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THERAPEUTIC POTENTIAL OF *EICHHORNIA CRASSIPES*: ANALGESIC AND ANTIDIARRHEAL EFFECTS IN PRECLINICAL AND *IN SILICO* STUDIES

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Abstract

Eichhornia crassipes is a plant known for its medicinal properties, and its active metabolites help explain its medicinal properties. Its pharmacological mechanisms and potential medical uses are being investigated through preclinical research and in silico analysis. In this study, we investigated the analgesic and anti-diarrheal properties of *E. crassipes* extracts in animal models and examined the effects of phytochemicals on cholinergic and COX-2 receptors by *In-silico* analysis. Animal models were used to assess the analgesic and anti-diarrheal effects of extracts from *E. crassipes*. *In-silico* experiments were also conducted to examine the impact of various phytochemicals on acetylcholine and COX-2 receptors. The effectiveness of 7,9-Di-tert-butyl-1-oxaspiro (4.5) deca-6,9-diene-2,8-dione and stigmasterol were compared to the standard drug Loperamide, and the pharmacokinetic characteristics of docked phytochemicals were also investigated. The research showed that extracts from *E. crassipes* have significant potential as pain relievers and anti-diarrheal agents in animals. Through computer simulations, certain plant compounds were found to interact well with cholinergic and COX-2 receptors. Specifically, stigmasterol and 7,9-Di-tert-butyl-1-oxaspiro (4.5) deca-6,9-diene-2,8-dione were just as effective as Loperamide. Additionally, the simulated plant compounds showed promising pharmacokinetic properties. The findings of this study traditional the conventional therapeutic practices of *E. crassipes* and illustrate the potential contribution of dynamic metabolites to its therapeutic pursuits. Considerable analgesic and anti-diarrheal potentials observed in animal models and *in silico* studies suggest that certain phytochemicals have promising impacts on cholinergic and COX-2 receptors.

Key words: *Eichhornia crassipes*; Antidiarrheal; Acute toxicity; Analgesic activity; Phyto-compounds; Molecular docking

Introduction

The symptoms of diarrhea are frequent wet feces, increased solutes in the intestinal canal, excess electrolyte release, the inability of the gut to absorb the recommended amounts of electrolytes, and enhanced gut motility (Wang *et al*., 2024). Diarrhea is a medical disorder that is brought on by bacteria, viruses, and other pathogens (Ahmad *et al.*, 2023; Naseer *et al*., 2025). Concerns regarding the impact of diarrhea on world health, particularly in young children under five, have been raised by the advent of antibiotic-resistant strains of the causative bacteria. This has the potential to hinder preventative efforts, especially when there are few treatment alternatives available (Afum *et al.*, 2022). The increased fatality rate among patients with secondary infections may be related to diarrheal illnesses, which may increase vulnerability to various diseases by negatively affecting the immune system (Balemba *et al.*, 2010). The investigation for possible plant products has become extremely important due to the negative side effects caused by synthetic medications and the markedly lower death rate associated with employing compounds derived from plants (Sadraei *et al.*, 2014; Mishra *et al.*, 2016).

Pain is an unpleasant sensation triggered in the nervous system and is associated with tissue damage or nerve damage. Pain can be acute (lasts for shorter intervals normally associated with injury) or chronic (lasts for longer intervals and normally associated with health conditions like diabetes and cancer) Pain is a global issue and approximately 20% of adults suffer from pain worldwide (Biswas *et al.*, 2011). Opiates, non-opioid analgesic agents, cannabinoids are more commonly used to treat both acute and chronic pains (Ashagrie *et al.*, 2023; Manan *et al*., 2025).

Acute toxicity testing has two primary objectives: to discover more about the biologic activity and mechanism of action of a chemical. In the context of chemical manufacture, handling, and usage, the data on acute toxicities produced by the tests is utilized for risk assessment and management (Walum, 1998; Sosa *et al.*, 2020). Acute systemic toxicity studies attempt to identify

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the dose-dependent adverse influence that may occur, and many suitable data may be obtained when evaluating a substance's full acute toxicity profile (Schaefer *et al*., 2022). *E. crassipes*is the free floating perennial aquatic plant growing to 0.3m, can flourish in a range of freshwater settings, including small ponds, marshes, and vast rivers (Gopal & Goel, 1993). *E. crassipes* has traditionally been used therapeutically in traditional healthcare systems for a variety of medical conditions, including easing digestive issues and acting as an antipyretic, anti-inflammatory, and antispasmodic agent (Shinwari, 2010; Kumar *et al.*, 2018), to relieve goitres, bleeding, swelling, burning, skin irritation, and as antiviral and anticancer agent (Jayanthi *et al.*, 2013).

The objectives of the current study were to assess the antidiarrheal and analgesic potentials and acute toxicity of *E. crassipes* using animal models and to identify potential phytochemicals using *In silico* approaches.

