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Anti-Inflammatory and Analgesic Activities of Aqueous Extracts from *Stigma maydis*: *In Silico* and *In Vivo* Investigations

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ABSTRACT

Chronic inflammation is a major global health concern, leading to diseases like cardiovascular disorders, cancer, diabetes, and kidney failure. The long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) to manage inflammation is associated with adverse effects. The present study evaluated the anti-inflammatory and analgesic properties of an aqueous extract from *Stigma maydis* (SAE), a traditional herb known for its medicinal benefits. Aqueous extract was prepared from *Stigma maydis* and subjected to phytochemical screening. Physicochemical and pharmacokinetic properties of SAE were predicted using pkCSM and Swiss-ADME platforms. *In silico* molecular docking was performed on SAE's phytoconstituents. The anti-inflammatory activity of SAE was evaluated in rats using carrageenan-induced paw edema, with doses of 125, 250, and 500 mg/kg and mefenamic acid as a reference. Analgesic activity was assessed through the Randall-Selitto assay. The results revealed the presence of flavonoids, saponins, alkaloids, terpenoids, and phenolic compounds. *In silico* analysis identified several active constituents, such as pelargonidin and apigenidin, which exhibited a high binding affinity for cyclooxygenase-2, a key target for anti-inflammatory drugs. The extracts at doses of 125, 250, and 500 mg/kg significantly ($p < 0.5$) reduced paw edema, with inflammation percentages of 21.63, 22.64, and 24.69%, respectively, compared to the negative control group. The 500 mg/kg dose of SAE exhibited the most pronounced effects, although it was less potent than the positive control. The Result of the study revealed that *Stigma maydis* aqueous extract exhibited anti-inflammatory and analgesic properties with minimal side effects, warranting further research to understand its mechanisms of action and clinical applications.

***Keywords*:** *Stigma maydis*, Inflammation, Phytochemical screening, Molecular docking, *in silico*, *in vivo*.

Introduction

Nowadays, chronic inflammatory disorders are acknowledged as a leading cause of mortality worldwide. Conditions such as coronary heart disease, stroke, cancer, diabetes mellitus, kidney failure, and metabolic dysfunction-associated steatotic liver disease all stem from persistent inflammation.[1] Pain is the primary symptom of inflammation,[2] which contributes to higher morbidity rates and significantly reduces patients' quality of life.[3] The use of nonsteroidal anti-inflammatory drugs (NSAIDs) has increased as a result of the increasing prevalence of inflammation. Prolonged use of NSAIDs increases the risk of adverse effects, including gastrointestinal issues, kidney disorders, and cardiovascular complications.[4,5] Therefore, researchers are investigating natural products with fewer side effects.

Stigma maydis is an herbal plant traditionally used to treat inflammation, hyperglycemia, kidney stones, and urinary tract infections.[6,7]

Based on several studies, *Stigma maydis* can treat diseases such as hypertension, diabetes mellitus, and inflammation.[8,9] *Stigma maydis* contains flavonoids, phenolic acids, alkaloids, anthocyanins, and polysaccharides, which have a role in reducing inflammation.[6,7,10] The extraction technique used significantly impacts the bioavailability of these compounds. Research has shown that water extraction, commonly used to obtain *Stigma maydis* extract, has significantly increased the amounts of flavonoids and phenolic compounds.[11] The method improves the extraction of beneficial phytochemicals and aligns with the traditional practice of recommending aqueous formulations for their safety and efficacy.[12] Research on the analgesic and anti-inflammatory properties of *Stigma maydis* is still limited, particularly regarding aqueous extracts obtained using aqueous solvents with the ultrasonic-assisted extraction (UAE) method and concentrated through freeze-drying. The present study evaluated the anti-inflammatory and analgesic activities of aqueous extracts from *Stigma maydis* through *in silico* modeling and *in vivo* experimentation.

Materials and Methods

Source and extraction of plant materials

Stigma maydis simplicial powder was obtained from the Laboratory of UPT Materia Medika Batu, Jl. Lahor 87, Pasanggrahan, Batu, East Java, Indonesia (-7.86754, 112.51924), with identification number 074/653/102.20-A/2022. The simplicial powder was extracted using the UAE method, employing water as a solvent for 15 minutes. The ratio of simplicial powder to water used was 1:10, and the solvent was removed using a freeze-dryer.

Source of animals

The study involved male Wistar rats that weighed between 150 and 200 grams and were 8 to 10 weeks old. All the animals were kept in standard

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husbandry conditions and fasted for 16 to 18 hours before treatment. The procedures used in this study were approved by the Ethical Research Commission of the Medical Faculty at the University of Islam Malang, Indonesia (049/LE.001/X/03/2022).

Phytochemical screening of Stigma maydis aqueous extract
The identification of various classes of phytoconstituents in *Stigma maydis* aqueous extract (SAE) was performed following standard procedures.[13] These tests aimed to detect the presence of alkaloids, flavonoids, phenols, triterpenoids, and saponins.

