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ORIGINAL ARTICLE

Insecticidal and Bactericidal Activities of *Cassia nigricans* Vahl and Molecular Docking Analysis of Insect Acetylcholinesterase

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ABSTRACT

Objectives: This study focused on the phytochemical, insecticidal, and bactericidal activities of *Cassia nigricans* Vahl, as well as molecular docking analysis of an acetylcholinesterase (AChE) inhibitor as a promising natural insecticide.

Materials and Methods: The leaves of *C. nigricans* were successively extracted with n-hexane, acetone, and methanol. Silica gel column chromatography of the methanol extract yielded compound 1. The insecticidal properties of the extracts and compound 1 were evaluated in terms of contact toxicity against *Sitophilus zeamais*. Bactericidal activity was achieved by photodynamic inactivation of fecal coliforms (FCs) and enterococci in water using extracts and compound one as natural photosensitizers. Compound 1 was analyzed for physicochemical and pharmacokinetic parameters and molecular docking against the AChE protein (6XYU).

Results: Compound 1 was characterized as emodin (1,3,8-trihydroxy-6-methylanthracene-9,10-dione) using 1D-2D-^{1}H-^{13}C nuclear magnetic resonance and mass spectrometry. Insecticidal properties showed that emodin exhibited the highest toxicity with an lethal concentration 50 (LC_{50}) = 5.00 mg/mL compared with all extracts. The *n*-hexane extract showed the highest insecticidal activity (LC_{50} = 177.48 mg/mL) compared with the methanol (LC_{50} = 195.08 mg/mL) and acetone (LC_{50} = 374.14 mg/mL) extracts. Complete inhibition of fecal enterococci by photosensitization was observed after 60 min of light exposure to emodin-treated water at all concentrations (1-5 mg/mL) and 120 minutes for FCs under the same conditions. Based on the docking score, the binding energy of emodin (-6.38 kcal/mol) was close to that of the marketed insecticide pirimiphos-methyl (-6.25 kcal/mol).

Conclusion: In addition, emodin was subjected to insecticide probability prediction and absorption, distribution, metabolism, excretion, and toxicity analysis and was found to be satisfactory as a natural insecticide. Emodin is a promising candidate for insecticidal pest control.

Keywords: *Cassia nigricans*, insecticidal, bactericidal, molecular docking

INTRODUCTION

Several methods are used to control stored grain insect pests: smoking, heating, and synthetic chemicals.[1] Synthetic insecticides have drawbacks, and their high cost limits their accessibility to farmers. To minimize post-harvest losses, plants containing alkaloids, terpenoids, and anthraquinones are used as natural insecticides and are of purely ecological interest as they are not harmful to the environment.[2] Some compounds can act as insecticides by inhibiting insect acetylcholinesterase (AChE) and preventing the breakdown of acetylcholine, the accumulation of which causes insect death. Many compounds such as the organophosphates used as insecticides for pest control exert their effects as AChE inhibitors (AChEI).[3] Numerous models are used for AChEI as part of the study of the insecticidal effect, such as the molecular docking analysis against insect AChE.[4]

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Some natural compounds used to protect stored foodstuffs can have a bactericidal effect.[5] The bactericidal effect may be due to the photodynamic inactivation of microorganisms by the photosensitizing effect of plants or compounds in the presence of light.[6] This photosensitizing effect is directly linked to the presence of substances that generate singlet oxygen, which can damage microorganisms present in the environment.[7] The alkaloids, coumarins, and anthraquinones present in plants are responsible for photosensitizing and bactericidal activities.[8]

Cassia nigricans Vahl (Caesalpiniaceae) was previously studied for its antimicrobial, insecticidal, analgesic, anti-inflammatory, and antiplasmodial activities.[9]

This work presents for the first time the insecticidal activity by contact toxicity against *Sitophilus zeamais*, the photosensitized inactivation of fecal coliforms (FC) and fecal enterococci (FE) by the leaves of *C. nigricans*, the molecular docking of the isolated anthraquinone against *Drosophila melanogaster* AChE (DmAChE) and the analysis of its absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties.

