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REVIEW

Review on *Cassia alata* Bioactive Compounds: In silico, in vitro, and in vivo Studies

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Abstract: *Cassia alata* Linn is a popular herbal remedy in many countries, and its activities have been studied through many studies, starting from in silico, in vitro, and in vivo. This narrative review will focus more on secondary metabolites that are responsible for certain pharmacological activities that have undergone in vivo, in vitro, and in silico testing to determine the underlying mechanism. Twenty pharmacological activities have been identified, with the flavonoid group (emodin, kaempferol, quercetin) as the most prevalent secondary metabolite found in *Cassia alata*. There have been numerous studies looking at the role of flavonoids about specific diseases, and flavonoid testing is quite thorough because it covers three different study types. However, there has not been significant progress accomplished in terms of the evaluation of the dosage form so that test results for promising activities like antidiabetic, antifungal, and antiviral can be carried out into further research. Additionally, several disorders lack comprehensive investigation, particularly in silico studies, therefore further study is required to fill any gaps in the knowledge.

Keywords: *Cassia alata*, pharmacological activity, chemical compound, in silico, in vitro, in vivo

Introduction

Cassia alata Linn is a blooming ornamental shrub known as a "candle bush" because the skeleton of the flower resembles that of a candle. This plant thrives in lowland and middleland environments.[1] *Cassia alata* Linn is a popular herbal remedy in many countries. In Cuba, Nigeria, and Egypt, all portions of the *Cassia alata* Linn plant are used as diuretics, laxatives, and remedies for skin illnesses such as herpes. In the Philippines and China, a decoction of the leaves stems, and bark is used to cure hemorrhoids, hernia, syphilis, intestinal parasites, and diabetes.[2] In Guatemala, Indonesia, and Brazil, all portions of *Cassia alata* Linn are utilized to cure influenza and malaria.[3]

Cassia alata Linn possesses a variety of pharmacological actions, including antibacterial, anti-inflammatory, antioxidant, anthelmintic and antifungal properties.[3–7] *Cassia alata* Linn is rich in phytochemical compounds, such as alkaloids, tannins, saponins, phenols, vedaflavonoids, and glycosides. These phytochemical components contribute significantly to the pharmacological effects of *Cassia alata* Linn.[8]

Cassia alata activities have been studied in silico, in vitro, and in vivo methods. In silico means the experiment that is performed by computer and can take part significantly in all stages from the preclinical stage to late-stage clinical development,[9] while in vitro method is a study that is not performed on complete living organisms. This method uses chemicals, cells, tissues, and even functioning isolated organs. This method is important because it provides important information, and it cannot harm living humans or laboratory animals. In vivo means a complete living animal. Studies in both humans and animals are called in vivo studies. In vivo studies are usually done after in vitro safety studies to make sure that living people or animals will not be harmed.[10] A comprehensive analysis of these studies (in silico, in vivo, and in vitro) is required to determine how far *Cassia alata* has been explored and how far it has progressed in terms of discovering substances that are useful in being responsible for pharmacological effects. The investigation of novel drugs that could be safer and more effective therapies for several diseases, such as diabetes, cancer, and malaria, must be

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conducted through comprehensive studies. From a bioinformatics perspective, knowing which isolate compound from *Cassia alata* is responsible for a particular activity can be useful for predicting interactions between protein structures and isolate compounds, thus becoming a tool for generating new knowledge and developing new drugs.

This narrative review aims to explore the pharmacological activities of *Cassia alata* as demonstrated by several studies, such as in vitro, in vivo, and in silico studies, as well as the substances underlying these features. By providing thorough studies, research gaps can be created, allowing for novel studies that have not previously been pursued to complete research. To achieve the aim, the review was conducted by selecting the article that was published in 2014–2024 with keywords "Cassia alata", "in silico", "in vitro", and "in vivo". By now, only one review discusses *Cassiaalata*, which is Fatmawati et al 2020. However, that review only revealed secondary metabolites found in every part of the plant, as well as various known pharmacological properties, without exposing the compound that is responsible for certain pharmacological activity, and the mechanism underlying has not been revealed yet. Thus, this research will focus more on secondary metabolites that are responsible for certain pharmacological activities that have undergone in vivo, in vitro, and in silico testing to determine the mechanism of action that made them beneficial as treatments for particular diseases. As a result, further research will be more focused on isolating specific secondary metabolite chemicals, resulting in specific results too. Understanding the mechanism will provide us the foundation for altering its pathway, to increase the effectiveness of therapy, lower the risk of adverse effects, and optimize the action of drugs in the body. Therefore, this review will be beneficial for the new drug discovery and development process in medical applications.

Results and Discussion

Twenty pharmacological activities of *Cassia alata* have been studied using in silico, in vitro, and in vivo methods. Results are divided into each activity and test method. Each of them will be described in the section below.

Antidiabetic/Anti Glycemic

In silico study by Thilak et al 2023 revealed that there were five compounds contained in *Cassia alata*, such as 5-methoxyhydnocarpin-D, quercetin 3-rhamnoside-7-glucoside, marimetin, kaempferol, and luteolin, showed a higher binding affinity than the voglibose and acarbose as the standard drug, when docked against alpha-glucosidase receptor.[11] Another study by Oso & Olaoye 2020 also discovered that emodin, quercetin, chrysoeriol, and kaempferol have a higher binding affinity than acarbose, docked with alpha-glucosidase and sucrase-isomaltase, but a lower affinity when docked with alpha amylase.[12] In vitro study by Kazeem et al 2015 found that the acetone extract of *S. alata* displayed the highest inhibitory activity against α-amylase (IC50 = 6.41 mg/mL) while hexane extract exhibited the highest inhibitory effect on α-glucosidase (IC50 = 0.85 mg/mL).[13] Another in vitro study by Uwazie et al also found that Emodin in *Senna alata* flowers produces maximum α-glucosidase and α-amylase inhibitory activities identified by FTIR and NMR instruments. These findings are also obtained from the successful in vivo testing of subfraction isolation on alloxan-induced diabetic male Wistar rats.[14] An in vivo study conducted by Laishram et al 2016 revealed that there was no sign of toxicity from the acute oral toxicity test and it was proved by OGTT that aqueous extract of *Cassia alata* Linn. enhanced glucose utilization.[15] Another study also found that the restoration of impaired glucose metabolism of *S. alata* in high-fat diet-induced obese mice may be associated with reduced hepatic gluconeogenesis and increased glucose uptake via AMPK activation.[16]

The study on *Cassia alata* for diabetics is one of the most comprehensive. According to a number of studies, the main substances that have the ability to cause anti-glycemic effects are kaempferol, quercetin, and emodin. These compounds have an IC50 value that is comparatively strong to that of acarbose, a standard medication that works to prevent the breakdown of carbohydrates into glucose, leading to better control over glucose levels.[11–14] These substances belong to a class of flavonoids that exhibit potent inhibitory effects on α-glucosidase due to hydroxylation at positions 5- and 7- or 8 of the A ring (functioning as hydrogen bond donors), at positions 3' and 4' of the A-ring, ring B, and position 3 of the C ring. In addition, the C2 = C3 double bond in the C ring plays a crucial role in the inhibitory activity of flavonoids. The stronger inhibitory impact that appears to encourage the interaction between α-glucosidase and flavonoids may also be attributed to the ortho-OH group.[17] The summary of antidiabetic activity results can be seen in (Table 1).