Materials and Methods

Plant collection: In the month of April 2021, fresh, healthy plants were obtained from Akbarpura (34.06°N, 71.72°E) Peshawar, Pakistan**.** The leaves, root, and bulbs of the *E. crassipes* plant were separated, washed to remove any adhesive materials and shade dried at room temperature. The plant specimens were identified using standard literature and further authenticated by Herbarium, Botany Department of Islamia Collage, Peshawar. The recognized plant species was preserved at the Islamia College Herbarium in Peshawar. Dried parts of plant were separately pulverized by an electric grinder and kept in airtight bottles.

Plant materials and extract preparation: Powdered *E. crassipes* of roots, bulbs, and leaves were each individually steeped in methanol for a week in an airtight glass container at a rate of 100.0g/250.0ml of solvent. The mixture was violently shaken periodically throughout the soaking period to ensure maximum extraction. Whatman filter paper No. 1 was then used to filter the mixture. The solubilized solvent in the crude drugs was concentrated under pressure of 50°C using a rotating vacuum evaporator. The filtrates were then gently heated on water bath to remove the remaining solvent and to obtain the *E. crassipes* root extracts of (ERE), bulbs (EBE) and leaves (ELE).

In vivo assays

Ethical committee approval: The ethical committee of biological sciences of botany department, Islamia college Peshawar, Pakistan, granted authorization for the current work, which includes in vivo experiments using mouse models, under approval number 2014/ICP-1180.

Experimental animals: Swiss-albino rats of males and females were purchased from the institutes of national of health, Islamabad, and the " A guide for caring for and usage of lab animals " was rigorously followed when using the animals in the experiments.

Antidiarrheal activity: Antidiarrheal potential of *E. crassipes* was assessed using charcoal meal assay. Test animals i.e., Swiss albino mice of either sex, aged 6-7 weeks or weighing 24-32 g were distributed among 11 groups (each group consisting of 6 animals). Animals in Group-I selected negative control, received normal saline (10 mg per kilogram of body mass). Atropine sulfate (5 mg/kg of body weight) was administered Intra-Peritoneally (IP) to the animals in Group II who were chosen as the positive control. The remaining 9 groups were given different dosages of ERE, EBE, and ELE (100, 150, and 200 mg/kg). Each group's animal subjects ingested 2 ml of an activated charcoal solution (charcoal meal) 30 minutes after the dosage delivery. Animals were slaughtered via cervical dislocation after receiving a charcoal diet for 50 minutes. The small intestine of each animal was then isolated and its total length was measured. The distance travelled by charcoal meal from the pylorus to the caecum was also measured for each animal. Percent inhibition in charcoal meal movement was calculated using the following formula (Awe *et al.*, 2011; Kamgang *et al.*, 2015; Suleiman *et al.*, 2017).

$$\% \text{ Inhibition} = \frac{\text{´D´ covered by charcoal in control - ´D´ covered by charcoal in treatment}}{\text{´D´ covered by charcoal in control}} \times 100$$

'D' = distance

Calculating the extract's percentage of inhibition can lead to Nunes Marona & Bastos Lucchesi (2004).

Analgesic activity: Analgesic potential of EBE, ERE and ELE was assessed using acetic acid induce writhings assay following standard protocol (Koster, 1959). Divide Swiss albino mice aged 6-7 weeks old or 24-32 g into divided into 11 groups. Prior to treatments all animals were fasted for 2 hours. Animals in Group-I, selected as negative control, received normal saline 10 ml/kg administered intraperitoneally. Group-II selected as positive control received 10mg/kg Diclofenac sodium administered intraperitonially. The remaining 9 groups were administered (IP) separately with 100 mg, 150 mg and 200 mg of EBE, ERE and ELE. After 30 min of doses administration 0.2 ml of acetic acid (1%) were injected (IP) to animals in all groups. After 5 min of acetic acid administration no. of writhings performed by treated animals were counted for 10 min. Percent inhibition in number of writhings were calculated using the following formula (Akuodor *et al.*, 2011).

$$\text{Percent inhibition} = \frac{A - B}{A} \times 100$$

where A is the average no. of writhings in control group, and B is the average no. of writhings in treatment group.

Acute toxic activity: The acute toxicity was assessed following Eumkeb *et al.*, 2010. Test animals (swiss albino mice) were distributed in 4 groups each consisting of six animals. Animal in Group-1, selected as control were administered 10 ml/kg normal saline (ip), while animals in the other 3 groups received separately a single dose of 5000 mg/Kg orally of ELE, EBE and ERE. Animals were

observed individually for symptoms of toxicity and lethality at the intervals of 60, 120, 240 and 360 min during the first 24 hours of dose administration and thereafter on daily basis for next 14 days.

In silico analysis

Molecular docking study: Using recognized software programs such as AutoDock Vina (version 1.1.2), Py-MoL 2.3, and B.I.O.V.A discovery studio version-4.5, molecular-docking investigation was achieved on the interaction sketch of twelve selected compounds from *E. crassipes* with their targeted proteins. To conduct an *in silico* analysis of the isolated chemicals from *E. crassipes*, the study adhered to the semi-flexible methodologies outlined in a number of other investigations (Hossain *et al.*, 2020; Hossain *et al.*, 2021; Khatun *et al.*, 2021).