MATERIALS AND METHODS

Plant material

The leaves of *C. nigricans* were collected in Dobem, Chad, in September 2021. The plant was identified at the National Herbarium of Cameroon, Yaounde, Cameroon where a Voucher Specimen 26339 SFR/Cam was deposited.

Extraction and isolation

Dried and ground *C. nigricans* leaves (1 kg) were extracted with 4 L of *n*-hexane for 48 hours and concentrated to produce the *n*-hexane extract (HE). The residues were successively extracted with acetone and methanol following the same procedure used previously to obtain the acetone extract (AE) and the methanol extract (ME).

Column chromatography (CC) of ME (40 g) on silica gel (60-120 Mesh) using a gradient of increasing polarity of *n*-hexane/EtOAc (1-100% EtOAC) yielded 83 fractions. Fractions 39-54, eluted with the Hexane/EtOAc (3/1) system formed a precipitate that was purified by recrystallization with 5% methanol/EtOAc yielding compound 1 (1 g). ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz using tetramethylsilane as reference (spectrometer Bruker AM-400 Darmstadt, Germany Company). Analytical thin layer chromatography (TLC) plates on silica gel 60 F254 TLC (Merck, Germany) were used for TLC analysis.

Obtaining and rearing insects

S. zeamais strains were collected from infested maize grains from Booster Soumian Entreprise, an agropastoral and quality control company in Chad. Mass rearing was performed with adult insects collected from already-infested maize grains at the Booster Soumian Entreprise rearing site. The insects were reared in the dark on white maize grains in a chamber (temperature 28 ± 2 °C, relative humidity 65 ± 5%).[10]

Contact toxicity tests

Contact toxicity tests were performed using Ndomo et al.[10] Five concentrations of crude extracts (25, 50, 100, 200, and 400 mg/mL) and compound 1 (5, 12.5, 25, 50, and 100 mg/mL) were prepared. One mL of each dose was added to 40 g of clean and undamaged maize grains. After evaporation of the solvent, each insect was infested with a batch of 20 two-day-old unsexed adult insects. The marketed insecticide pirimiphos-methyl was used as a positive control. The number of dead insects was estimated daily for 3 days. Abbott's (1925) formula was used to calculate the corrected insect mortality rate.[11]

% Mortality = (Number of dead insects / Total number of insects) x 100

Mc (%) = (Mt - Mo) / (100 - Mo)

Mc: Corrected mortality (%); Mt: Mortality in treated batches (%); Mo: Mortality in untreated controls (%)

LC_{50} values were determined by Probit analysis.[12]

Microorganisms

The microorganisms used for the photosensitization tests were FC and FE from an open well for consumption by the population of Dobem, Chad. This water contained 25.10^2 colony-forming units (CFU) FC/100 mL and 15.10^2 CFU FE/100 mL.

Photosensitized inactivation of bacteria

Five concentrations of 1, 2, 3, 4, and 5 mg/mL of ME and compound 1 were used for photosensitization experiments according to the method of Sunda et al.[13] A batch of treated water samples (with ME and compound 1) and another batch of untreated water samples (blank) were exposed to light (ultraviolet lamp, brand B-100 AP, emitting between 320 and 400 nm, with a maximum at 365 nm). The lamp was placed 15 cm from the water samples for 0, 30, 45, 60, 120, and 180 min. A batch of treated and untreated samples was kept in the dark. For each dataset, the standard error was calculated (mean ± standard deviation).

Bacteriological analysis

Bacteriological analysis was performed via culture in rapid *Escherichia coli* and Bile Esculin agar media for FC and FE. After the photosensitization experiments, 1 mL of water from the samples was inoculated into the culture medium, and the number of colonies formed after 24 h of incubation at 44.5 °C was counted.[12] The number of germs in CFU was determined in water samples before and after the application of the photosensitizer.