Prediction of the physicochemical and pharmacokinetic properties of Stigma maydis phytoconstituents
The physicochemical and pharmacokinetic properties of *Stigma maydis* phytoconstituents were carried out using the pkCSM website (https://biosig.lab.uq.edu.au/pkcsm/) and the Swiss-ADME website (http://www.swissadme.ch/).

In silico analysis
The macromolecular protein target, cyclooxygenase-2 (COX-2) (PDB ID: 5IKR), was obtained from the Protein Data Bank website (https://www.rcsb.org/). Data on the chemical compounds of *Stigma maydis* were sourced from Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/). The PubChem website (https://pubchem.ncbi.nlm.nih.gov/) was used to retrieve the 3D structure of the ligands. Molecular docking analysis was conducted using PyRx 0.8 software (Sarkis, USA), with grid dimensions set to x: y: z = 10.3861: 7.9228: 11.1667. The molecular docking results were visualized using Discovery Studio Visualizer V21.1.0.20298 (Dassault Systèmes Biovia Corp., France). The analysis of the molecular docking results included bond energy (ΔG) and the inhibition constant (Ki). The inhibition constant was derived from the binding energy (ΔG) using Equation 1.

$$Ki = \exp(\frac{\Delta G}{RT}) \quad \text{..... (Equation 1)}$$

Where T is the temperature (298.15 K) and R is the universal gas constant (1.985×10^{-3} kcal $mol^{-1}K^{-1}$).[14]

Assessment of the anti-inflammatory effect of Stigma maydis aqueous extract
The evaluation of the anti-inflammatory effect was conducted using rats with carrageenan induction.[15,16] Male Wistar rats (n = 6 per group) were administered distilled water as the negative control, mefenamic acid (45 mg/kg) as the positive control, and SAE at doses of 125, 250, and 500 mg/kg as the treatment groups. All treatments were administered orally one hour before 0.1 ml of 1% carrageenan (w/v) was injected into the right hind paw. The paw edema was measured before induction and 1 hour after carrageenan injection using a plethysmometer for 5 hours (Ugo Basile 57140, Stoelting, Italy). The percentage inhibition of paw edema was calculated using Equation 2.

$$A\% = \left(\frac{P_t - P_C}{P_t}\right) \times 100 \quad \text{..... (Equation 2)}$$

Where A% is the percentage inhibition of paw edema, P_t is the mean of AUC paw edema of the treatment group, and Pc is the mean of AUC paw edema of the control group; AUC: Area under curve.

Evaluation of the analgesic potency of the Stigma maydis aqueous extract
The analgesic potency of SAE was evaluated using the Randall-Selitto assay.[16] Five groups, each consisting of six animals, were established. The groups received treatments orally, including SAE (at doses of 125, 250, and 500 mg/kg), mefenamic acid (at 45 mg/kg), and distilled water as the negative control. The pain threshold was measured in all groups using a Randall-Selitto analgesiometer (Ugo Basile 57215, Stoelting, Italy) before, 30, 60, 120, 150, 180, 210, 240, and 300 minutes after treatment. Pain threshold values in the treatment groups were compared to those in the negative control group. The percentage inhibition of pain was calculated using Equation 3.

$$P\% = \left(\frac{T_t - T_C}{T_t}\right) \times 100 \quad \text{..... (Equation 3)}$$

Where; P% is the percentage of pain inhibition, T_t is the mean of the pain threshold of the treatment group, and Tc is the mean of the AUC pain threshold of the control group; AUC: Area under control.

Statistical analysis
Data were statistically analyzed and presented as mean ± standard deviation (SD). Analysis of Variance (ANOVA) was performed using Statistical Package for Social Sciences (SPSS; version 16) software, followed by the LSD post hoc test. Statistical significance was set at $p < 0.05$.

Results and Discussion

The phytochemical contents of the Stigma maydis aqueous extract
The results (Table 1) of the phytochemical screening of SAE revealed flavonoids, saponins, alkaloids, terpenoids, and phenol. The presence of flavonoids in SAE may suggest potential antioxidant activity, which can help reduce oxidative stress and neutralize free radicals. Flavonoids are also known for their cardiovascular protective and anti-inflammatory effects. Phenols, a class of antioxidants, further enhance the extract's capacity to combat oxidative damage and potentially contribute to its anti-aging effects.[17,18] Saponins are recognized for their cholesterol-lowering effects, immune-supporting benefits, and antimicrobial properties. Alkaloids have many pharmacological effects, like pain relief, anti-oxidant, and anti-inflammation.[19,20] These compounds indicate that SAE may offer a range of therapeutic benefits, including antioxidant, antimicrobial, anti-inflammatory, and potential cardiovascular effects. Further studies are required to explore the specific mechanisms of action and possible uses of these phytochemicals in medicine.