Target protein selection: The identified compounds were subjected to computational docking to assess their potential bioactivities, such as antidiarrheal, acute toxicity, and analgesic properties. The M3 muscarinic acetylcholine receptor crystal structure (P.D.B I-D: 4.U-14) and cyclooxygenase 2 (C.O.X-2) crystal structure (P.D.B I-D: 1.C.X2) proteins were chosen for this purpose established on their biological processes and available data. These three-dimensional structures were stored in P.D.B format and taken via the R.C.S.B Proteins Data Bank (https://www.rcsb.org; retrieved on March 3, 2023) database. All of the collected biomolecules were subjected to processing using Py-MoL 2.3, which also removed any unwanted protein residues and water molecules. Using the Swiss P.D.B viewer, which employs an energy minimization program, non-polar hydrogen atoms were introduced to the purified proteins, and they were changed to their lowest energy state. The proteins were stored in the P.D.B file format after they had been cleaned and optimized.

Ligand preparation: Thirteen major compounds were obtained from the Pub-Chem compound-repository (https://pubchem.ncbi.nlm.nih.gov/; retrieved on 3 March 2023), and their 3D conformers and those of standard drugs loperamide (Pub-Chem CID: 3955) for antidiarrheal activity and diclofenac (Pub-Chem C.I.D: 3033) for analgesic activity were downloaded in S.D.F format. The ligands were then put sequentially into discovery-studio version-4.5, and a ligand library in P.D.B format was made using the Pub-Chem C.I.Ds of the ligands.

Ligand protein interaction: Molecular docking was carried out using the Auto-Dock Vina program (version 1.1.2) to ascertain the probable interaction profiles and binding affinities of the separated plant-based constituents with the target macromolecules. The computer-aided docking process employed a semi-flexible modeling approach. The target protein was selected as the macromolecule and loaded into the software. The target receptor of binding sites were observed utilizing computed atlas for surface topology of proteins (CASTp: http://sts.bioe.uic.edu/castp/). CASTp used for measuring and identification of the active pockets on surface of protein within the 3D structure interior. This analysis for accurate mapping and prediction of protein regions and key residues that interact with ligands. Hence, it has become an important resource for predicting protein sites involved in ligand binding (Dundas *et al.*, 2006). The amino acid residues reported by CASTp includes ILE116 ASP147 TYR148 SER151 ASN152 VAL155 TRP199 LEU225 THR231 THR234 ALA235 ALA238 PHE239 TRP503 TYR506 ASN507 VAL510 TYR529 CYS532 TYR533 for 4U14 protein and ASN34 CYS36 ASN39 PRO40 CYS41 GLN42 ARG44 GLY45 GLU46 CYS47 MET48 TYR130 GLY135 LEU152 PRO153 PRO154 GLN461 GLU465 for 1CX2 protein.

Import the 3D conformers of the ligand in SDF format into Auto-Dock Vina software and convert them to pdbqt format using the Open Babel tool to determine the best fit.

A grid box was then created with aspects of 58, 58, and 40 in the x, y, and z directions, respectively, with a grid size of 0.375 Å and the protein binding site in the middle of the box. For the COX-2 protein, the center grid box was located at coordinates 33.5096 Å, 9.09222 Å, and -5.14251 Å, while for the M3 muscarinic acetylcholine receptor, the coordinates were 7.47608 Å, 24.44 Å, and 344.944 Å. For each ligand, nine variants were generated and scored using the Auto-Dock-Vina scoring function. These conformations were then rated based on their binding energies, with the ones with the most advantageous (least) free binding energy selected for further study. PyMOL was used to examine the interactions between the target receptor and ligands, and Discovery Studio Visualizer (version 4.5) was utilized to create 3D and 2D figures for visualization purposes.

In silico toxicity assessment

To assess drug-likeness, molecular properties, and toxicity of the ligands, Molinspiration (Rhee and Tanaka, 1999) and pkCSM server (Pires *et al.*, 2015) were utilized (accessed on 5 March 2023). Table 4 displays the molecular weight, Log P, number of H2 bond acceptors, number of H2 bond donors, total surface area, rotatable bonds, and bioactivity of the ligands. Acute oral toxicity was determined based on toxicity class, and predictions were made for mutagenicity, carcinogenicity, hepatotoxicity, and nephrotoxicity. Additionally, drug likeness parameters and Lipinski's rule, were evaluated.

Results

Acute toxicity: Animals were observed on the first day after short intervals and then regularly for the next 14 days for toxicity or mortality. No visible symptoms of toxicity, behavioral changes, negative body changes or mortality were recorded for EBE, ERE and ELE at dose of 5000 mg/kg (Table 1).

Analgesic activity: Acetic Acid-Induced Writing Test: EBE, ERE, and ELE showed significantly significant peripheral analgesic activity in a dose-dependent manner compared to the negatives control at the selected dosages (100 mg/kg, 150 mg/kg, and 200 mg/kg) (p 0.01). As shown in Table 2, the incidence of writhings at ERE doses of 100 mg/kg, 150 mg/kg, and 200 mg/kg were 54.66±02.51, 46.00±03.00, and 30.33±01.52, respectively. For EBE, the reduction in writhings was measured at 100 mg/kg, 150 mg/kg, and 200 mg/kg as follows: 52.66±02.51, 47.33±01.52, and 37.33±0.57. ELE also exhibited significant activities at selected doses. At selected dosages of ELE (100 mg/kg, 150 mg/kg, and 200 mg/kg), the number of writhings was counted as 56.00±01.00, 45.66±1.52, and 33.66±4.04 correspondingly.