Table 1 Antidiabetic Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Molecular docking	α-glucosidase (5NN8)	5- methoxyhydnocarpin-D, quercetin 3-rhamnoside-7-glucoside, marimetin, kaempferol and luteolin	Not mentioned in the article	Thilak et al 2023[11]
	Molecular docking	α-amylase (1HNY), α-glucosidase (5ZCB), sucrase-isomaltase (3LPO).	Emodin, quercetin, chrysoeriol, and kaempferol	Not mentioned in the article	Oso & Olaoye et al 2020[12]
In vitro	Inhibition of α-glucosidase, α-amylase, and sucrase activity	α-glucosidase, α-amylase sucrase	Not mentioned in the article	IC50: 9.67±0.88, 65.54±0.34, 48.35±1.45	Oso & Olaoye et al 2020[12]
	α-amylase and α-glucosidase inhibitory assay	α-amylase α-glucosidase	Not mentioned in the article	IC50: 6410 850	Kazeem et al 2015[13]
	NMR and FTIR	Gamma receptor	Emodin	Not mentioned in the article	Uwazie et al 2020[14]
In vivo	In vivo bioactivity-guided isolation procedures	Not mentioned in the article	Not mentioned in the article	Not mentioned in the article	Uwazie et al 2020[14]

In summary, the research on the antidiabetic properties of *Cassia alata* is thorough, indicating significant promise. However, there is a shortage of in vivo studies. The current ones only demonstrate its effectiveness in animal tests without establishing the IC50 value. Further investigation is necessary to ascertain this value, crucial for determining the appropriate dosage for medicinal purposes.

Antifungal

There have been various investigations on *C. alata*'s antifungal activities, including in silico, in vitro, and in vivo research. Ahmed et al 2019 and Saptarini et al 2024 found that secondary metabolite chemicals in *Cassia alata* leaves had an antifungal effect on the lanosterol 14-α-demethylase receptor (Cyp51). Ahmed et al 2019 discovered that according to the Glide docking score of the Schrödinger Maestro program, aloe-emodin (−7.81), chrysophanol (−7.493), and rhein (−8.518) scored higher than the native medication fluconazole (−6.856), while the other compound emodin (−6.717) had a slightly lower docking value. The findings revealed that all four compounds exhibited considerable antifungal capabilities, as assessed by computer simulations.[18] Saptarini et al 2024 discovered that the secondary metabolites 9-Ene-methyl palmitate, stearidic acid, and trichosanic acid in the n-hexane fraction had a high affinity for the lanosterol 14-alpha demethylase enzyme. Molecular docking revealed that stearidonic acid has the highest binding affinity (−7.2 kcal/mol). In silico investigations support the conclusion that molecules in the n-hexane fraction have antifungal activity against M. furfur.[19] Based on these two studies, secondary metabolite chemicals from *C. alata* leaves show antifungal action by blocking the lanosterol 14-alpha demethylase enzyme.

In vitro research by Pham et al 2021 discovered that rhein chemicals identified in *C. alata* plants inhibit Phytophthora species mycelial growth with an IC50 of 675.9 µg/mL. The substances methyl 2,4,6-trihydroxybenzoate, aloe emodin, kaempferol, (-)-epiafzelechin, rhein, kaempferol-3-(O)-gentiobioside, and aloe-emodin-8-O-β-D-glucoside have antifungal activity against Phytophthora species in vitro. Rhein inhibited the growth of the bacteria Acidovorax avenae subsp. cattlvae with an IC50 of 2.5 µg/mL.[20]

In vivo research by Alpapara et al 2020 showed the potential of *C. alata* leaf decoction to treat tinea imbricata using a community-prepared, with results lowering severity in 40% of participants. *S alata* leaf decoction improved tinea imbricata lesions in 28 days based on subjective (VAS for itching) and objective (disease severity and KOH conversion) clinical indicators. The majority of patients (95%) had alleviation from pruritus. However, in this investigation, it was unclear which chemical was responsible for the observed impact.[1] The result of the antifungal activity test that was already done can be seen in (Table 2).

Based on existing in silico, in vitro, and in vivo investigations, *C. alata* has a high antifungal potential. However, in vivo studies are currently insufficient to explain *C. alata*'s antifungal activity. More in-vivo study on test animals is required to assess the efficacy and IC 50 value of *C. alata*, which may be used to calculate safe medication dosages.

Antiviral

There have been three types of studies investigated for antiviral activity based on Table 3, but the results still tend to be small, so research can be carried out on other viruses to maximize the potential of *C. alata*. Further in vitro research needs to be done to determine the secondary metabolites that play a role in antiviral activity. In vivo testing can be carried

Table 2 Antifungal Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Molecular docking	Lanosterol 14-α-demethylase (1EA1).	Aloe-emodin, chrysophanol, and rhein	Not mentioned in the article	Ahmed et al 2019[18]
	Molecular docking	Lanosterol 14-Alpha Demethylase (CYP51)	9-Ene-methyl palmitate, stearidic acid, and trichosanic	Not mentioned in the article	Saptarini et al 2024[19]
In vitro	Poisoned-food technique.	Not mentioned in the article	Kaempferol	IC50: 413.7 ± 89.4	Pham et al, 2021[20]
In vivo	Clinical case series	Not mentioned in the article	Not mentioned in the article	Not mentioned in the article	Alpapara et al, 2020[1]

Table 3 Antiviral Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Molecular docking	Main protein (Mpro) of SARS-CoV-2 (6LU7)	Emodin, chrysoeriol, quercetin, kaempferol, and rhein	Not mentioned in the article	Turista et al 2023[1]
	Molecular docking	Mpro (6LU7) (2GTB), 3CLpro (3M3V) (Swiss-Model homology modeling)	Quercetin, kaempferol and aloe-modin	Not mentioned in the article	Ernawati et al 2020[22]
In vitro	MTT and karber method	Not mentioned in the article	Not mentioned in the article	IC50: 740	Shaheen et al 2015[22]
	DENV inhibition	Not mentioned in the article	Not mentioned in the article	IC50: 0.0256	Angelina et al 2017[23]
	Half-leaf method	Not mentioned in the article	Alkaloids, alataindoleins A–C, alatachromone A, alataindolein D	IC50 TMV: 55.3, 22.6, 14.2, 17.9, 43.4	Yang et al 2022[24]
In vivo	Induced rotavirus (RV) mice	Not mentioned in the article	Not mentioned in the article	Not mentioned in the article	Shaheen et al 2015[25]

out on other viruses to understand the reaction in the living body and to ensure safety and whether other effects are observed. For antiviral activity, in silico, in vitro, and in vivo testing has been carried out. In silico testing was carried out on the SARS-CoV-2 virus with the results that the combination of *Cassia alata* bioactive compounds has the potential to be used as a candidate for anti-SARS-CoV-2 supported by the result of drug-likeness, ADMET, pharmacokinetics, binding affinity, and antiviral activity prediction. Kaempferol has the lowest binding affinity to Mpro than other compounds.[21] The other in silico method was revealed by Ernawati et al 2020 that quercetin, kaempferol, and aloe-emodin have lower free energy Gibbs and low kinetic inhibition compared to other sample compounds, and these two parameters of the virtual filtering simulation can predict SARS-CoV-2 inhibitory activity.[22]