Table 1: Phytochemical screening of *Stigma maydis*t aquoeus extract

Phytochemical	SAE
Flavonoid	+
Alkaloid	+
Saponin	+
Phenol	+
Terpenoid	+

+: presence; -: absence of tested phytochemicals; SAE: *Stigma maydis* aqueous extract

Prediction of physicochemical and pharmacokinetic properties of the phytoconstituents
Table 2 presents the predicted properties of the phytoconstituents found in the chemical composition of SAE. Out of the 44 compounds in SAE, 42 met the Lipinski criteria, while 2 did not. The profile of the physicochemical properties was assessed based on Lipinski criteria, namely less than 500 Daltons for molecular mass, high lipophilicity (expressed as Log P <5), hydrogen bond donors <5, and hydrogen bond acceptors <10.[21,22,23] Compounds with a molecular mass of more than 500 Daltons have low permeability to the intestinal tract and blood-brain barrier (BBB). The log P value affects a compound's ability to cross the plasma membrane, its distribution, and its affinity for plasma proteins, thereby influencing the drug's bioavailability. The most frequent cause of low bioavailability of drugs through the oral route is low permeability. The optimal log P value of a drug candidate is <5. The hydrogen bond donor value is <5, and the acceptor is <10, indicating that the molecule can be well absorbed. The score exceeded the criteria, indicating that the chemical dissolves in polar solvents via hydrogen bonding.[23,24] In drug candidate research, Lipinski's Rule of Five helps predict and exclude molecules likely to exhibit poor pharmacological properties, thereby conserving valuable drug

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Table 2: Physicochemical properties of *Stigma maydis* phytoconstituents

Phytoconstituents	Physicochemical properties				Lipinski requirement
	MW ≤ 500	Log P ≤ 5	HBA ≤ 10	HBD ≤ 5	
Alpha-terpineol	154.25	2.51	1	1	Yes
Apiforol	274.27	1.64	5	4	Yes
Apigenidin	290.70	3.24	4	3	Yes
Betaine	117.15	-2.19	2	0	Yes
Beta-ionone	192.30	2.77	1	0	Yes
Beta-sitosterol	414.71	5.05	1	1	Yes
Butan-1-ol	74.12	1.57	1	1	Yes
Carvacrol	150.22	2.24	1	1	Yes
Chlorogenic-Acid	354.31	-0.42	9	6	Yes
Cinnamic-acid-ethyl-ester	176.21	2.23	2	0	Yes
Cyanidin	287.24	0.77	6	5	Yes
Daucosterol	576.85	5.17	6	4	No
Decan-1-ol	158.28	2.99	1	1	Yes
Decan-2-ol	158.28	3.08	1	1	Yes
Ergosterol	396.65	4.81	1	1	Yes
Gamma-nonalactone	156.22	2.33	2	0	Yes
Geosmin	182.30	2.66	0	1	Yes
Geraniol	154.25	2.52	1	1	Yes
Hept-4-en-2-ol	114.19	2.22	1	1	Yes
Heptan-2-ol	116.20	2.29	1	1	Yes
Hex-1-en-3-ol	100.16	1.94	1	1	Yes
Hordenine	165.23	2.11	3	1	Yes
Limonene	136.23	2.72	0	0	Yes
Luteoforol	290.27	1.28	6	5	Yes
Malic-acid	134.09	-0.01	5	3	Yes
Oleanolic-acid	456.70	3.94	3	2	Yes
Palmitic-acid	256.42	3.85	2	1	Yes
Pelargonidin	271.25	3.2	4	4	Yes
Pyrrole	67.09	0.00	0	1	Yes
Rhamnose	138.21	2.63	1	0	Yes
Stigmasterol	412.69	5.08	1	1	Yes
Tartaric-acid	150.09	-0.29	6	4	Yes
Thymol	150.22	2.32	1	1	Yes
Vitexin	432.38	1.63	10	7	Yes
1,2,3-trimethyl-benzene	120.19	2.22	0	0	Yes
1,2,4-trimethyl-benzene	120.19	2.28	0	0	Yes
1,2-dimethyl-4-ethyl-benzene	134.22	2.51	0	0	Yes
1,8-cineol	154.25	2.58	1	0	Yes
2-methyl-butan-1-ol	88.15	1.80	1	1	Yes
2-methyl-pentan-3-one	100.16	1.84	1	0	Yes
2-methyl-propan-1-ol	74.12	1.56	1	1	Yes
2-pentyl-furan	138.21	2.63	1	0	Yes
3'-methoxymaysin	590.53	1.76	14	7	No
3-methyl-butan-1-ol	88.15	1.78	1	1	Yes

MW: molecular weight; Log P: calculated logarithm of the octanol-water partition coefficient; HBA: hydrogen bond acceptor; HBD: hydrogen bond donor

development resources. Additionally, pharmacokinetic profiling is essential to identify potentially promising drug candidates.[22,23,24]