Table 1. Effects of extracts from *Echhornia crassipes* plant material at various concentrations on mortality rates and death rates in acutely poisoned mice.

Treatment	Dosage	No. of animals died	Percent mortality
Normal saline	10.00 ml/kg	00	00.00
E. crassipes root extract (ERE)	5000 mg/kg	00	00.00
E. crassipes bulb extract (EBE)	5000 mg/kg	00	00.00
E. crassipes leaf extract (ELE)	5000 mg/kg	00	00.00

Table 2. Effects of varying concentrations of extracts from *E. crassipes* plant parts on the quantity of writhing in analgesic mice.

Treatment	Dosage (mg/kg)	Number of writhing	Percent (%) inhibition
Normal saline	10.00ml/kg	72.00 ± 01.00	NA
Diclofenac sodium	10.00	19.00 ± 01.00*	73.61
ERE	100.00	54.66 ± 02.51*	24.08
	150.00	46.00 ± 03.00*	36.11
	200.00	30.33 ± 01.52*	58.87
EBE	100.00	52.66 ± 02.51*	26.86
	150.00	47.33 ± 01.52*	34.26
	200.00	37.33 ± 00.57*	48.15
ELE	100.00	56.00 ± 01.00*	22.22
	150.00	45.66 ± 01.52*	36.58
	200.00	33.66 ± 04.04*	53.25

The data has been expressed in Mean ± Standard deviation, * = Significantly different at $p<0.01$

Table 3. The efficiency of *E. crassipes* to treat diarrhea in a test using charcoal meal.

Treatment	Dose (mg/kg)	Overall intestinal length	Distance that charcoal covers	Percent inhibition
Control	10.00 ml/kg	57.00 ± 01.00	12.00 ± 01.00	78.94
Castor oil	10.00 ml/kg	52.00 ± 01.00	29.00 ± 01.00	44.23
ERE	100.00	41.00 ± 01.00	09.00 ± 01.00	78.04
	150.00	45.33 ± 01.52	08.66 ± 01.52	82.22
	200.00	41.33 ± 01.52	06.66 ± 01.52	85.36
EBE	100.00	46.33 ± 03.51	14.33 ± 01.52	69.56
	150.00	50.33 ± 01.52	10.33 ± 02.51	80.00
	200.00	55.33 ± 05.03	10.66 ± 01.52	81.81
ELE	100.00	44.66 ± 05.03	11.00 ± 01.00	75.00
	150.00	45.00 ± 02.00	08.66 ± 01.52	82.22
	200.00	43.00 ± 03.60	09.00 ± 1.00	79.06

Antidiarrheal assay: Use of all extracts/fractions at certain doses significantly reduced the motility of the charcoal meals in the small intestine. The highest concentration (200mg/kg) of ERE produced the highest activity among all extracts/fractions (Table 3). The highest activity was recorded for ERE with % inhibition values of 85.36%, whereas EBE had the lowest level of inhibition, at 69.56%. The average distance travelled by charcoal in the intestine was greatly enhanced in a dose-dependent manner by EBE (14.33±1.52) and all other fractions. Maximum reduction at the highest concentration was documented for ERE (6.66±1.52), followed by ELE (8.66±1.52) and ELE (9.00±01.00). Generally, EBE extracts were often determined to be the least efficient treatment.

Anti-diarrheal molecular docking study: The findings of the docking analysis for antidiarrheal efficacy are shown in Tables 4-5 and Figures 1-2. The study focused on exploring the potential antidiarrheal activity of *E. crassipes* plant using one major receptor (M3 muscarinic acetylcholine receptor, PDB: 4U14) involved in intestinal motility. When compared to loperamide, a common analgesic, the binding affinity of 7,9-Di-tert-butyl-1-oxaspiro (4.5) deca-6,9-diene-2,8-dione towards the M3 muscarinic acetylcholine receptor was shown to be greater (-9.3 kcal/mol) (PDB: 4U14). The compounds were docked against M3 muscarinic acetylcholine receptor, and the docking scores were ranked as follows: 7,9-.Di-.tert-butyl-1-.oxaspiro (4.5) deca-6,9-.diene-2,8-.dione > loperamide > Stigmasterol > Phenol, 3,5-bis(1,1-dimethylethyl)- > Dibutyl phthalate > 9,12,15 Octadecatrienoic Acid > Phytol > Palmitic acid vinyl ester > 2-Bromooctadecanal = Hexadecanoic acid, methyl ester > 7 Oxabicyclo. [4.1.0] heptan.-1-ol, acetate > Propiloc acid > Dodecamethyl cyclohexasiloxane (Table 4). The selected phytoconstituents from the plant extracts were discovered to interact with the sites that are active of the M3 muscarinic acetylcholine enzyme, including TYR 148, ALA 238, and TRP 503, through alkyl and pi-alkyl interactions. Moreover, one hydrogen bond was formed when 7,9-Di-tert-butyl-1-oxaspiro (4.5) deca-6,9-diene-2,8-dione interacted with the M3 muscarinic acetylcholine receptor. Figure 1 shows the active binding sites of the M3 muscarinic acetylcholine receptors upon interaction with certain phytoconstituents from the extract of the *E. crassipes* plant.