In vitro research, Shaheen et al 2015 investigated the antiviral activity of five extracts (methanol, chloroform, ethyl acetate, n-butanol, and aqueous) from *Cassia alata* leaves against rotavirus (RV). In vitro, all extracts prevented the cytopathic effect (CPE) of RV, as demonstrated in an MTT colorimetric and karber methods, with therapeutic index (IT) ranging from 22.8 to 0.02 and reduction in virus titers ranging from 4.25 and 0.25 log10 TCID50. From all extracts, methanol extract was the strongest than the other extracts against RV replication.[25] Angelina et al 2017 discovered that *Cassia alata* leaves extract and butanol subfraction are potent candidate antiviral against dengue virus serotype-2 (DENV-2) with the IC50 0.0256 and 6.47 ug/mL and CC50 323.45 and 645.8 ug/mL.[26] Yang et al 2022 shows that Alataindolein B, alataindolein C, and alatachromone A of stem bark extract have high anti-tobacco mosaic virus (TMV) activities with inhibition rates of 44.4%, 66.5%, and 52.3%.[24]

In vivo, research was carried out by Shaheen et al 2015 by testing anti-rotavirus in mice. It was found that 50 mg and 100 mg/kg body weight of methanol extract of *Cassia alata* leaves significantly reduced rotavirus (RV) yield in the small intestine as well as reduced mortality, severity, and duration of diarrhea after infection for 7 days in mice.[25] Angelina et al 2022 stated that ethanol extract of *Cassia alata* leaves decreased the DENV-2 titer and increased platelet count significantly compared to the control group in balb/c strain mice infected by dengue virus.[22] The review for antiviral activity results can be seen in (Table 3).

There is quite a lot of research that has been done on *C. alata* for its antiviral activity. This shows that this plant has the potential to be further developed as a therapeutic agent. However, there has not been much testing of antiviral activity against other viruses, so further research needs to be done to maximize the potential of *C. alata*. More in vivo testing also needs to be done to research safety and the effects on living things.

Antimicrobial

Cassia alata's antibacterial activity has not been investigated in silico, however numerous in vitro investigations have been conducted. In vitro study has shown that agar-well diffusion analysis revealed the strong antimicrobial activities against the clinical strain of S. aureus produced from ethyl acetate root extracts of *C. alata* with soxhlet extraction, shown by large inhibition zone with MIC 0.312 and MBC 0.625. Further GC–MS analysis also revealed the presence of 88 phytochemicals, 32 of them have previously been shown to possess antibacterial, antioxidant, and anti-inflammatory properties. These phytochemicals include phenolics, steroids, fatty acids, alcoholics, esters, and alkane hydrocarbons. Many fatty acid components found in *Cassia alata*, including linoleic acid, palmitic acid, stearic acid, neophytadiene, and methyl palmitate, have been shown to exhibit antibacterial properties against both gram-positive and gram-negative bacteria. The antibacterial effect of fatty acids is regulated by their structure, morphology, carbon-chain length, and the presence, number, position, and orientation of double bonds. Methyl palmitate, containing a methyl ester group, functions as a defense mechanism against bacterial infections by acting on the membranes of the bacteria and causing lysis of the bacteria directly, inhibiting enzyme activity, and disrupting cell energy production.[27] Further studies also revealed that the *Sennaalata* extract exhibited strong antimicrobial activities due to the presence of hydrocarbon sesquiterpenes and monoterpene as well as the monoterpene lactone by way of their synergistic effects.[28]

Another study by Sharma et al 2015 also found significant antibacterial activity in the acetone extract of root against clinically isolated P. vulgaris and B. subtilis, and the chloroform extract of root against S. aureus.[29] The crude extracts for both leaves and roots showed concentration-dependent *Neisseria gonorrhea* inhibition, with ether root extract showing the highest potency.[30] The dye of *Cassia alata* flower was also found to be highly active against all pathogens after extraction in methanol, with maximum inhibition observed against Pseudomonas sp. with a MIC value of 1.56 mg/mL.[31]

The antimicrobial study of *Cassia alata* is rather extensive and primarily utilizes the zone of inhibition determined from the MIC and MBC values using disc-diffusion and agar-well diffusion methods. The strong antibacterial activity was demonstrated by the results against clinical strains of *Neisseria gonorrhoea, Pseudomonas sp., P. vulgaris, B. subtilis*, and *S. aureus*.[27,29–31] Due to their synergistic actions, hydrocarbon sesquiterpenes, monoterpenes, and monoterpene lactone are thought to be responsible for the significant antimicrobial effect.[28] Aloe emodin is one of the substances that showed the strongest antibacterial action against Shigella flexneri and MDR *Vibrio cholerae*.[32] Aloe-emodin also reduces pathogenicity by interfering with the oligomerization of α-toxin and prevents the formation of biofilms in Staphylococcus aureus by inhibiting the production of extracellular proteins.[33,34]

Potential causes for antimicrobial activity include the creation of irreversible damage to bacterial cell membranes, which can result in cytoplasmic material losses, ion leakage, and the loss of energy substrates like glucose and ATP. These events can then cause the bacteria to lyse (cytolyze) and eventually die. Inhibiting the synthesis of amylase and protease, which halts the generation of toxins, and electron flow, and causes the cell content to coagulate, is another possible course of action.[28] The *Cassia alata* formulation in herbal hydrogel has also been developed and evaluated to have a favorable effect in killing bacteria on the surface of the skin of rats with surgical site infection, even better than the commercial formulation.[5] The review for antimicrobial activity results can be seen in (Table 4).

Table 4 Antimicrobial Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Never been done	Never been done	Never been done	Never been done	Never been done
In vitro	Agar-well diffusion, colorimetric broth, and grid culture from soxhlet extraction and GC-MS	Not mentioned in the article	Linoleic acid Palmitic acid Stearic acid Neophytadiene Methyl palmitate	MIC: 313	Toh et al 2023[24]
	Disc diffusion and GC-MS	Not mentioned in the article	β-farnesene αα-selinene Dihydroactinidiolide β-citronellene Methyl palmitate Palmitic acid Methyl strearate	Not mentioned in the article	Igwe & Onwo 2015[28]
	Agar well diffusion method	Not mentioned in the article	No specific compound mentioned, only secondary metabolite group: alkaloids, cardioglycosides, fats and oils, flavonoid, glycosides, phytosterols, quinone, resins, tannins, and terpenoid	Not mentioned in the article	Sharma et al 2015[28]
	Microdilution method	Not mentioned in the article	Aloe emodin	4–128	Tatsimo et al 2017[32]
	Agar well diffusion method	Not mentioned in the article	Not mentioned in the article	MIC: 11000	Otto et al 2014[30]
	Agar double dilution method	Not mentioned in the article	No specific compound was mentioned, only a secondary metabolite group: anthraquinone	MIC 156000	Muruganandham et al 2023[31]
In vivo	Visual examination of healing of infected wound	Not mentioned in the article	Not mentioned in the article	80–120	Iraqui et al 2019[5]

The research on *Cassia alata* has been thoroughly explored through in vitro and in vivo investigations of anti-microbial activity, yet no in silico research has been undertaken. However, it remains imperative to pursue in silico studies as they can aid in forecasting interactions with biological targets, assessing potential side effects or toxicity, and providing a deeper understanding of the mechanism of action of particular compounds or molecules. In silico studies, more cost-effectively than direct experimental methods, can yield comprehensive insights into both the chemical and biological attributes of a substance, guiding future research or the molecular refinement of potential drug candidates.