The predicted pharmacokinetic profile of compounds in SAE is presented in Table 3. Among them, 30 compounds showed high intestinal absorption, indicating good absorption and permeability within the gastrointestinal tract.[22,25] Twenty-seven compounds can permeate the BBB. A BBB is a microvascular unit that selectively regulates drug permeability in the brain. With the growing number of drug targets for central nervous system (CNS) diseases, it is essential to prioritize and accurately predict which compounds in the company's database should be screened for desirable properties.[25,26] Thirty-seven SAE compounds did not bind to the P-glycoprotein substrate (P-gp). P-gp is a transmembrane glycoprotein that transports hazardous compounds from the cell to the extracellular space. It also actively exports various compounds, which can significantly reduce or eliminate their activity.[25,27] Due to its ability to alter drug penetration rates, the level of P-gp expression correlates with the degree of drug resistance.[25] The metabolic parameters analyzed included the potential inhibition of cytochrome P450, an enzyme responsible for metabolizing a wide range of drug compounds. Inhibition of cytochrome P450 enzymes can alter a drug's pharmacokinetics, making it essential to evaluate whether a compound affects these enzymes. The five major cytochrome P450 isoforms involved in drug metabolism are CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A.[25,28] Thirty-three compounds in SAE did not inhibit the five main isoforms of cytochrome P450. Total clearance reflects the excretion profile and is a pharmacokinetic indicator that measures the rate at which compounds are eliminated from the body. For all phytoconstituents, the clearance rate was low. A clearance rate above 15 ml/min/kg is considered high, while a rate between 5 and 15 ml/min/kg is considered moderate.[29]

Table 3: Prediction of pharmacokinetic properties of *Stigma maydis* phytoconstituents

Ligand	Intestinal absorption	BBB permeation	P-gp Substrate	Cyp1A2 inhibitor	Cyp2C19 inhibitor	Cyp2C9 inhibitor	Cyp2D6 inhibitor	Cyp3A4 inhibitor	Clearance Total
Alpha-terpineol	High	Yes	No	No	No	No	No	No	1.219
Apiforol	High	No	Yes	No	No	No	No	No	0.181
Apigenidin	High	No	Yes	Yes	No	No	No	No	0.626
Betaine	Low	No	Yes	No	No	No	No	No	0.326
Beta-ionone	High	Yes	No	No	No	No	No	No	1.315
Beta-sitosterol	Low	No	No	No	No	No	No	No	0.628
Butan-1-ol	High	Yes	No	No	No	No	No	No	0.375
Carvacrol	High	Yes	No	Yes	Yes	No	No	No	0.207
Chlorogenic-Acid	Low	No	No	No	No	No	No	No	0.307
Cinnamic-acid-ethyl-ester	High	Yes	No	No	No	No	No	No	0.843
Cyanidin	High	No	Yes	Yes	No	No	No	No	0.532
Daucosterol	Low	No	No	No	No	No	No	No	0.689
Decan-1-ol	High	Yes	No	No	No	No	No	No	1.641
Decan-2-ol	High	Yes	No	No	No	No	No	No	1.598
Ergosterol	Low	No	No	No	No	Yes	No	No	0.564
Gamma-nonalactone	High	Yes	No	No	No	No	No	No	1.363
Geosmin	High	Yes	No	No	No	No	No	No	1.112
Geraniol	High	Yes	No	No	No	No	No	No	0.437
Hept-4-en-2-ol	High	Yes	No	No	No	No	No	No	0.406
Heptan-2-ol	High	Yes	No	No	No	No	No	No	1.483
Hex-1-en-3-ol	High	Yes	No	No	No	No	No	No	0.416
Hordenine	High	Yes	No	Yes	No	No	No	No	0.907
Limonene	Low	Yes	No	No	No	Yes	No	No	0.213
Luteoforol	High	No	Yes	No	No	No	No	No	0.023
Malic-acid	High	No	No	No	No	No	No	No	0.81
Oleanolic-acid	Low	No	No	No	No	No	No	No	-0.081
Palmitic-acid	High	Yes	No	No	No	No	No	No	1.763
Pelargonidin	High	No	Yes	Yes	No	No	Yes	No	0.569
Pyrrole	High	Yes	No	No	No	No	No	No	0.665
Rhamnose	High	No	Yes	No	No	No	No	No	0.577

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Stigmasterol	Low	No	No	No	No	Yes	No	No	0.618
Tartaric-acid	Low	No	No	No	No	No	No	No	0.885
Thymol	High	Yes	No	Yes	No	No	No	No	0.211
Vitexin	Low	No	No	No	No	Yes	No	No	0.444
1,2,3-trimethyl-benzene	Low	Yes	No	No	No	No	No	No	0.283
1,2,4-trimethyl-benzene	Low	Yes	No	No	No	No	No	No	0.28
1,2-dimethyl-4-ethyl-benzene	Low	Yes	No	No	No	No	Yes	No	0.304
1,8-cineol	High	Yes	No	No	No	No	No	No	1.009
2-methyl-butan-1-ol	High	Yes	No	No	No	No	No	No	0.386
2-methyl-pentan-3-one	High	Yes	No	No	No	No	No	No	0.45
2-methyl-propan-1-ol	High	No	No	No	No	No	No	No	0.33
2-pentyl-furan	High	Yes	No	Yes	No	No	No	No	0.456
3'-methoxymaysin	Low	No	No	No	No	No	No	No	-0.217
3-methyl-butan-1-ol	High	Yes	No	No	No	No	No	No	0.36