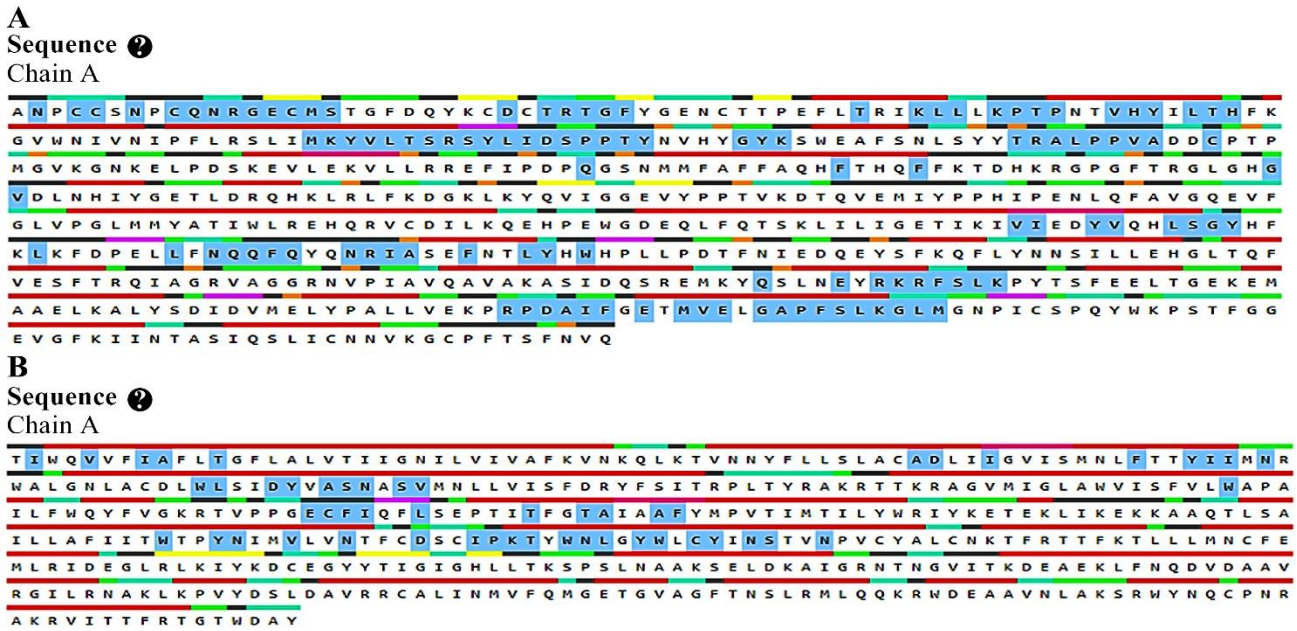

Fig. 1. Active sites finding of M3 muscarinic acetylcholine receptor (P-D-B I-D: 4-U-14) and cyclooxygenase 2 (C.O.X-2) (P.D.B. I-D: 1.C.X2) proteins through C.A.S.Tp server.