Molecular docking and dynamics analysis

Molecular Operating Environment (MOE, 2013) software[14] was used to dock a DmAChE protein against compound 1 and two marketed insecticides into the protein's active site. The crystal structure of DmAChE (PID: 6XYU, resolution: 2.51 Å) was downloaded from the Protein Data Bank (https://www.rcsb.org/). The water molecules and heteroatoms in the proteins were removed. Compound 1 and two marketed insecticides: emodin (PubChem CID 3220), cypermethrin (PubChem CID 91691), and pirimiphos-methyl (PubChem CID 34526), were collected from the chemical database (https://pubchem.ncbi.nlm.nih.gov/), input into the MOE program, and subjected to 3D protonation and energy minimization. The MMFF94X force field

was used to minimize the number of ligands and the protein structure. After docking, the best and top conformations were determined based on the S-scores and the interacting residues.[15] The physicochemical, pharmacokinetic, and ADMET properties of compound 1 were predicted using the SwissADME web tool.[16]

Statistical analysis

Experiments were performed in triplicate, and mean values were obtained. All statistical analyses were performed using SPSS version 21.0. Data on corrected mortality were subjected to analysis of variance using the Waller-Duncan test.

RESULTS

Phytochemical studies

Extraction of *C. nigricans* leaves with HE, AE, and ME gave yields of 28.6 g (2.9%), 36.5 g (3.9%), and 95.3 g (11.2%) of crude extracts respectively.

Characterization of the isolated compound

Chromatographic fractionation of ME yielded compound 1, whose structure (Figure 1) was established by spectral data and by comparison with literature data.[17]

Compound 1: Orange needles; melting point 260-263 °C. Solubility in dimethyl sulfoxide (DMSO); HRESIMS at m/z 270.3 (calculated for $C_{15}H_{10}O_5$). ESI-MS (70 ev): m/z (rel. Int.) 271.3 (5), 226.5 (10), 224.4 (15), and 222.6 (20). ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6) (Table 1).

Mortality of S. zeamais

A time- and concentration-dependent increase in the mortality rate of adult *S. zeamais* was observed for all extracts and emodin (Table 2). The highest dose (400 mg/mL) caused 95.7, 22.2, and 55.7% mortality in insects on the second day of exposure for HE, AE, and ME, respectively. The insecticidal activity of emodin was found to be higher than that of HE, which was the most active extract tested. Similarly, the activity of emodin (LC_{50}, 5 mg/mL) was higher than that of the positive control, Pirimiphos-methyl (LC_{50}, 1.25 mg/mL) (Table 3).

Photosensitizer inactivation by bacteria

Results of the light exposure of water samples treated and untreated with ME and emodin showed complete inactivation of FC after 3 h of exposure for ME treatment and 2 h for emodin treatment (Table 4). Complete inhibition of EF was observed after 2 h of light exposure in water treated with ME and after 1 h of treatment with emodin for all tested concentrations. No inactivation of FC et FE in the water was observed after 3 h of light exposure in the untreated water samples and all treated water samples exposed to darkness. The photo disinfection efficiency increased as a function of concentration and irradiation time (Figures 2, 3).

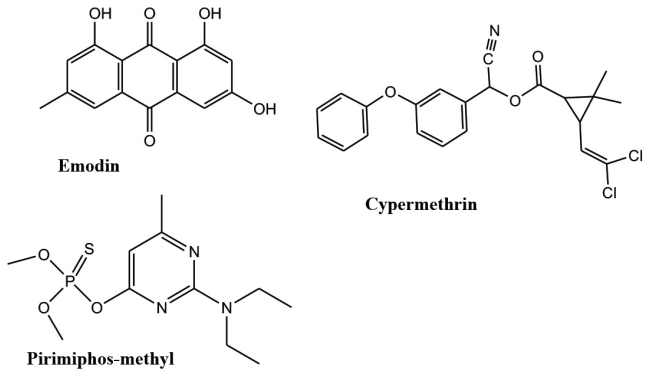

Figure 1. Structures of the isolated compounds and drugs

Molecular docking

A molecular docking analysis of emodin was performed to study its binding to DmACHE and to compare it with marketed insecticides used to control pests in crops, fruit trees, and ornamental plants. The crystal structure of DmAChE was used as a model to study insecticidal potential due to the unavailability of DmAChE from *S. zeamais* in the PDB. The binding energy of emodin (-6.38 kcal/mol) was close to that of pirimiphos-methyl (-6.25 kcal/mol) and lower than that of cypermethrin (5.52 kcal/mol) (Table 5). Emodin has two types of interaction bonds (pi-pi) with residues TRP83 and TYR370, similar to those of tacrine-derived insecticides used as ligands.[18] Emodin and pirimiphos-methyl have a common amino acid TRP83 residue for binding to the 6XYU active site (Table 5).