Molecular docking of phytoconstituents of Stigma maydis on COX-2

Cyclooxygenase-2 (PDB ID: 5IKR) is the target receptor to assess anti-inflammatory and analgesic activity (Figure 1). Molecular docking was validated to assess the accuracy of the docking method used. The validation parameter, RMSD (Root Mean Square Deviation), measures the difference in the ligand pose before and after redocking. The validation process was successful if the program could return the ligand pose from redocking to the original pose with an RMSD value <2 Å.[14,29] In the present study, the validation process was considered successful, as the RMSD value obtained was 1.852 Å, as shown in Figure 1. The computational docking studies revealed that no compounds had a free binding affinity (ΔG) and inhibition constant (Ki) equal to or greater than the positive control drug. The ΔG and Ki of SAE compounds ranged from -8.1 to 62.7 kcal/mol and 1.191 to $2519.692x10^{45}$ µM. Table 4 summarizes the docking results for the phytoconstituents and reference drug. Pelargonidin exhibited the best binding conformation with the cyclooxygenase-2 receptor, showing a ΔG of -8.1 kcal/mol. It was followed by apigenidin (-8.0 kcal/mol), apiforol (-7.8 kcal/mol), luteoforol (-7.7 kcal/mol), cyanidin (-7.3 kcal/mol), and chlorogenic acid (-6.8 kcal/mol) (Table 4). The ΔG reflects the strength of the interaction between the ligand and receptor. A lower ΔG indicates a more stable compound-receptor complex.[14,22,29] In the present study, the ΔG of the top 6 compounds was close to those of the positive control drug (-8.1 kcal/mol), which confirmed that these phytochemicals might have potential analgesic and anti-inflammatory activities.

Other parameters analyzed were amino acid interactions between ligands and receptors. Amino acid interactions influence the bond energy between the ligand and receptor, thereby affecting the stability of the molecule's geometric configuration.[22,30] Six SAE compounds with free binding energies close to positive control drugs were checked for similarities in their amino acid interactions. According to Table 5 and Figure 4, pelargonidin, apigenidin, apiforol, luteoforol, cyanidin, and chlorogenic acid have similar residual interactions with amino acid residues in the positive control drug (mefenamic acid). The interaction of luteoforol (with residues 385 TYR, 349 VAL, 352 LEU, and 527 ALA) closely resembles that of mefenamic acid (which involves residues 385 TYR, 530 SER, 349 VAL, 352 LEU, 527 ALA, and 531 LEU), followed by interactions with pelargonidin, apigenidin, apioforol, and cyanidin (which all involve residues 349 VAL, 352 LEU, 527 ALA, and 531 LEU). Similar interactions with binding site residues suggest that the compound may exhibit inhibitory activity comparable to the reference drug.[30,31]

The anti-inflammatory activity of Stigma maydis aqueous extract

The anti-inflammatory effect of SAE was evaluated by the AUC of rat paw edema and the percentage of edema inhibition (Table 6 and Figure 2). A significant difference ($p < 0.05$) was observed in the total AUC of rat paw edema between the negative control group, reference drug, and treatment group. However, no significant difference was observed among the treatment doses of 125, 250, and 500 mg/kg. This comparison of total AUC indicated that a smaller AUC value for rat paw edema reflects a stronger anti-inflammatory effect of the compound. Although SAE did not show significant differences between the tested doses, all doses were still more effective than the negative control. These results are consistent with other studies that demonstrated natural compounds could exert significant anti-inflammatory effects by modulating various biochemical pathways in the body.[15,16,32]

Table 4: Binding energy and inhibition constant of the ligand

Protein	Ligand	Free affinity energy (kcal/mol)	Inhibition Constant (µM)
Cyclooxygenase-2 (ID 5IKR)	Mefenamic acid	-9.1	0.210
	Pelargonidin	-8.1	1.191
	Apigenidin	-8.0	1.474
	Apiforol	-7.8	2.154
	Luteoforol	-7.7	2.657
	Cyanidin	-7.3	5.177
	Chlorogenic-Acid	-6.8	11.915
	Beta-ionone	-6.7	14.077