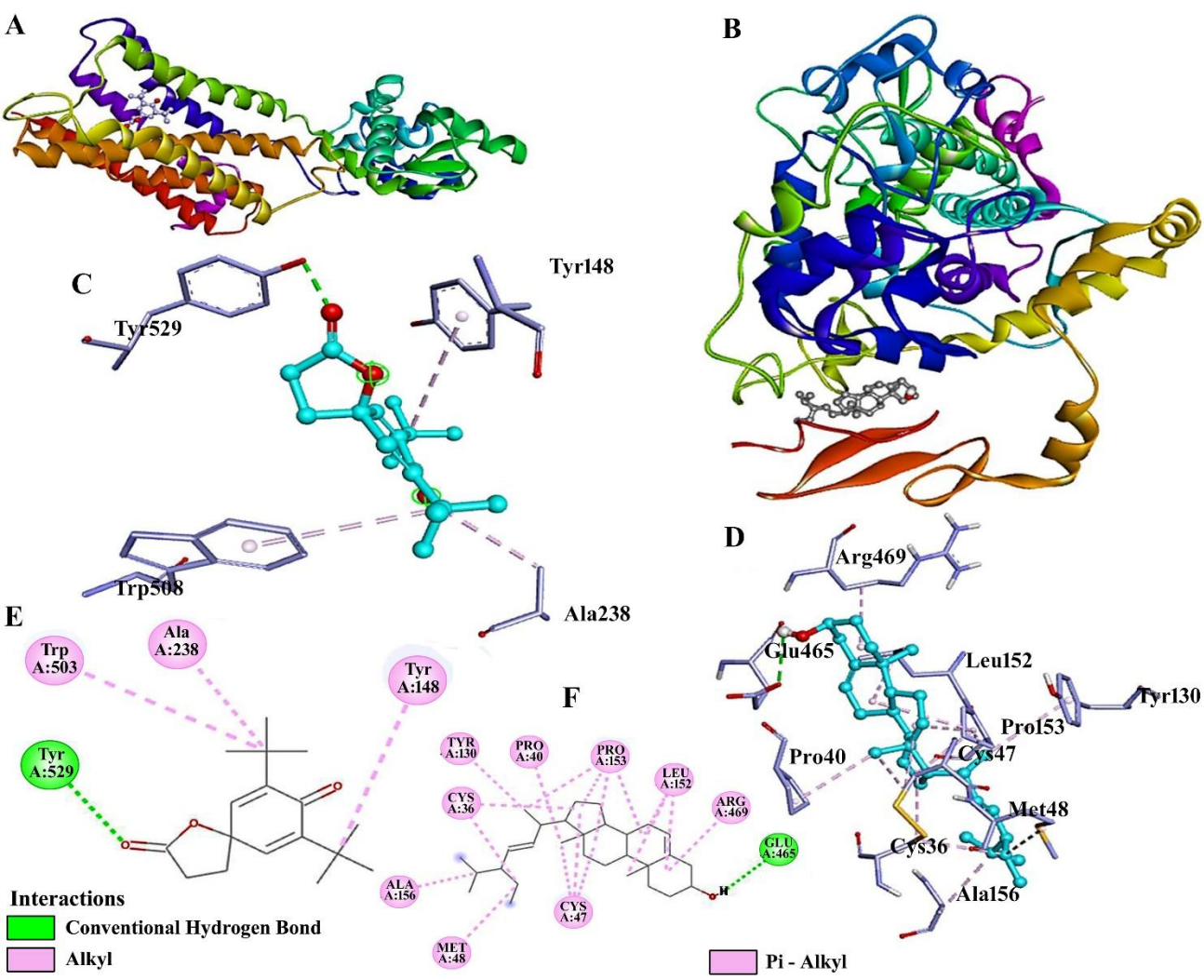

Fig. 2. Demonstrates the intricate three-dimensional structures of M3 muscarinic acetylcholine receptor, P.D.B: 4.U.14 and C.O.X-2 protein, P.D.B I-D: 1.C.X-2; **(A-B, C, E)** depict the incredibly intricate 2D contacts between the 4, U14 receptor and the 7,9-Di-tert-butyl-1, oxaspiro (4.5) deca-6,9-diene-2,8-dione, as well as their complicated 3D structural relationships; and **(D, F)** illustrate the extremely intricate 2D interactions between the 1CX2 receptors and the stigmasterol molecule.

Table 4. Prediction of toxicity of compounds computed by pkCSM server.

S. No.	Ligand	AMES toxicity	Max. tolerated dose (human)	hERG I inhibitor	hERG II inhibitor	Oral rat acute toxicity (LD50)	Oral rat chronic toxicity (LOAEL)	Hepatotoxicity	Skin sensitization	T. Pyriformis toxicity	Minnow toxicity
1.	DODECAMETHYLCYCLOHEXASILOXANE	No	0.62	No	No	4.111	-0.157	No	No	0.235	0.951
2.	PROPIOLIC ACID	No	1.215	No	No	1.844	1.4	No	No	-0.949	2.603
3.	Phytol	No	-0.301	No	Yes	1.848	1.232	No	Yes	1.714	-1.137
4.	2-Bromooctadecanal	No	-0.06	No	Yes	2.266	0.943	No	Yes	1.061	-2.042
5.	Hexadecanoic acid, methyl ester	No	0.178	No	No	1.635	2.998	No	Yes	1.935	-1.373
6.	Dibutyl phthalate	No	1.536	No	No	1.806	2.326	No	No	1.1	0.09
7.	9,12,15 Octadecatrienoic Acid	No	-0.84	No	No	1.441	3.115	Yes	Yes	0.722	-1.183
8.	Stigmasterol	No	-0.664	No	Yes	2.54	0.872	No	No	0.433	-1.675
9.	7,9-Di-tert-butyl-1-oxaspiro (4.5) deca-6,9-diene-2 ,8-dione	No	0.219	No	No	1.75	1.913	No	No	1.333	0.509
10.	Phenol, 3,5-bis(1,1-dimethylethyl)-	No	0.409	No	No	2.346	1.736	No	Yes	1.667	-0.108
11.	7 Oxabicyclo [4.1.0]heptan-1-ol, acetate	Yes	0.783	No	No	2.249	2.174	Yes	Yes	-0.888	2.36
12.	Palmitic acid vinyl ester	No	0.205	No	No	1.689	3.051	No	Yes	1.83	-1.546

Table 5. Drug-likeness predictions of compounds and molecular properties of compound computed by Molinspiration.