Antimalaria

There are various antimalarial investigations in vitro and in vivo, but there is no in silico study. The crude extract of *C. alata* leaves has antimalarial efficacy against Plasmodium falciparum strain 3D7 parasites, with an IC50 value of 17.270 g/mL and a maximum inhibition percentage of 65.62. This action is generated by phytochemicals contained in *C. alata* leaves, such as alkaloids, tannins, anthraquinones, flavonoids, steroids, and terpenoids.[35] In vivo investigations also showed that mice infected with P. berghei and treated with plant extract from *Cassia alata* saw a parasitemia reduction equal to that seen with the standard drug chloroquine. The greatest chemosuppression of 84.36% was seen when *S. alata* extract was treated at 400 mg/kg/day. Based on these two trials, it is known to have antimalarial properties.[36] However, the antimalarial mechanism of *C. alata* leaves is yet unknown, thus more study is needed.

Plasmodium falciparum strain D10 utilizing the parasite lactate dehydrogenase assay. A 4-day suppression experiment with Plasmodium berghei in mice was utilized to assess the extract's in vivo antiplasmodial efficacy. The dichloromethane/methane (1:1, v/v) extract of *Cassia alata* was highly effective against Plasmodium falciparum.[37] The antiplasmodial capabilities of the ethanol extract of *Cassia alata* leaves were substantial, resulting in a reduction in parasitemia.[38] It is unclear how *C. alata*'s antiphasic action works. The review for antimalarial activity results can be seen in (Table 5).

Results from in vitro and in vivo investigations *C. alata* have significant antimalarial properties (Table 5). However, in silico research is still needed in the future to determine how *C. alata* bioactive chemicals interact with biological targets from malaria, with the intention of developing targeted therapies for the disease. Furthermore, additional in vivo experiments are required to estimate IC50 so that an effective dosage may be established.

Anticancer/Antitumor

The anticancer activity has never been tested in silico, however in vitro testing has been carried out by Salamatullah et al 2021 that the methanol extract of *Cassia alata* flower suppresses the oxidative stress-induced protumorigenic and inflammatory signaling pathway causing DNA damage and epithelial cell stress. This mechanistic action increased antioxidant capacity in HT-115 human colon cancer cell growth cells which arrested mitochondrial oxidative stress.[37] Ali et al 2017 tested the

Table 5 Antimalarial Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Never been done	Never been done	Never been done	Never been done	Never been done
In vitro	The WHO Mark III Microtest assay	Not mentioned in the article	Alkaloids, flavonoids, tannins, anthraquinone, steroid, and terpenoid.	IC 50: 17.270	Yaw, 2015[35]
In vivo	Curative test.	Not mentioned in the article	Alkaloids, flavonoid, phenolics, tannins, terpenoids, saponins, and steroids	Not mentioned in the article	Atanu et al, 2022[36]
	Peter's 4 day test	Not mentioned in the article	Alkaloids, tannins, anthraquinones, flavonoids, steroids, and terpenoids.	Not mentioned in the article	O Da et al 2016[37]
	Peter's 4 day test	Not mentioned in the article	Sennosides, anthraquinones and kaempferol.	Not mentioned in the article	Okoro et al 2019[37]

chloroform fraction of *Cassia alata* L leaves and showed remarkable cytotoxicity against HepG2 cells possibly because of anthraquinones content.[39] Khoerunisah et al 2022 showed that the compounds contained in *C. alata* L. leaves namely aloe-emodin, emodin, and kaempferol showed better cytotoxic activity against breast cancer (MCF-7) cells than the extract and fractions of *C. alata* L. leaves with IC50 values of 12.7 ppm, 18.1 ppm, and 131.3 ppm, respectively.[40]

The other research done by Kittiwattanokhun et al 2021 found that the ethanol extract of *Senna alata* could reduce MMP-2 and MMP-9 expression by downregulation of NF-κB which is downstream of MAPKs and PI3K/Akt signaling pathway in SW1353 cells resulting in reduced cancer cell migration and invasion.[41] Chahardehi's et al 2020 study shows that ethanolic leaf extract of *Cassia alata* has activity on breast cancer anticancer agents (IC50 < 100 μg/mL), which did not exhibit toxicity on normal cell lines as well. Interestingly, in contrast to SRB assay results, the *S. alata* extract/fractions exhibited non-toxic activity (LC50 > 1000 μg/mL) was assessed using the brine shrimp lethality test as a primary assay for anticancer activity.[42]

Then, in in vivo testing, lung anticancer testing was carried out on mice using methanolic extract of *Cassia alata* leaves, and the results showed a decreased lung index and restoration of almost normal lung histoarchitecture and decreased lung inflammation, mucin production, cell proliferation.[43,44] The review for anticancer activity results can be seen in (Table 6).

Compared to other activities, much research has been done on *C. alata* as an anticancer agent (Table 6). This shows that this plant has the potential to be further developed as a therapeutic agent. However, in silico research has never been carried out, where it is important to know the mechanism of interaction between substances and receptors and to determine the profile of the substance to be developed. In vivo research is also relatively small, namely only on the lungs, so further research can be carried out by looking at existing in vitro research results.

Table 6 Anticancer/Antitumor Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Never been done	Never been done	Never been done	Never been done	Never been done
In vitro	MTT Assay	Not mentioned in the article	Cyclotrisiloxan, b-Sitosterol, thiophene, tocopherol, beta carotene and cyclotrisiloxan derivative	1 in 48 h and 2 in 24h	Salamatullah et al 2021[38]
	MTT Assay	Not mentioned in the article	Anthraquinones	37.4 in 48 h.	Ali et al 2017[39]
	MTT	Not mentioned in the article	Aloe-emodin, emodin, kaempferol	12.7, 18.1, and 131.3 respectively	Khoerunisah et al 2022[40]
	MTT Assay	Not mentioned in the article	Not mentioned in the article	325.28 ± 0.75	Kittiwattanokhun et al 2021[41]
	Sulforhodamine B (SRB) cytotoxicity assay	Not mentioned in the article	Not mentioned in the article	0.013, 47.11, 57.61, and > 100 at 72 h	Chahardehi's et al 2020[42]
In vivo	Lung index	Not mentioned in the article	Not mentioned in the article	Not mentioned in the article	Mohamed et al 2022[39]
	Lung histoarchitecture, lung inflammation, mucin production, cell proliferation.	Not mentioned in the article	Not mentioned in the article	Not mentioned in the article	Fathalla et al, 2023[43]

Antioxidant

There have been numerous investigations on *Cassia alata*'s antioxidant properties since antioxidants are the activity that underlies the mechanisms of other pharmacological actions, making them crucial to comprehend. Most of the compounds found in it belong to the phenolics class (anacardic acid triene, caffeic acid, ferulic acid, o-coumaric acid) and flavonoid class (epigallocatechin gallate, agathisflavone, amentoflavone, kaempferol 3-O-gentiobioside, routine), particularly the tetramer of proanthocyanidin, which is known to have potent antioxidant activity demonstrated by its ability to scavenge DPPH and ABTS. It can also effectively chelate Fe^{2+} ions and have good reducing powers. *Senna alata* roots are rich in phenolics and flavonoids, phytoconstituents known to have antioxidant activity. Phenolic compounds are potent anti-oxidants that can scavenge free radicals, chelate transition metals, act as hydrogen donors, and prevent peroxide formation. Therefore, they could protect against diseases associated with elevated levels of free radicals in the body.[44]