Table 1. Spectra of compound 1 in DMSO-d6

N°	1 δ_c en ppm	DEPT-135	Published[16] δ_c en ppm	1 H-multiplicity (J in Hz)
1	166.0	C	166.03	
2	108.3	CH	108.39	1Hd (2.4)
3	161.8	C	161.87	
4	109.2	CH	109.43	1Hd (2.4)
5	120.8	CH	124.59	1Hs
6	148.6	C	148.71	
7	124.5	CH	120.93	1Hs
8	164.8	C	164.90	
9	190.0	C	190.19	
10	181.6	C	181.85	
4a	135.4	C	135.58	
8a	113.6	C	113.85	
9a	109.2	C	109.22	
10a	133.1	C	133.29	
Ar-CH_3	21.9	CH_3	21.96	3Hs
1-OH	–			1Hs
3-OH	–			1Hs
8-OH	–			1

DMSO: Dimethyl sulfoxide, DEPT: Distortionless enhancement by polarisation transfer

ADMET analysis

Evaluation of the pharmacokinetic parameters of emodin and other marketed insecticides enabled us to assess insecticide-likeness as well as intestinal absorption and brain permeation, which are key toxicokinetic parameters that determine insecticide toxicity, including neurotoxicity. The insecticide-likeliness of emodin was evaluated based on Tice's rule of five, which helps identify herbicides and insecticides.[19] The pharmacokinetic properties (Table 6) show that all ligands have hydrogen bond donors ≤2 and hydrogen bond acceptors between 1 and 8. The molecular weights of these ligands ranged from 150 to 500 g/mol, and the ClogP values ranged from 0 to 5. The number of notable bonds for all ligands is <12. The same is true for the bioavailability radar of the predicted physicochemical and pharmacokinetic properties (Figure 4).

Table 2. Effects of the extracts and emodin on the mortality of *S. zeamais*

Samples	Concentration (mg/mL)	Mean percentage mortality ± standard error at 24 to 72 h post-treatment		
		24 h	48 h	72 h
HE	25.0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]
	50.0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]
	100.0	0.0 ± 0.0[a]	12.6 ± 0.4[b]	17.4 ± 0.5[b]
	200.0	12.3 ± 0.3[b]	32.3 ± 0.3[c]	50.3 ± 0.3[c]
	400.0	95.7 ± 0.2[c]	100.0 ± 0.0[d]	100.0 ± 0.0[d]
AE	25.0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]
	50.0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]
	100.0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]
	200.0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]
	400.0	22.2 ± 0.9[b]	42.3 ± 0.2[b]	58.3 ± 0.3[b]
ME	25.0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]
	50.0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]
	100.0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	13.6 ± 0.4[b]
	200.0	0.0 ± 0.0[a]	17.6 ± 0.6[b]	32.6 ± 0.4[c]
	400.0	55.7 ± 0.5[b]	76.6 ± 0.3[c]	100.0 ± 0.0[d]
Emodin	5.0	18.0 ± 0.4[a]	24.1 ± 0.6[a]	58.3 ± 0.5[a]
	12.5	24.6 ± 0.6[a]	38.6 ± 0.8[a]	63.5 ± 0.9[a]
	25.0	38.6 ± 0.4[b]	52.9 ± 0.7[b]	73.0 ± 0.5[b]
	50.0	70.7 ± 0.6[c]	85.5 ± 0.4[c]	100.0 ± 0.0[c]
	100.0	100.0 ± 0.0[d]	100.0 ± 0.0[d]	100.0 ± 0.0[c]
Control (untreated)	0.0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]