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Carvacrol	-6.6	16.631
1,2-dimethyl-4-ethyl-benzene	-6.5	19.648
Palmitic-acid	-6.5	19.648
Alpha-terpineol	-6.4	23.213
Thymol	-6.4	23.213
1,2,3-trimethyl-benzene	-6.3	27.425
Limonene	-6.3	27.425
Geosmin	-6.3	27.425
Cinnamic-acid-ethyl-ester	-6.3	27.425
1,2,4-trimethyl-benzene	-6.2	32.401
Gamma-nonalactone	-5.9	53.430
Geraniol	-5.8	63.124
Hordenine	-5.8	63.124
Decan-2-ol	-5.7	74.577
2-pentyl-furan	-5.7	74.577
Rhamnose	-5.4	122.980
Decan-1-ol	-5.2	171.655
Heptan-2-ol	-4.9	283.066
Hept-4-en-2-ol	-4.8	334.425
2-methyl-pentan-3-one	-4.7	395.102
Malic-acid	-4.7	395.102
Hex-1-en-3-ol	-4.4	651.539
Tartaric-acid	-4.4	651.539
3-methyl-butan-1-ol	-4.1	1074.414
2-methyl-butan-1-ol	-4.1	1074.414
2-methyl-propan-1-ol	-3.8	1771.753
Betaine	-3.7	2093.214
1,8-cineol	-3.6	2473.000
Butan-1-ol	-3.5	2921.693
Pyrrole	-3.4	3451.795
Stigmasterol	-0.6	3677.370×10^{2}
Beta-sitosterol	-0.4	5132.845×10^{2}
Vitexin	1.8	2010.888×10^{4}
Ergosterol	3.2	2075.554×10^{5}
Oleanolic-acid	21.3	2651.089×10^{15}
Daucosterol	25.2	1767.793×10^{18}
3'-methoxymaysin	62.7	2519.692×10^{45}

Additionally, data analysis of the percentage of inflammation inhibition revealed that the positive control exhibited inhibition of 41.18%, SAE at doses of 125, 250, and 500 mg/kg showed inhibition percentages of 21.63, 22.64, and 24.99%, respectively. These inflammation inhibition percentages suggest that although SAE was not as effective as the reference drug, it still holds potential as a viable anti-inflammatory agent. *Stigma maydis* aqueous extract contains flavonoid compounds (apiforol, apigenidin, cyanidin, pelargonidin, vitexin), alkaloids (betaine, hordenine, pyrrole), phenols (carvacrol, chlorogenic acid, cinnamic acid, ethyl ester, thymol), triterpenoids (beta-sitosterol, oleanolic acid, stigmasterol, ergosterol), and saponins. Flavonoids, alkaloids, and triterpenoids can act as anti-inflammatory agents. The mechanism of action of flavonoids, alkaloids, and triterpenoids in reducing inflammation involves several complex biochemical pathways. One of the main mechanisms is to suppress cyclooxygenase (COX-2) enzyme activity, which plays an important role in synthesizing prostaglandins, compounds that trigger inflammation[33,34,35]. Flavonoids, alkaloids, and triterpenoids also modulate inflammatory signalling pathways by inhibiting the transcription factor NF-κB, a central regulator of pro-inflammatory genes, whose activation is often triggered by cytokines like TNF-α and IL-1β.[35,36,37]

Meanwhile, phenolic compounds employ various mechanisms of action in addressing inflammation. Phenol has a strong antioxidant potential that protects cells from oxidative stress, often occurring during inflammatory processes. Oxidative stress frequently contributes to the inflammatory process, so reducing oxidation can alleviate inflammation. Phenol can also affect signalling pathways involved in inflammatory responses by inhibiting the activation of the mitogen-

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activated protein kinase (MAPK) pathway, which plays a role in pro-inflammatory signal transduction. By inhibiting this pathway, phenols can reduce the expression of inflammatory cytokines and other mediators.[39,40]

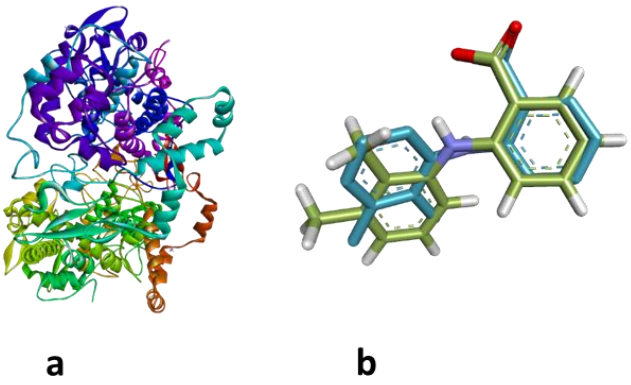

Figure 1: Crystal structure of human COX-2 (ID 5IKR) (a) and overlay of the native ligand (green) and those of the experimental ligands (blue) (b).