Chemiluminescence measurements showed that *Cassia alata* has the best scavenger activity and highest reducing power compared to Eleusine indica, Polyscias fulva, Carica papaya, and Eremomastax speciosa. The ferric-reducing power may be attributed to the phenolic and flavonoid contents of the extracts. Both groups could scavenge reactive oxygen species such as superoxide free radicals, singlet oxygen, and hydroxyl radicals; phenolic compounds have the potential to be antioxidants. The ability to reduce Fe (III) can be attributed to hydrogen donation from phenolic compounds, which is associated with the presence of a reducing agent. Furthermore, the quantity and orientation of hydroxyl groups in phenolic substances also determine their antioxidant capacity by scavenging dangerous-free radicals and chelating metal ions to prevent the generation of hazardous radicals that damage vital biomolecules.[45] Numerous studies conducted in vitro have also indicated that the extract from *Cassia alata* exhibited strong antioxidant properties, with strong IC50 values indicating good DPPH free radical scavenging action compared to conventional drugs or other medicinal plants.[46,47]

Flavonoids work as antioxidants in two ways: directly, by giving hydrogen ions to counteract the harmful effects of free radicals, and indirectly, by raising the production of endogenous antioxidant genes via various pathways. Meanwhile, phenolics efficiently block the action of lipid peroxides, superoxide anions, H_2O_2, and the hydroxyl radical as free radical scavengers.[47] The review for antioxidant activity results can be seen in (Table 7).

Table 7 Antioxidant Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Molecular docking	Elastase (3HGP) Tyrosinase (2Y9X)	Epigallocatechin gallate, agathisflavone, amentoflavone, kaempferol 3-O-gentiobioside, rutin, tetramer of proanthocyanidin, anacardic acid triene, caffeic acid, ferulic acid, and o-coumaric acid	Not mentioned in the article	Chaikhong et al 2023[48]
In vitro	Evaluation of DPPH and ABTS Activity	Not mentioned in the article	No specific compound mentioned, only secondary metabolite group: phenolics and flavonoids	45.18 39.14	Ita & Ndukwe 2017[49]
	Chemiluminescence measurements	Not mentioned in the article	No specific compound mentioned, only secondary metabolite group: phenolics and flavonoids	Not mentioned in the article	Sagnia et al 2014[45]
	DPPH	Not mentioned in the article	No specific compound mentioned, only secondary metabolite group: alkaloids, flavonoids, saponins, tannins, triterpenoids, steroids and glycosides.	66.01–138.8	Rahmawati et al 2022[46]
	DPPH and GC-MS	Not mentioned in the article	3-Methylmannoside, Neophytadiene, Campesterol, and Vitamin E	29.81	Karki et al 2023[47]
In vivo	Never been done	Never been done	Never been done	Never been done	Never been done

According to findings from in silico and in vitro research, *Cassia alata* demonstrates considerable antioxidant capabilities (Table 7). Nonetheless, there has been no exploration through in vivo trials on animal models thus far, presenting a significant avenue for investigation. In vivo experiments offer the opportunity to confirm the antioxidant properties of *Cassia alata* under conditions more akin to human physiology, utilizing animals or more sophisticated models. Furthermore, such studies can shed light on the toxicity and possible adverse effects of *Cassia alata*. It is crucial to grasp the potential risks and advantages before human application.

Anti-Inflammation

C. alata's anti-inflammatory efficacy has not been studied in silico, although there have been some in vitro and in vivo investigations. The anti-inflammatory effects of rhein and *C. alata* leaf extract on tert-butyl hydroperoxide-induced oxidative stress in HaCaT cells were assessed. *C. alata* leaf extract inhibited ROS formation and TNF-α and IL-8 production, demonstrating anti-inflammatory activity compared to rhein standard. The extract of *C. alata* leaves contained 0.1225% w/w of rhein. Rhein (1–50 μM) decreased ROS formation in a concentration-dependent manner while also decreasing TNF-α and IL-8 production. At the same concentration, *C. alata* leaf extract outperformed rhein regarding anti-inflammatory activity. Research suggests that rhein and *C. alata* leaf extract can decrease inflammation by lowering TNF-α and IL-8 levels through ROS reduction. This activity might be because other components in the extract, such as flavonoids and other anthraquinones, also have anti-inflammatory properties.[47]

C. alata extract significantly suppressed IPP-induced TNF-a production in a dose-dependent manner. *Cassia alata*, at 1 mg, provided the most effective inhibition. It inhibits IPP-induced TNF-a generation. Chemical research of *Cassia alata* revealed the presence of Chrysarobin, tannins, Kaempferol, Isochrysophanol, Chrysophanol glycosides, and Chrysarobin, which explains its anti-inflammatory action.[45]

Methanol and water extracts of *C. alata* effectively reduced inflammation by lowering IL-8 levels. The methanol and water extracts (6.25–100 μg/mL) significantly inhibited COX-1 and 5-LOX enzymes compared to standard indomethacin and nordihydroguaiaretic acid, respectively. However, there was reduced suppression of the COX-2 enzyme compared to Indomethacin. At a dosage of 6.25 μg/mL, the methanol extract significantly inhibited COX-1 and 5-LOX enzymes compared to the control treatments at the same dose. The methanol extract of *C. alata* possesses anti-inflammatory effects.[50]

The percentage of protection at 100μg/mL was discovered to be 36.51% and 23.32% for the standard medication diclofenac sodium and *C. alata* extract, respectively. This action occurs because *C. alata* contains phenols and flavonoids, which are plant secondary metabolites with anti-inflammatory characteristics.[47]

In vivo, methanol extract of *Cassia alata* bark (100 mg/kg, i.p). significantly reduced rat paw edema (P < 0.01) across all evaluation periods. The methanol extract inhibited carrageenan-induced paw oedema by 51.58*% at a 100 mg/kg dosage after 3 hours of treatment, while the conventional medication inhibited it by 54.70*%. According to Kumari (2020), flavonoids and tannins decrease prostaglandin production throughout the inflammatory phase.[51] The review for anti-inflammation activity results can be seen in (Table 8).

According to the previous study (Table 8), *C. alata* functions in inflammation relief. However, further in silico study is needed to determine the mechanism of action between C. alata chemicals and target receptors, aiming to accelerate the process of generating medications from this plant. Flavonoid molecules are also known to possess anti-inflammatory properties. Further study is needed to determine which chemicals are responsible for this action.

Hepatoprotective

There has been no in silico testing on hepatoprotective activity, but there have been in vitro and in vivo tests. In vitro testing carried out by Mary et al (2019) showed that the ethanolic extract of *Cassia alata* flower showed the highest hepatoprotective activity that was induced by CCl4 toxicity when compared to standard due to the presence of alkaloid and flavonoid in the extract.[53] Meanwhile, research by Sugumar et al (2016) conducted in vivo stated that ethanolic extract of *Senna alata* leaves tends to protect the liver and renal tissues from the damage caused by the oxidative stress during diabetes in streptozotocin-induced experimental rats.[54] The review for hepatoprotective activity results can be seen in (Table 9).