Each value is the mean ± standard error of three replicates. Means followed by the same letter within a column are not significantly different ($p < 0.05$) from each other using the new Duncan multiple range test. HE: n-hexane extract, AE: Acetone extract, ME: Methanol extract

Table 3. LC_{50} values of the extracts and emodin at 72 h

Extract	LC_{50} (mg/mL)	95% CL[a] (mg/mL)	Pearson chi-square	Slope ± SE	Intercept ± SE
HE	177.48	(146.59-217.43)	2.23	5.12 ± 1.00	-11.52 ± 2.24
AE	374.14	(310.86-513.55)	0.71	6.77 ± 2.14	-17.43 ± 5.45
ME	195.08	(160.72-241.84)	5.18	4.88 ± 0.94	-11.19 ± 2.15
Emodin	5.00	(1.39-8.50)	5.25	1.55 ± 0.40	-1.08 ± 0.49

LC: Lethal concentration, CL: Confidence limit, SE: Standard error, HE: *n*-hexane extract, AE: Acetone extract, ME: Methanol extract, [a]Confidence limit

DISCUSSION

The high yield of ME (11.2%) compared to HE (2.9%) and AE (3.9%) could be due to the presence of many more polar compounds in the leaves of *C. nigricans*. Terpenoids, flavonoids, anthraquinones, and quinones were detected in all extracts. However, coumarins, glycosides, alkaloids, and tannins were not detected in HE compared with AE and ME. These results are similar to those of previous studies on the plant.[9] Compound 1 was identified as emodin, a known compound, by MS and 1D and 2D NMR analyses, and its structure was confirmed by literature data.[17] The analytical TLCs performed on the three extracts: HE, AE, and ME) showed that only ME had a spot (R_f = 0.56) corresponding to emodin. The yield of emodin obtained from ME was 1g (1.04%).

Insect mortality tests showed that HE, AE, and ME were active against *S. zeamais*, the most destructive insect pest of stored maize. Results are similar to those of previous studies on the insecticidal activity of *C. nigricans* extracts on mosquito larvae (*Anopheles gambiaea*) and whiteflies (*Bemisia tabaci*).[20,21] Emodin exhibited very high toxicity against *S. zeamais* compared to all extracts. The lowest concentration (100 mg/mL) necessary to achieve 100% insect mortality on the first day of exposure using emodin was recorded. HE (LC_{50} of 177.48 mg/mL) was more active than ME (LC_{50} = 195.08 mg/L) and AE (LC_{50} = 374.14 mg/mL). The efficacy of HE and ME against *S. zeamais* could be due to the presence of terpenoids and phenolic compounds, which are highly toxic to insects.[22] Previous reports have shown that emodin inhibits AChE and Glutathione S-transferase activities in insects, resulting in their death.[23] Previous studies have shown that emodin may be useful as a new natural larviciding agent against mosquitoes.[24] Our study revealed for the first time the insecticidal activity of *C. nigricans* leaf extract and emodin against *S. zeamais*.

The minimum concentration of emodin resulting in complete inactivation was 4 mg/mL for FC and 3 mg/mL for FE for an irradiation time of 30 min (Table 4). Therefore, the FE (Gram+) is more sensitive to ME than FC (Gram-). These results are similar to those of previous studies showing that emodin under visible light was more likely to penetrate the

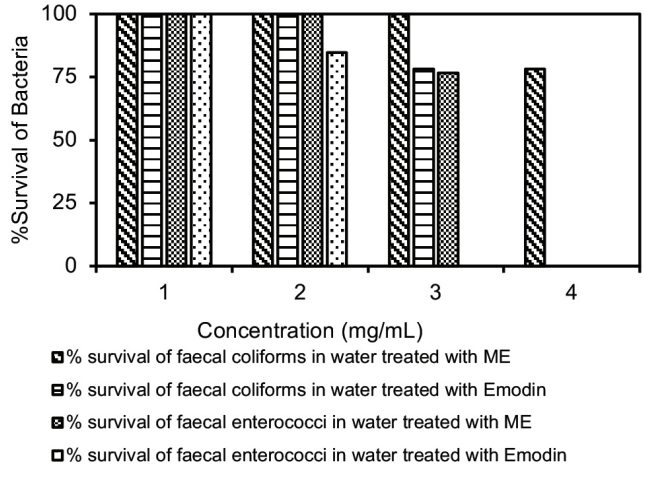

Figure 2. Bacterial survival (%) as a function of photosensitizer concentration. Irradiation time: 0.5 h

