, Glucomannan and Chlorogenic acid.

Two of the compounds didn't have molecular weight within the acceptable range of ≤500. (Glucomannan - 666.58 Daltons, Chlorogenic acid- 510.4 Daltons). The logP values of all the constituents were within the acceptable range of ≤5. The number of hydrogen bond donors in the case of Glucomannan and Chlorogenic acid was more than 5 and hence it was beyond the acceptable limit of ≤5. Isoquercetin, Glucomannan and Chlorogenic acid possessed more than 10 acceptor hydrogen bonds and hence were not in the acceptable range. In totality, Isoquercetin has 2 violations, while Glucomannan and Chlorogenic acid both possessed 3 violations each.

3.3. ADMET properties

ADMETSAR was used to determine the ADMET properties and deliver the data about the onset of action and penetration of the drug.

3.3.1. Prediction of Molar refractivity

Molar refractivity gives a measure of the polarizability and size of a mole of a substance. It depends on numerous factors such as pressure, refractive index, temperature, etc. It is determined by the MR values and its acceptable range is from 40-130. All the active bio constituents of Diplocyclos palmitus as well as Luliconazole & Terbinafine have molar refractivity values within the recommended range indicating that the volume of the constituents is favorable and that they can undergo considerable drug-receptor interaction through London dispersive forces.

3.3.2. Prediction of the total polar surface area

PSA is regarded as the capability of a molecule to undergo hydrogen bond formation. The tPSA values of Luliconazole, Terbinafine, Goniothalamin, Gallic acid, Protocatechuic acid, and Punicic acid were within the limit of 140 Å indicating good cell permeability. It also gives an insight into the hydrogen bonding potential of the compounds. In the case of Isoquercitrin, Glucomanan and Chlorogenic acid, the tPSA values were>140 Å, indicating that these compounds have poor absorption.

3.3.3. Prediction of the flexibility of the molecule

The number of rotatable bonds (Nrobs) measures the molecule's flexibility and serves as a good indicator of drug absorption and bioavailability. The value of Nrobs was within the acceptable limit of 10 for all the compounds except Punicic acid.

3.3.4. Prediction of blood-brain barrier (BBB) penetration

BBB penetration score assessed the access to the central nervous system. According to the prediction of the software, Goniothalamine penetrates the Blood-brain barrier. All the other active constituents of Diplocyclos palmitus, namely, Isoquercitin, Glucomannan, Gallic acid, Chlorogenic acid, Protocatechunic acid and Punicic acid do not penetrate the blood-brain barrier. The standard drug Luliconazole as well as the marketed standard, Terbinafine also do not penetrate the BBB.

3.3.4. Prediction of skin permeability

The ability of the drugs to penetrate the skin membrane, increases the amount of the drug reaching the blood circulation through topical application, thereby affecting drug efficiency. The skin permeability is determined by the logKp values (recommended range is -8 to -1). Isoquercitin, Glucomannan, and Chlorogenic acid have greater values and do not comply with this property. The other constituents of Diplocyclos palmitus as well as Luliconazole & Terbinafine are likely to penetrate the skin and be available at the target site.

3.3.5. Prediction of bioavailability

Bioavailability scores help predict the bioavailability of the compounds. The BAS rule states that for a drug to be physiologically active, the scores have to be >0. A score of 0 to -5.0 indicated moderate activity and <-5.0 indicates inactivity.

3.3.6. Gastro-intestinal absorption

The GI absorption of all the compounds was predicted and it was found that except Isoquercitin, Glucomannan, and Chlorogenic acid, all the active

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

constituents of Diplocyclos palmitus as well as the standard drugs have high GI absorption.

In vitro Anti-Microbial Activity:

The plant extracts were subjected to in-vitro anti-fungal assay at concentrations 10 µg/ml, 30 µg/ml, and 50 µg/ml against Candida albicans.

The results of the anti-fungal activity (Table-2) depicted that most of the tested compounds showed good activity against the tested organisms.

Upon testing against the fungal strain of Candida albicans, the ethanolic extract of Diplocyclos palmatus showed more anti-fungal potential compared to chloroform extract (diameter of zone of inhibition: 20±0.43 mm in 50 µg/ml, 16±0.43 in 30 µg/ml and 15±0.43 in 10 µg/ml), followed by chloroform extract (diameter of zone of inhibition: 13±0.43 mm in 50 µg/ml, 11±0.43 in 30 µg/ml and 9±0.43 in 10 µg/ml).

5. Conclusion:

In conclusion, extraction of the leaves of Diplocyclos palmatus was carried out using various solvents such as Chloroform, Methanol, Ethanol, and water. The phytochemical evaluation of each of these extracts was carried out and the extracts were compared for their practical yields. The results depicted that the highest yield was obtained from the ethanolic extract of the leaves. target protein, Orotidine 5'-phosphate decarboxylase (PDB ID:2CZD). With the aid of molecular docking studies, it was established that the compounds have an imperative interaction with 2CZD. Various concentrations of the ethanolic & chloroform leaf extract were evaluated for their In-Vitro antifungal activity against Candida albicans. Substantial antifungal efficacy upon comparison to the standard (Luliconazole) and the marketed standard, (Terbinafine), was demonstrated by the ethanolic extracts. It was evident from the results that, as the concentration increased, the antifungal efficacy also increased..