Table 8 Anti-Inflammatory Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Never been done	Never been done	Never been done	Never been done	Never been done
In vitro	ELISA kit	Not mentioned in the article	Flavonoids and anthraquinones	Not mentioned in the article	Wadkhien et al 2018[52]
	Flow cytometry	Not mentioned in the article	Not mentioned in the article	Not mentioned in the article	Sagnia et al 2014[45]
	COX and LOX Inhibition Assay	Not mentioned in the article	Not mentioned in the article	Methanol extract: 6.25 Water extract: 100	Agampodi and Collet et al 2022[50]
In vivo	Carrageenan-induced rat paw oedema method	Not mentioned in the article	Flavonoids and tannins	Not mentioned in the article	Kumari et al 2020[51]
	Human red blood cell (HRBC) membrane stabilizing method	Not mentioned in the article	Flavonoids and phenolic	9.93	Karki et al 2023[46]

Table 9 Hepatoprotective Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Never been done	Never been done	Never been done	Never been done	Never been done
In vitro	Malondialdehyde, GOT(AST), GPT (ALT) and reduced glutathione (GSH).	Not mentioned in the article	Alkaloid and flavonoid	Not mentioned in the article	Mary et al 2019[53]
In vivo	The levels of plasma insulin, glucose, urea, uric acid, creatinine, vitamin C, vitamin E, reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione-s-transferase	Not mentioned in the article	Not mentioned in the article	Not mentioned in the article	Sugumar et al, 2016[50]

Based on the research that has been carried out, *C. alata* has potential as a hepatoprotective agent (Table 9). However, there is still little research on this activity both in vitro and in vivo. In silico research has never been done, so we need to determine the toxicity and the interactions with receptors. We also need to know the secondary metabolites that play a role in this activity for further drug development.

Antidepressant

Research conducted in vivo has demonstrated that the aqueous extract of *Cassia alata* leaves has potent antidepressant effects. The results of the TST and FST, which demonstrated a reduction in immobility and an increase in climbing activity comparable to imipramine in TST and fluoxetine in FST, provided evidence for this. The key mechanisms responsible for this decrease in immobility rate include enhanced 5-hydroxytryptamine (5-HT) and catecholamine neurotransmission, resulting in antidepressant effects. The antidepressant effect of *Cassia alata* leaf extract may be related to its flavonoid content, particularly kaempferol and quercetin, which appear to exist as conjugated forms in

circulation. Recent reports have detailed how these metabolites cross the blood–brain barrier to reach the brain as well as their effects on the central nervous system.[55] Antidepressant activity has also been demonstrated in some animal models, where it was discovered to enhance the availability of serotonin and norepinephrine in the synaptic cleft by inhibiting the MAO enzyme. This resulted in an increase in dopamine, serotonin, and nor-epinephrine levels with a decrease in serotonin metabolism.[56] The review for antidepressant activity results can be seen in (Table 10).

In silico and in vitro examinations retain significance despite the completion of in vivo trials as they offer distinct and supplementary perspectives. In silico assessments of antidepressants can uncover the drug's molecular mechanisms, its interactions with specific receptors, and its toxicity profile, aiding in the development of safer medications, particularly to mitigate central nervous system (CNS) side effects commonly associated with antidepressants. In vitro investigations are also crucial for assessing the direct impacts of compounds on biological systems within a controlled setting, including the determination of efficacy and toxicity of antidepressant compounds at the cellular level. Furthermore, ongoing in vivo testing can be pursued to establish LC50 and IC50 values, serving as benchmarks for dosage calculations.

Antianxiety

There has only been one in vivo investigation on the antianxiety activity of *C. alata*. Administration of an aqueous leaf extract (200 mg/Kg) of *S. alata* substantially enhanced the mean number of entries (2.25 ± 0.98) and time spent in the open arm (2.23 ± 0.04) compared to the control group, indicating anti-anxiety action. The extract has somewhat more activity than the usual medication, Diazepam. The antidepressant action of *S. alata* leaf extract may be due to its flavonoid concentration. *S. alata* leaves contain kaempferol, which appears conjugated in the circulation, as do other flavonoid glycosides like quercetin. This metabolite's delivery to the brain across the blood–brain barrier and its effects on the CNS have been recently documented. However, more research is needed to determine the phytoconstituents responsible for the antidepressant action of these taxa.[55] The review for antianxiety activity results can be seen in (Table 11).

There has been limited research on anti-anxiety in *C. alata* (Table 11). In silico research is required as a preliminary study to anticipate the interactions between *C. alata* and anxiety target proteins, which will aid in discovering compounds

Table 10 Antidepressant Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Never been done	Never been done	Never been done	Never been done	Never been done
In vitro	Never been done	Never been done	Never been done	Never been done	Never been done
In vivo	Tail suspension test (TST) and Forced swim test (FST)	Not mentioned in the article	Kaempferol Quercetin	Not mentioned in the article	Pamulaparthi et al 2016[55]

Table 11 Antianxiety Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Never been done	Never been done	Never been done	Never been done	Never been done
In vitro	Never been done	Never been done	Never been done	Never been done	Never been done
In vivo	Tail suspension test (TST) and Forced swim test (FST)	Not mentioned in the article	Kaempferol Quercetin	Not mentioned in the article	Pamulaparthi et al 2016[51]

that may be used as anti-anxiety medication candidates. Therefore, in the future, more in-depth studies may be conducted in vitro and in vivo to identify various characteristics such as LC50 and IC50.

Anticonvulsant and Sedative

There is one in vivo test on the anticonvulsant and sedative effect where aqueous extract of *Cassia alata* leaves. The result is that at 200 and 400 mg doses, the extract of *Cassia alata* significantly increases and reduces the time to onset and the duration of convulsions. At the same therapeutic dose, this extract significantly increases and reduces, respectively, the time to onset and barbiturate sleep. The extract significantly reduces motor activity at 200 and 400 mg/kg doses. The chemical screening made it possible to highlight the presence of alkaloids, tannins, saponosides, flavonoids, and free anthraquinones in this extract.[57] The review for anticonvulsant and sedative activity results can be seen in (Table 12).

Research on *C. alata* as an anticonvulsant and sedative agent has been conducted in vivo, but little information has been obtained. So, three types of studies still need to be carried out to find out whether *C. alata* can be developed in the future as an anticonvulsant and sedative herbal therapy that is expected to be safer and have fewer side effects.

Hyperlipidaemia

Only a few studies have been conducted on *Cassia alata*'s hyperlipidaemic effect. An in vivo investigation revealed that the *Senna alata* (L) Roxb aqueous leaf extract on palm oil exhibits significant hypolipidemic features such as better lipid profile (total Cholesterol, triglycerides, LDL, and HDL) but impact negatively on the liver (ALT, AST, and ALP) probably causing bile duct obstruction. The total and conjugated bilirubin levels show a reduction within the normal range.[57] The review for hyperlipidemia activity results can be seen in (Table 13).

Hyperlipidemia presents a significant risk for cardiovascular conditions like heart attacks and strokes, making it a crucial area for further investigation. There is limited in silico research on hyperlipidemia, primarily focusing on HMG CoA reductase. Expanding in silico exploration of this aspect could pave the way for new avenues in the research of hyperlipidemia therapy. In vitro examination of *Cassia alata* can be conducted by characterizing its constituent compounds acting as antihyperlipidemic agents. It utilizes analytical tools, such as GC-MS and FTIR, resulting in the name of the compound or secondary metabolite responsible for the effect.