ME: Methanol extract

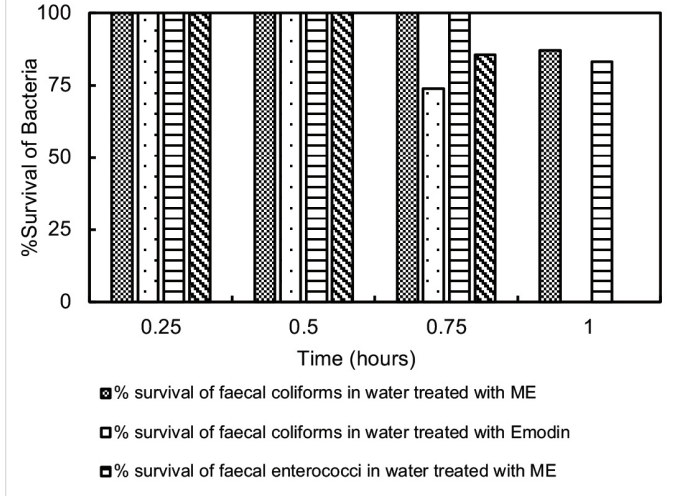

Figure 3. Bacterial survival (%) as a function of irradiation time. Sensitizer concentration: 1 mg/mL

ME: Methanol extract

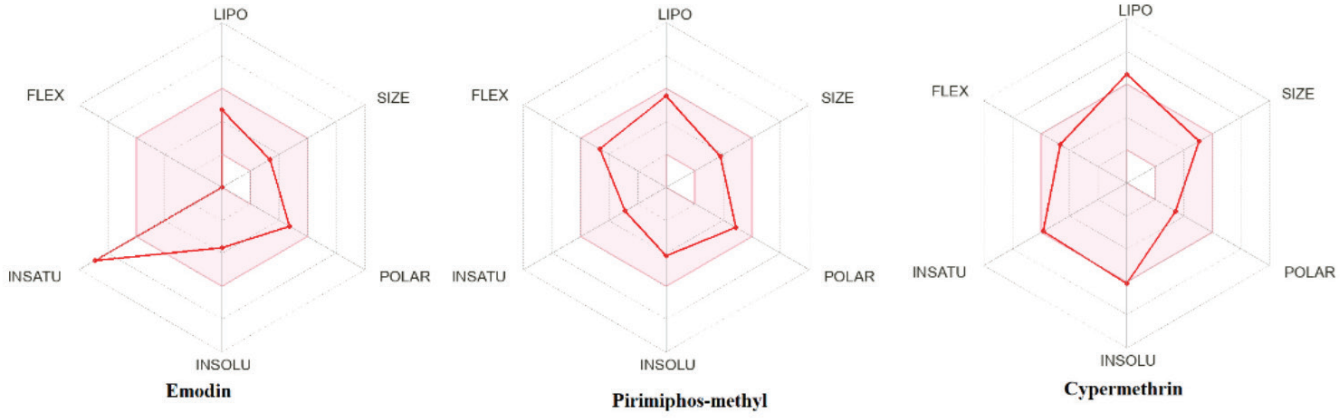

Figure 4. Bioavailability radar plots of the drugs and emodin

intracellular environment of Gram-positive bacteria permeable to bioactive compounds, thus enhancing the local killing effect on the bacteria. In contrast to Gram-positive bacteria, Gram-negative bacteria are more resistant to photodynamic effects because of the different surface structures of the bacterial cells.[25,26] The photosensitizing activity of ME may be due to a combination of several factors: photosensitizer, sunlight, and oxygen.[6] This photoreactivity is mainly due to the presence of photoactivatable molecules (anthraquinones and quinones), which are natural dyes capable of storing light energy that is then transferred to stable oxygen to generate singlet oxygen.[8] When emodin is exposed to light, visible light photons are excited and transfer energy to the oxygen molecules as they return to their ground state, generating reactive oxygen species with cytotoxic properties that cause irreversible damage to cell membranes, DNA, and proteins in bacterial cells.[25]