S. No.	Phytochemicals	Molecular weight	NHD	NHA	miLogP	NoR	TPSA	No of violations	GPCR ligand	Ion channel modulator	Kinase inhibitor	Protease inhibitor	Enzyme inhibitor
1.	DODECAMETHYLCYCLOHEXASILOXANE	444.93	6	0	2.27	0	55.40	0	0.17	0.16	0.11	0.25	0.23
2.	PROPIOLIC ACID	54.05	1	0	0.29	0	17.07	0	-3.75	-3.67	-3.82	-3.21	-3.53
3.	Phytol	296.54	1	1	6.76	13	20.23	1	0.11	0.16	-0.32	0.00	0.31
4.	2-Bromooctadecanal	347.38	1	0	8.62	16	17.07	1	-0.16	-0.25	-0.43	-0.04	0.09
5.	Hexadecanoic acid, methyl ester	270.46	2	0	7.37	15	26.30	1	-0.11	-0.05	-0.34	-0.13	0.04
6.	Dibutyl phthalate	278.35	4	0	4.43	10	52.61	0	-0.16	-0.09	-0.27	-0.25	-0.07
7.	9,12,15 Octadecatrienoic Acid	278.44	2	1	5.84	13	37.30	1	0.33	0.23	-0.19	0.13	0.42
8.	Stigmasterol	412.70	1	1	7.87	5	20.23	1	0.12	-0.08	-0.48	-0.02	0.53
9.	7,9-Di-tert-butyl-1-oxaspiro (4.5) deca-6,9-diene-2 ,8-dione	276.38	3	0	2.31	2	43.38	0	-0.17	-0.26	-0.39	-0.25	0.15
10.	Phenol, 3,5-bis(1,1-dimethylethyl)-	206.33	1	1	4.80	2	20.23	0	-0.31	0.08	-0.43	-0.56	-0.09
11.	7 Oxabicyclo [4.1.0]heptan-1-ol, acetate	156.18	3	0	1.68	2	38.83	0	-0.74	-0.23	-1.09	-0.59	-0.05
12.	Palmitic acid vinyl ester	282.47	2	0	7.67	16	26.30	1	0.04	0.06	-0.32	0.02	0.07

NHD stands for number of hydrogen donor; NHA = Number of hydrogen acceptor; NoB = Number of rotatable bonds; and TPSA = Total polar surface area

Inhibition of COX-2 proteins: analgesic activity: To investigate the molecular mechanism underlying the analgesic activity of the *E. crassipes* plant extract, molecular docking was performed on the compounds that isolated with COX-2 protein (P.D.B.-ID: 1-C-X-2). The results showed that all the compounds had higher binding affinity towards COX-2 enzyme. Notably, Stigmasterol outperformed the standard medication Loperamide (-7.7 kcal/mol) in terms of docking score (-9.0 kcal/mol). Analysis of the docking fits revealed that each compound interacted with the target enzyme in various ways. The compounds in this study were tested for their affinity towards a receptor, and their order of affinity was found to be Stigmasterol > loperamide > Phenol, 3,5-bis(1,1-dimethylethyl)-> Dibutyl phthalate > Phytol = 7,9-Di-tert-butyl-1-oxaspiro (4.5) deca-6,9-diene-2,8-dione > 9,12,15 Octadecatrienoic Acid > 2-Bromooctadecanal = Hexadecanoic acid, methyl ester = Palmitic acid vinyl ester > 7 Oxabicyclo [4.1.0] heptan-1-ol, acetate > Propiloc acid > Dodecamethylcyclohexasiloxane. With the exception of Stigmasterol, which established a bond of hydrogen with the GLU 465 amino acid at an offset of 2.95869 from the protein, the bulk of the binding contact sites were hydrophobic in character (Table 4). The receptor's active binding amino acids were CYS-36, PRO-40, CYS-47, MET-48, TYR-130, LEU-152, PRO-153, ALA-156, and ARG-469, which interacted with the substance stigmasterol in an alkyl and pi-alkyl fashion. Figure 2 provides a list of associated amino-acids (A.A) together by their three-letter I-Ds.

ADMET and toxicity prediction

We calculated ADMET parameters and toxicity using *In silico* techniques like pkCSM and Molinspiration to get insight into the pharmacokinetics characteristics of the drugs. Table 5 lists the physico-chemical and pharmacokinetic properties of each substance. The compounds' physicochemical characteristics were determined to be in accordance with the applicable criteria because of their good oral bioavailability and molecular weight range of 54.05-444.93 g/mol. The compounds showed a high ability to penetrate into the cellular plasma membrane since their topological polar area (TPSA) values were likewise within a desirable range (below 140 A2). For hydrogen bond donors (HBD) (1-6), acceptors of hydrogen bond (HBA) (0-1), and bonds that are rotatable (0-16), all target compounds showed acceptable values. Additionally, Lipinski's rule of five was perfectly satisfied by the compounds' medicinal qualities. Results also showed that the chemicals weren't harmful or carcinogenic. Overall, the outcomes show that the compounds have strong drug-like characteristics.