Table 12 Anticonvulsant and Sedative Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Never been done	Never been done	Never been done	Never been done	Never been done
In vitro	Never been done	Never been done	Never been done	Never been done	Never been done
In vivo	Time to onset and the duration of convulsions	Not mentioned in the article	Alkaloids, tannins, saponosides, flavonoids and free anthraquinones	Not mentioned in the article	Nkundineza et al 2020[53]

Table 13 Hyperlipidaemia Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Never been done	Never been done	Never been done	Never been done	Never been done
In vitro	Never been done	Never been done	Never been done	Never been done	Never been done
In vivo	Determination of Lipid Profile, Liver Enzymes, and Bilirubin	Not mentioned in the article	Not mentioned in the article	Not mentioned in the article	Onyegeme - Okerenta et al 2017[63]

Anthelmintic

Regarding anthelmintic activity, there are only tests carried out in vitro. From the test results, it was found that freeze-dried aqueous extract of *Cassia alata* leaves has the anthelmintic activity on two stages of life of the Haemonchus contortus parasite at concentration 6.25–100 mg/mL. The phytochemical analysis revealed the presence of steroidal and triterpenoid compounds, anthracenosides, saponosides, and polyphenols (tannins).[57] Anbu et al 2015 found that ethanol extract of *Cassia alata* leaves exhibited significant anthelmintic activity against Pheretima posthuma and Ascardia galli at the highest concentration of 100 mg/mL.[57] Roy and Lyndem 2019 discovered that ethanolic extract of *Cassia alata* leaves has strong antitrematodal activity against the ruminant parasite Paramphistomum gracile.[57] Roy et al 2016 found that exposure of the worm Hymenolepis diminuta to the alcoholic leaves extract of *Cassia alata* leads to disruption in intracellular calcium homeostasis. A significant increase (44.6% and 25%) of efflux in Ca2+ from the tissue to the incubated medium was observed. *Senna alata* showed a high rate of efflux (5.32 mg/g).[57] Ahmed et al 2021 found that the ethanol extract of *Cassia alata* leaves showed the quickest time for paralysis (4 min) at its 50 mg/mL concentration against aquarium worms (*Tubifex tubifex*).[59] The review for anthelmintic activity results can be seen in (Table 14).

Based on several in vitro studies that have been conducted (Table 14), *C. alata* is known to have anthelmintic activity, but in silico and in vivo studies are still needed. In silico evaluation of anthelmintic may identify its molecular mechanism, interaction with specific receptors, and toxicity profile, thereby assisting in developing safer drugs. Continuing in vivo is especially important for analyzing the efficacy and toxicity of anthelmintics at the molecular level. Furthermore, in vivo testing may provide LC50 and IC50 values, which will be used as standard dosage estimations.

Anticoagulant

Studies on *Cassia alata*'s anticoagulant effect are rare; the only available studies are in vitro investigations that demonstrate the plant's ability to prolong coagulation time as measured by the activated partial thromboplastin time (aPTT) and the prothrombin time (PT) test. For the PT test, the time of clotting for normal blood samples without applying anticoagulant was 12–15 s. While the extracts were added, the time of clotting increased to 28s for EE and 26s

Table 14 Anthelmintic Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Never been done	Never been done	Never been done	Never been done	Never been done
In vitro	Egg hatching inhibition test and mobility inhibition of adult worms	Not mentioned in the article	Steroidal and triterpenic compounds, anthracenosides, saponosides, polyphenols (tannins) and reducing compounds	Aqueous: 60.924 (20.242–86.643) Hydroacetonic: 17.651 (0.460–59.785)	Fidele et al, 2020[60]
	Paralysis and mortality time	Not mentioned in the article	Glycosides, flavonoids	Not mentioned in the article	Anbu et al 2015[61]
	Motility and time of mortality	Not mentioned in the article	Not mentioned in the article	Not mentioned in the article	Roy and Lyndem 2019[62]
	Intracellular calcium homeostasis.	Not mentioned in the article	Not mentioned in the article	Not mentioned in the article	Roy et al 2016[62]
	The time for paralysis and death of the worm	Not mentioned in the article	Alkaloids, coumarins, glycosides, flavonoids, phenols, resins, saponins, tannins, terpenoids	Not mentioned in the article	Ahmed et al 2021[59]
In vivo	Never been done	Never been done	Never been done	Never been done	Never been done

for AE at 1 mg/mL extract concentration. For an aPTT test, the time of clotting for a normal blood sample without applying an anticoagulant was 25–39s. While the extracts were added, the time was extended to 47s for EE and 41s for AE at 1 mg/mL extract concentration. The result shows that the times were higher than the sample containing no extracts, therefore it is effective as an anticoagulant.[59] The review for anticoagulant activity results can be seen in (Table 15).

In silico investigations of anticoagulant activity are necessary to uncover the compounds involved, underlying mechanisms, and toxicity profile, serving as a foundation for modifying drug compounds. Identifying the specific compound will enable a more targeted and focused approach to drug development. Furthermore, in vivo anticoagulant research is essential to assess the body's reaction to the physiological impacts of specific compounds or molecules. Exploring its development through studies evaluating dosage forms also presents a promising research avenue.

Antivenom

There has been one in-vivo investigation on *C. alata*'s antivenom activity. Ethanolic extracts of *Cassia alata* plants were investigated for antivenom action against Daboia russelii venom. In mice, leaf extract at doses of 200 and 400 mg/kg demonstrated considerable hemorrhagic neutralization action, as well as strong necrotic activity. However, the mechanism is unknown, as are the chemicals that have antitoxic action.[63] The review for antivenom activity results can be seen in (Table 16).

C. alata's anti-venom activity has been the subject of limited study. In silico study is required to determine how the mechanism occurs. Further phytochemical screening study is required to determine which compounds have a role in this function. Furthermore, more extensive in vitro and in vivo investigations should be undertaken to assess the efficacy of *C. alata* as an anti-venom based on LC50 and IC50 levels.

Analgesic

There is only one test on analgesic activity, namely the in vivo test carried out by Kumar et al 2019. The results showed that hydro-alcoholic extract of *Cassia alata* Roxb. leaves possess very significant analgesic activity when screened through acetic acid-induced writhing and formalin-induced paw licking in mice. The analgesic activity exhibited by the

Table 15 Anticoagulant Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Never been done	Never been done	Never been done	Never been done	Never been done
In silico	Never been done	Never been done	Never been done	Never been done	Never been done
In vitro	Prothrombin time (PT) test and activated partial thromboplastin time (aPTT) test	Not mentioned in the article	Not mentioned in the article	Not mentioned in the article	Ahmed et al 2021[59]
In vivo	Never been done	Never been done	Never been done	Never been done	Never been done

Table 16 Antivenom Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Never been done	Never been done	Never been done	Never been done	Never been done
In vitro	Never been done	Never been done	Never been done	Never been done	Never been done
In vivo	Neutralization of Lethality	Not mentioned in the article	Not mentioned in the article	Not mentioned in the article	Bhat et al 2016[55]

test extract was less significant in the radiant heat method and hot plate method. The test extract was found to possess more peripheral analgesic activity than central-mediated analgesia.[51] The review for analgesic activity results can be seen in (Table 17).

From in vivo research, *C. alata* has analgesic potential. However, the research is still limited. In silico research needs to be carried out to determine the interaction mechanism and receptors that play a role. In vitro research is also needed to determine the IC50 value as a reference for determining dose. In addition, the secondary metabolites for analgesic activity have not been known, so further research is needed.