The energy score results showed that the lowest values were obtained with emodin and pirimiphos-methyl, which is an insecticide used as AChEI. Results indicate that all ligands are non-violent and conform to the rules of Hao et al.,[27] and Clarke et al.[28] The ADMET evaluation showed that emodin and the selected insecticides predicted high intestinal absorption but were not expected to penetrate the brain (Figure 5). All ligands did not inhibit the human ether-a-go-go gene growth enzyme (Table 6). The acute oral toxicity of emodin (2.01 mol/kg) was higher than that of the marketed insecticides pirimiphos-methyl (3.10 mol/kg) and cypermethrin (3.19 mol/kg). The aqueous solubilities of emodin is -3.91, that of pirimiphos-methyl -3.16, and 6.24, respectively. MD results revealed that emodin created a high-affinity pi-pi bond with TRP83 in the DmAChE active site (distance: 3.63 Å) similar to that created by iodobenzyltacrine with TRP83.[18] Additionally, emodin established a pi-pi bond with the same TYR370 at a distance of 3.84 Å (Figure 6). *In silico* molecular docking studies provide more detailed information on the interactions between emodin and DmAChE (6XYU).

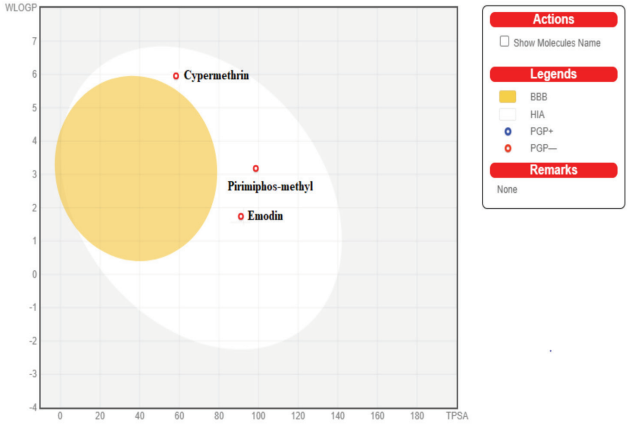

Figure 5. Egan BOILED-egg plot prediction model for intestinal and brain permeation. The white and yellow regions are the physicochemical spaces of compounds predicted to exhibit high intestinal absorption and permeation, respectively

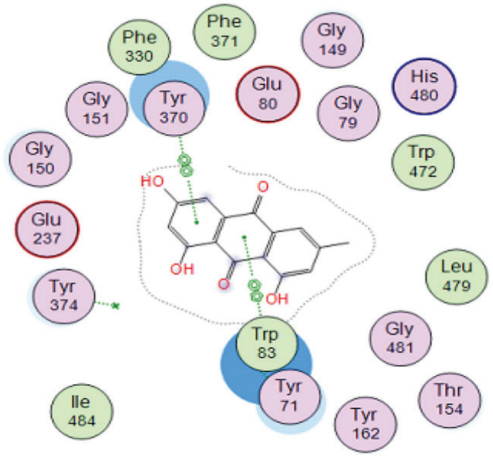

Figure 6. 2D visualization of the best pose of Emodin_6XYU for docking with MOE

MOE: Molecular Operating Environment

Table 4. Inhibitory concentrations of ME and emodin

Microorganisms	Irradiation time (minute)	Methanol concentration (mg/mL)				
		1.0	2.0	3.0	4.0	5.0
ME						
FC	30	–	–	–	–	+
	45	–	–	–	+	+
	60	–	–	+	+	+
	120	–	+	+	+	+
	180	+	+	+	+	+
FE	30	–	–	–	+	+
	45	–	–	+	+	+
	60	–	+	+	+	+
	120	+	+	+	+	+
	180	+	+	+	+	+
Emodin						
FC	30	–	–	–	+	+
	45	–	–	+	+	+
	60	–	+	+	+	+
	120	+	+	+	+	+
	180	+	+	+	+	+
FE	30	–	–	+	+	+
	45	–	+	+	+	+
	60	+	+	+	+	+
	120	+	+	+	+	+
	180	+	+	+	+	+