References

[1] Van Burik JA, Magee PT. Aspects of fungal pathogenesis in humans. Annual Reviews in Microbiology. 2001 Oct;55(1):743-72. doi: 10.1146/annurev.micro.55.1.743. PMID: 11544373.

[2] Casadevall A. Fungal diseases in the 21st century: the near and far horizons. Pathogens & immunity. 2018;3(2):183. doi: 10.20411/pai.v3i2.249.

[3] Turner SA, Butler G. The Candida pathogenic species complex. Cold Spring Harbor perspectives in medicine. 2014 Sep 1;4(9):a019778. doi: 10.1101/cshperspect.a019778.

[4] Elbossaty WF. The Black Fungus is One of the Bad Conse-quences of COVID 19. Clin Onco. 2021;5(2):1-4.

[5] Mahomoodally MF. Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. Evidence-Based Complementary and Alternative Medicine. 2013 Oct;2013. doi: 10.1155/2013/617459.

[6] N Gokulakrishnan, Rubalakshmi G, Pugalendhi P, Vijayakymar N, Nirubama K, Prabhakar yellanur konda. Evaluation of pharmacological activities of diplocyclos palmatus. The International journal of analytical and experimental modal analysis. 2019;11(11):80-108. https://doi.org/10.1016/j.matpr.2020.09.435

[7] Sen S, Chakraborty R. Toward the integration and advancement of herbal medicine: a focus on traditional Indian medicine. Botanics: Targets and Therapy. 2015 Feb 13;5:33-44. DOI:10.2147/BTAT.S66308

[8] Balkrishna A, Singh A, Shankar R, Kumar R, Mishra AS, Joshi B, Chauhan A. Establishing the correct botanical identity of Śivaliñgī plant in India: A critical analysis based on various literatures. Journal of Medicinal Plants. 2021;9(3):156-67.

[9] Gautam VP, Aslam PR, Bharti KU, Singhai AK. Diplocyclos palmatus: a phytopharmacological review. International Journal of Research in Pharmacy and Chemistry. 2013;3(1):157-9.

[10] Venkateshwarlu G, Shantha TR, Shiddamallayya N, Ramarao V, Kishore KR, Giri SK, Sridhar B, Pavankumar S. Physicochemical and preliminary phytochemical studies on the fruits of "Shivalingi"[Diplocyclos palmatus (Linn.) Jeffrey]. Int J Ayur Med. 2001;2(1):20-6.

[11] Parveen-Bano D, Singh N. Preliminary Phytochemical Investigation on Leaves, Seeds Extract of Diplocyclos palmatus (L.) C. Jeffrey Medicinal plant. Int. J. Adv. Res. 2015;3:501-5

[12] Patel DK. Diplocyclos palmatus (L.) Jeffry: Morphological variations and medicinal values. J. Med. Plants. 2018;6:3-5.

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

[13] Venkateshwarlu G, Shantha TR, Shiddamallayya N, Ramarao V, Kishore KR, Giri SK, Sridhar B, Pavankumar S. Physicochemical and preliminary phytochemical studies on the fruits of "Shivalingi"[Diplocyclos palmatus (Linn.) Jeffrey]. Int J Ayur Med. 2001;2(1):20-6.

[14] Khan H, S Mubarak M, Amin S. Antifungal potential of alkaloids as an emerging therapeutic target. Current drug targets. 2017 Dec 1;18(16):1825-35. doi: 10.2174/1389450117666160719095517. PMID: 27440186.

[15] Wolters, B. 1966. Antimicrobial activity of plant steroids and triterpenes. Planta Med. 14:392-401. DOI: https://doi.org/10.1128/AAC.50.5.1710-1714.2006

[16] Dharajiya D, Pagi N, Jasani H, Patel P. Antimicrobial activity and phytochemical screening of Aloe vera (Aloe barbadensis Miller). Int J CurrMicrobiol App Sci. 2017;6(3):2152-62.doi: https://doi.org/10.20546/ijcmas.2017.603.246

[17] "Schrodinger. Schrodinger Release 2020-4." https://www.schrodinger.com.

[18] Bakht J, Islam A, Ali H, Tayyab M, Shafi M. Antimicrobial potentials of Eclipta alba by disc diffusion method. African Journal of Biotechnology. 2011;10(39):7658-67. DOI: 10.5897/AJB11.454

[19] Zaidan MR, Noor Rain A, Badrul AR, Adlin A, Norazah A, Zakiah I. In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. Trop biomed. 2005 Dec 1;22(2):165-70. PMID: 16883283.

[20] Iqbal E, Salim KA, Lim LB. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of Goniothalamusvelutinus (Airy Shaw) from Brunei Darussalam. Journal of King Saud University-Science. 2015 Jul 1;27(3):224-32. http://dx.doi.org/10.1016/j.jksus.2015.02.003

[21] Parveen-Bano D, Singh N. Preliminary Phytochemical Investigation on Leaves, Seeds Extract of Diplocyclospalmatus (L.) C. Jeffrey Medicinal plant. Int. J. Adv. Res. 2015;3:501-5.

[22] PDB: 1EIX; Harris P, Navarro Poulsen JC, Jensen KF, Larsen S (April 2000). "Structural basis for the catalytic mechanism of a proficient enzyme: orotidine 5'-monophosphate decarboxylase". Biochemistry. 39 (15): 4217–24. doi: 10.1021/bi992952r. PMID: 10757968.