During the drug discovery process, toxicity assessment of compounds is a crucial step (Maliehe *et al.*, 2020). To evaluate their potential toxicity to both humans and the environment, various toxicities were assessed for each molecule (Table 5). The Ames test was used to assess a compound's mutagenic potential, and all compounds were categorized as non-Ames hazardous except for 7 Oxabicyclo. [4.1.0.] heptan. -1-ol, acetate, which is considered to be of uncertain carcinogenicity. The degree of toxicity of a substance is determined by the human dosage that is most well tolerated, and compared to other compounds, PROPIOLIC ACID and Dibutyl phthalate had a significantly higher MTD. Furthermore, only compounds 3,4,5,7,10,11,12 were found to cause skin sensitivity, while compounds 3,4,8 were predicted to be hEGR II inhibitors but did not show any hEGR I inhibition results. However, compounds 7 and 11 were predicted to be hepatotoxic and may cause drug-induced liver damage, while all other phytochemicals were predicted to be non-hepatotoxic.

Discussion

There have been centuries of usage of both traditional and complementary medicine. Some of the well-liked and successful methods to treat the sickness is herbal-based therapy. It is also an evidence-based medication due to its prevalence and widespread utilization (Catarino *et al.*, 2016). *E. crassipes* is an important medicinal, traditionally used to cure and manage several human ailments, specially known for its Pain-relieving and antibacterial potentials. The plant is also used as antidiabetic, antioxidant and anticancer agent (Ben Bakrim *et al.*, 2022).

In acute toxicity test a single dosage of 5000 mg/kg of EBE, ERE and ELE did not result in any deaths, clinical symptoms of fever increase or reduction, changes in skin colour, eye colour, overall appearance, diarrhea, or drowsiness. The intake of food and water was unaffected after 14 days of oral EBE, ERE and ELE treatments. It shows that there were no negative impacts on the animals' development or ability to eat because of the extracts. *E. crassipes* is also one of the ancient medicinal plants that are usually employed as a kind of treatment for inflammatory conditions and discomfort (Golshani *et al.*, 2004; Ayanda *et al.*, 2020). The acetic acid-induced writhing experiment was utilized to ascertain the plant extract's peripheral analgesic effects. When compared to the negative control, the three doses of *E. crassipes* extracts (100, 150, and 200 mg/kg) considerably ($p<0.05$ and $p<0.01$) decreased the amount of writhing. The most probable processes by which the extract in this model generated peripheral analgesia may include suppression of production and release of various endogenous inflammatory mediators as well as a decrease in the sensitivity of peripheral nociceptors to chemically induced pain (Yimer *et al.*, 2020; Khan *et al.*, 2024). These suggested pathways are consistent with the guiding principles, which claim that any substance that reduces writhing will exhibit analgesia by preventing the production and release of PGs as well as the transmission of peripheral pain (Debebe *et al.*, 2007).

Diarrhea-related morbidity and death have become significant worldwide health issues. The scientific community prefers herbal techniques to treating ailments because of the side effects of pharmaceutical treatments. Although there are several anti-diarrheal traditional plants that have been shown to be effective, little emphasis has been paid to using these Phyto-preparations for treating diarrhea and related illnesses (Rawat *et al.*, 2017). In the castor oil-induced diarrhoea model and the charcoal meal test, extracts of *E. crassipes* dramatically decreased the rate of defection, which may be related to the extracts' capacity to block prostaglandin synthesis. According to Adeyemi *et al.*, 2011, the effect of *Talinum triangulare's* aqueous root extract on the mice's gastrointestinal system was assessed using their regular

intestinal transit. In mice treated with castor oil. The herb extract at 2000 mg per kilogram significantly ($p<0.05$) reduced the frequency of defecation and also cruelled diarrhea. At 200 mg/kg, the methanolic extract of *E. crassipes* also produced notable results. Stigmasterol, Phenol, 3,5-bis(1,1-dimethylethyl), Dibutyl phthalate, Phytol = 7,9-Di-tert-butyl-1-oxaspiro, deca-6,9-diene-2,8-dione, Octadecatrienoic Acid, 2-Bromooctadecanal, Hexadecanoic acid, methyl ester, Palmitic acid vinyl ester, Propiloc acid, Dodeca methyl cyclohexasiloxaneare are important secondary metabolites that have been discovered from *E. crassipes* (Lalitha *et al.*, 2012), which most likely account for the plant's observed antidiarrheal properties (Ahmad *et al.*, 2023). *In silico* studies of the phytochemicals confirmed the proposed potentials of these phytochemicals.

Conclusions

The findings of the study confirmed the traditional therapeutic applications of *E. crassipes* which may be attributed to the active metabolites present in the plant. The plant extracts demonstrated notable analgesic and antidiarrheal properties in animal models. *In silico* studies of the selected phytochemicals showed promising analgesic and antidiarrheal potentials by altering acetylcholine and COX-2 receptors. The potentials of 7,9-Di-tert-butyl-1-oxaspiro (4.5) deca-6,9-diene-2,8-dione, Stigmasterol are comparable with standard drugs (Loperamide). Furthermore, the docked phytochemicals showed promising pharmacokinetic properties. Detailed investigation and clinical trials are needed to confirm the possible mode of action of the docked phytochemicals for their future pharmaceutical applications.

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