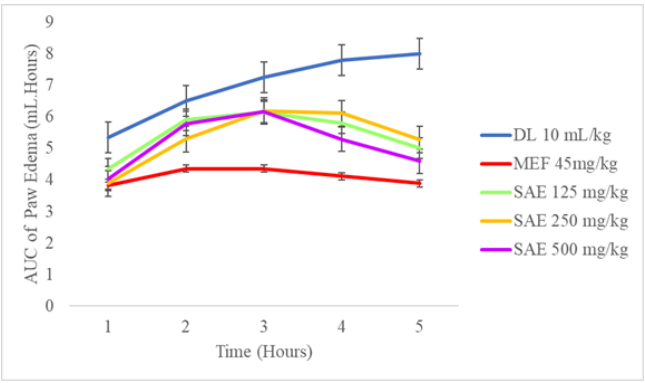

Figure 2: Area under the curve of paw edema on SAE.
DL: Distilled water; MEF: Mefenamic acid; SAE: *Stigma maydis* aqueous extract

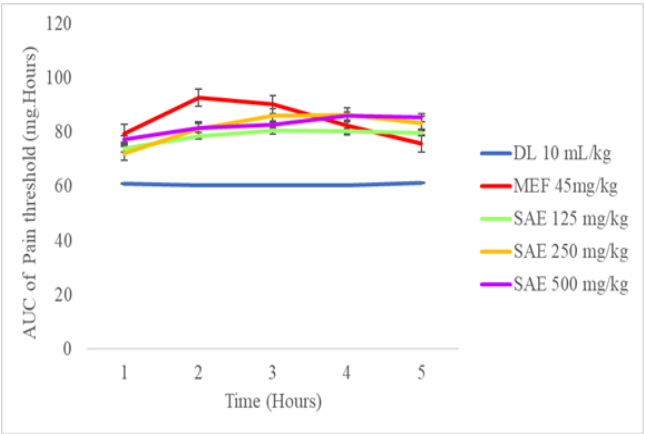

Figure 3: Area under the curve of pain threshold of SAE.
DL: Distilled water; MEF: Mefenamic acid, SAE: *Stigma maydis* aqueous extract

The analgesic potential of Stigma maydis aqueous extract
The results in Table 7 and Figure 3 showed a significant difference ($p < 0.05$) in the total AUC pain threshold between the control and treatment groups. A significant difference ($p < 0.05$) was found in the total AUC pain threshold between SAE at a dose of 125 mg/kg and SAE

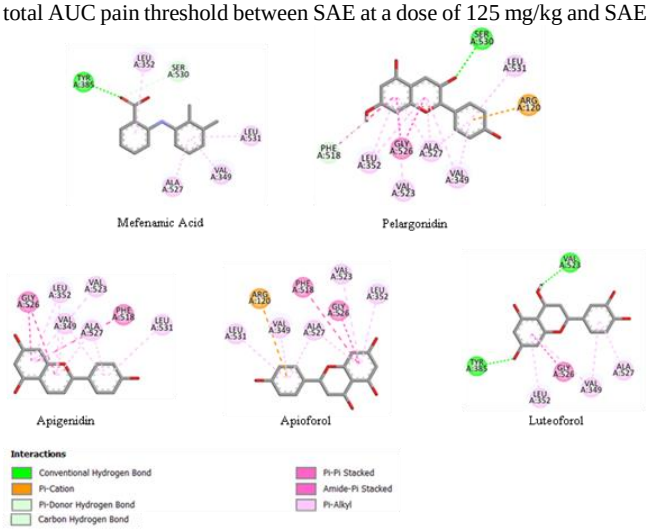

Figure 4: Receptor-ligand interactions of four top hits of *Stigma maydis.*

at doses of 250 and 500 mg/kg, whereas no significant difference ($p > 0.05$) was observed between the 250 and 500 mg/kg doses. The analgesic potential of a compound can be assessed using various methods, one of which is analyzing the AUC pain threshold. The AUC is a key parameter in evaluating analgesic potential, with a lower AUC value indicating a stronger analgesic effect of the tested compound.[16] The percentage of pain inhibition is also an essential parameter in assessing analgesic potency. Based on the pain inhibition percentage (Table 7), SAE demonstrated potential for pain relief compared to the control group. The analgesic effect of SAE is likely influenced by its composition of secondary metabolites, including flavonoids, alkaloids, triterpenoids, saponins, and phenols. Alkaloids, flavonoids, and terpenoids have effects as analgesics through several pathways, including their impact on the central and peripheral nervous systems. Alkaloids, flavonoids, and terpenoids can inhibit signalling pathways involved in pain transmission, such as the COX and LOX pathways, which are key factors in synthesizing prostaglandins that cause pain.[33,34,41]

Alkaloids, flavonoids, and terpenoids also have anti-inflammatory properties that reduce pain. These compounds can reduce pro-inflammatory cytokines levels, such as IL-1β and TNF-α, contributing to inflammation and pain. By lowering the levels of these cytokines, flavonoids can help relieve pain caused by inflammation.[36,37,38] Alkaloids and triterpenoids also affect the nervous system, modulating neurotransmitter receptors in the brain. They can also interact with opioid receptors in the brain, which reduces pain perception.[41,42] Meanwhile, saponins also exhibit analgesic activity through a different mechanism. They can influence the nervous system by altering cell membrane permeability and modulating neurotransmitter signalling. Saponins promote the release of endorphins, which act as natural analgesics. By boosting endorphin levels, saponins help reduce pain and enhance pain tolerance.[41]