-: No inhibition, +: Inhibition, ME: Methanol extract, FC: Fecal coliform, FE: Fecal enterococci

Table 5. Results of docking score

Ligand names	Role of receptor residues	Types of interaction bonds	Distance (Å)	Docking score (kcal/mol)
Emodin	TRP 83 TYR 370	Pi-pi Pi-pi	3.63 3.84	-6.3836
Pirimiphos methyl	TYR 71 TYR 370	H-acceptor Pi-H	2.78 4.46	-6.2595
cypermethrin	THR 154 GLY 155	H-donor H-acceptor	3.40 3.30	-5.5267

TRP: Tryptophane, TYR: Tyrosine, THR: Threonine, GLY: Glycine

Table 6. Druglikeness and pharmacokinetic parameters of the ligands

	Emodin	Pirimiphos-methyl	Cypermethrin
Molecular weight g/mol	270.24	305.33	416.30
Number of heavy atoms	20	19	28
Number of aromatic heavy atoms	12	6	12
Fraction Cps3	0.07	0.64	0.27
Number of rotatable bonds	0	7	7
Number of H bond acceptors	5	5	4
Number of H bond donors	3	0	0
TPSA ($Å^2$)	94.83	98.61	59.32
Log *P*o/w (XLogP3)	2.72	4.20	6.05
LogP		2.52732	6.17798
Refractivity molar	70.78	79.86	108.87
LogS (SILICOS-IT)	-3.91	-3.16	-6.24
Blood-brain barrier (log BB)	-0.948	0.049	0.147
Human intestinal absorption (%)	87.671	94.716	92.464
Human ether-a-go-go-related gene inhibition	No	No	No
Acute oral toxicity (LD_{50}, mol/kg)	2.021	3.108	3.195
Oral rat chronic toxicity (LOAEL, log mg/kg_bw/day)	1.575	0.683	0.832

TPSA: Topological polar surface area, LD_{50}: Lethal dose 50, LOAEL: Lowest-observed-adverse-effect level

The labeled insecticides pirimiphos-methyl and cypermethrin give the possibility of using emodin as a promising insecticide. The results clearly show that emodin is promising in terms of binding affinity and pharmacokinetic properties. These results are consistent with in vitro studies on the inhibition of human AChE, which showed the inhibitory activity of emodin at an IC_{50} = 15.215.21 ± 3.52 µM.[29]

CONCLUSION

Extracts of *C. nigricans* as well as emodin isolated from the ME of the leaves were toxic to *S. zeamais* and can be used as natural insecticides to protect stored products. Additionally, ME exhibited bactericidal activity due to the presence of photoactivatable molecules, including emodin, which is a photoreaction site that can lead to the inactivation of FC and FE present in polluted waters. Molecular docking confirmed the binding positions of emodin in the active center of AChEI. Emodin does not passively cross the BBB but is passively absorbed from the HIA, making it a promising natural insecticide for pest control.

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Ethics

Ethics Committee Approval: Our research project does not require ethical approval, as we have not conducted any animal or human experiments.

Informed Consent: Not required.

Authorship Contributions

Concept: A.F., F.B.H., M.G., G.B.N., J.M., L-N.M., S.G., Design: A.F., F.B.H., M.G., G.B.N., J.M., L-N.M., S.G., Data Collection or Processing: A.F., F.B.H., G.B.N., L-N.M., Analysis or Interpretation: A.F., F.B.H., M.G., G.B.N., J.M., L-N.M., S.G., Literature Search: A.F., M.G., J.M., L-N.M., Writing: A.F., F.B.H., G.B.N., L-N.M.

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