The mechanism of action of phenols as analgesics can be explained through several pathways, including their effects on the nervous system and modulation of inflammatory processes. Phenols can inhibit inflammatory signalling pathways, such as NF-κB and MAPK, which contribute to pain.[39,40] They also enhance the production of anti-inflammatory compounds in the body, helping to reduce pain. Phenols increase anti-inflammatory mediators like interleukin-10 (IL-10), which counteracts pro-inflammatory effects.[41,43] Additionally, phenols and flavonoids possess significant antioxidant activity, protecting nerve cells from oxidative stress damage. By boosting the properties of antioxidant enzymes, phenols help reduce cell damage and alleviate pain associated with oxidative stress.[39,40,41,44]

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Table 5: Amino acid interaction of COX-2 with phytoconstituents of *Stigma maydis.*

Ligand	Amino Acid Interactions					
	Conventional hydrogen bond	Carbon hydrogen bond	Hydrophobic interactions			Electrostatic interactions
			Unfavourable donor-donor	Pi alkyl/alkyl	Pi-pi stacked	Pi -Cation
Mefenamic acid	385 TYR	530 SER		349 VAL 352 LEU 527 ALA 531 LEU		
Pelargonidin	530 SER	518 PHE		349 VAL 352 LEU 523 VAL 527 ALA 531 LEU	526 GLY	120 ARG
Apigenidin				349 VAL 352 LEU 523 VAL 527 ALA 531 LEU	518 PHE 526 GLY	
Apiforol				349 VAL 352 LEU 523 VAL 527 ALA 531 LEU 532 LEU	518 PHE 526 GLY	120 ARG
Luteoforol	385 TYR 323 VAL			349 VAL 352 LEU 527 ALA	526 GLY	
Cyanidin	120 ARG		TYR 385	349 VAL 352 LEU 523 VAL 527 ALA 531 LEU	518 PHE 526 GLY	
Chlorogenic-Acid	355 TYR 530 SER	523 VAL 527 ALA 353 SER		352 LEU	526 GLY	522 MET

Table 6: Effects of SAE against carrageenan-induced paw edema model in rats

Treatment	AUC of Paw Edema (mL.Hours)					Total AUC	Percentage of inflammation inhibition (%)
	1 h	2 h	3 h	4 h	5h		
Carr + DL 10 mL/kg	5.35 ± 0.1	6.50 ± 0.13	7.25 ± 0.13	7.80 ± 0.13	8.00 ± 0.15	35.38[a]	-
Carr + MEF 45 mg/kg	3.82 ± 0.07	4.36 ± 0.09	4.35 ± 0.07	4.12 ± 0.05	3.89 ± 0.04	20.81[b]	41.18
Carr + SAE 125 mg/kg	4.35 ± 0.09	5.91 ± 0.07	6.15 ± 0.11	5.81 ± 0.23	5.01 ± 0.21	27.73[c]	21.63
Carr + SAE 250 mg/kg	3.89 ± 0.16	5.29 ± 0.12	6.18 ± 0.19	6.11 ± 0.29	5.28 ± 0.22	27.51[c]	22.24
Carr + SAE 500 mg/kg	4.04 ± 0.16	5.79 ± 0.13	6.16 ± 0.22	5.29 ± 0.19	4.59 ± 0.14	26.54[c]	24.99

SAE: *Stigma maydis* aqueous extract; Values are expressed as mean ± SD; The letters a, b, and c indicate differences between treatments ($p < 0.05$).

Table 7: Analgesic effect of SAE and mefenamic acid in Randall-Selitto assay

Treatment	Total AUC of Pain Threshold (mg.Hours)	Percentage of pain inhibition (%)
DL 10 mL/kg	304.00[a]	-
MEF 45 mg/kg	420.75[b]	27.75
SAE 125 mg/kg	393.08[c]	22.66
SAE 250 mg/kg	409.33[d]	25.73
SAE 500 mg/kg	412.83[d]	26.36

SAE: *Stigma maydis* aqueous extract; The letters a, b, and c indicate differences between treatments ($p < 0.05$).

Conclusion

The present study demonstrated that the aqueous extracts of *Stigma maydis* possess notable anti-inflammatory and analgesic activities. Both *in silico* and *in vivo* findings support the potential of *Stigma maydis* compounds, including flavonoids, alkaloids, and phenols, in modulating inflammation-related pathways, especially COX-2 inhibition. While the anti-inflammatory effects were moderate compared to standard NSAIDs, the analgesic properties showed significant promise, especially at higher doses. These findings suggest *Stigma maydis* as a viable candidate for natural anti-inflammatory and pain-relief therapies, warranting further research into its mechanisms and clinical applications.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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