Antinociceptive

There is only one in vivo study that addresses *Cassia alata*'s antinociceptive activity, and it was conducted on mice utilizing the Hotplate Test and the Acetic Acid-induced Writhing Method. The study's findings indicated that the presence of tannins and flavonoids, including routine, quercetin, luteolin, hesperidin, and biflavonoids, which are mediated through both central and peripheral mechanisms, resulted in a significant antinociceptive effect of the methanolic extract of *Cassia alata* bark that is comparable to that of the standard, pentazocine.[51] Flavonoids possess the ability to bind to opioid receptors. Studies have demonstrated that the systemic administration of flavonoids can cause a dose-dependent reduction in the nociceptive behavioral response. This effect is linked to the activation of the μ-opioid system, an essential component in the regulation of pain.[64] The review for antinociceptive activity results can be seen in (Table 18).

Although in vivo research offers valuable insights into how an organism reacts to specific compounds or molecules, in silico and in vitro investigations offer a more targeted, streamlined, and regulated method for comprehending the biological and pharmacological characteristics of these substances. Integrating data from these three study types allows researchers to achieve a thorough grasp of the potential clinical uses and impacts of the substances under examination. More in silico exploration is needed to see the interactions and mechanisms underlying these activities. In vitro studies will be needed to evaluate the cellular response and direct impact on certain specific cells. Further in vivo studies are needed to determine the LC50 and IC50 in terms of dosage calculation.

Chondroprotective

There has only been one investigation on the chondroprotective activity of *C. alata* in vitro. The ethanolic extract of *S. alata* prevented the breakdown of cytokines (IL-1β and IL-17A) via blocking matrix degradation, including proteoglycans. The researchers believe that the strong chondroprotection of *S. alata*'s ethanolic extract is due to the synergistic/additive activities of additional anthraquinones, such as emodin and chrysophanol, or other phytoconstituents (phenolic compounds).[65] The review for chondroprotective activity results can be seen in (Table 19).

The research on the chondroprotective activity of *C. alata* Amish is relatively limited. In silico studies are required to determine how chemicals in C. alata interact with disease receptors, which will help future in vitro or in vivo research. An in vivo study is also needed to determine the effectiveness of *C. alata* as a chondroprotective. Deeper in vitro and in vivo research is required to determine the LC 50 and IC 50 values of *C. alata* to assess the effective dosage.

Table 17 Analgesic Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Never been done	Never been done	Never been done	Never been done	Never been done
In vitro	Never been done	Never been done	Never been done	Never been done	Never been done
In vivo	Radiant heat method, hot plate method, acetic acid induced writhing, and formalin induced paw licking	Not mentioned in the article	Not mentioned in the article	Not mentioned in the article	Kumari et al 2020[51]

Table 18 Antinociceptive Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Never been done	Never been done	Never been done	Never been done	Never been done
In vitro	Never been done	Never been done	Never been done	Never been done	Never been done
In vivo	Acetic Acid-induced Writhing method and Hotplate Test	Opioid receptor	Rutin, quercetin, luteolin, hesperidin, bioflavonoids.	Not mentioned in the article	Kumari et al 2020[48]

Table 19 Chondroprotective Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Never been done	Never been done	Never been done	Never been done	Never been done
In vitro	Bar-HRM analysis	Not mentioned in the article	Senna, rhein and aloe-emodin	Not mentioned in the article	Ongchai et al 2019[65]
In vivo	Never been done	Never been done	Never been done	Never been done	Never been don

Thrombolytic

There has been no in silico or in vivo testing of the thrombolytic activity of *Cassia alata*. However, from the in vitro method, Ahmed et al 2021 discovered that the thrombolytic activity of ethanol extract leaves of *Cassia alata* was 10 mg/mL concentration, achieving the highest (37%) clot lysis activity.[59] Based on Karki's et al 2023 study, the thrombolytic activity of methanolic leaves extract of *Cassia alata* showed clot lysis percentages of 7.89% and 10.13% at concentrations of 10 mg/mL and 25 mg/mL.[47] The review for thrombolytic activity results can be seen in (Table 20). In vitro testing using the lyse blood clot method has been carried out, but the secondary metabolites that play a role have not been determined. Apart from that, in silico and in vivo research has never been done so there is still little information on thrombolytic activity in *C. alata* plants. Further research is needed to determine whether thrombolytic *C. alata* can be developed as a therapeutic agent.

Previous studies by Fatmawati et al 2020 have reviewed *Cassia alata*'s pharmacological activity obtained from any methods but not focused on the secondary metabolites that are involved in resulting the activity like this study did.[66] This

Table 20 Thrombolytic Activity Result

Type of Studies	Methods	Receptor	Secondary Metabolite Responsible for the Effect	Inhibition Value (ug/mL)	References
In silico	Never been done	Never been done	Never been done	Never been done	Never been done
In vitro	Lyse blood clots	Not mentioned in the article	Alkaloids, coumarins, glycosides, flavonoids, phenols, resins, saponins, tannins, terpenoids	Not mentioned in the article	Ahmed et al, 2021[59]
	Lyse blood clots	Not mentioned in the article	Tannin, flavonoids, saponin, carbohydrates, terpenoids, and cardiac glycosides	Not mentioned in the article	Karki's et al, 2023[47]
In vivo	Never been done	Never been done	Never been done	Never been done	Never been done

study will confirm and expand on the therapeutic potential of *Cassia alata* that could be developed into new treatments for many diseases. The findings hopefully will support the development of plant-based drug as an alternatives or complements to existing medications, particularly in the face of rising antibiotic resistance since *Cassia alata* has antimicrobial properties. According to previous studies by Angelina et al 2021, the content of *Cassia alata* varied based on geographical conditions, which may affect the reproducibility results[23] Therefore, further research needs to be specific in certain specific locations. Another limitation of this study is the lack of in silico investigation compared to in vitro and in vivo studies. In silico studies frequently fail to account for living organism complexities. As a result, conclusions drawn from these investigations might not always correspond to direct results in clinical applications or in vivo studies. However, in silico studies are equally important as a basis for comprehending the mechanisms and interactions driving activity, as well as the receptors involved. These studies serve as a foundation for refining drug molecules to amplify therapeutic benefits while minimizing side effects.

Conclusion

Twenty pharmacological activities of *Cassia alata* were comprehensively reviewed in this study, including in silico, in vitro, and in vivo studies. The greatest research has been done on *Cassia alata*'s pharmacological activity as an antioxidant, antiviral, antimicrobial, antifungal, and antidiabetic; nevertheless, there is still a dearth of information about other activities, therefore more investigation is required. The type of study that is most commonly found is in vitro studies, while in silico studies are still very few, even though it is important to know the interaction with the receptor so that the mechanism underlying the pharmacological effect can be revealed. The flavonoid group (emodin, kaempferol, quercetin) is regarded to be particularly effective for diverse pharmacological functions. However, there has not been significant progress accomplished in terms of the evaluation of the dosage form as an alternative therapy so that test results for promising activities of these groups, such as antidiabetic, antifungal, and antiviral, can be carried out into further research. Several activities lack comprehensive investigation, particularly in silico studies, therefore further study is required to fill any gaps in the knowledge. Even though in silico studies frequently fail to account for living organism complexities and do not always correspond to clinical applications, further in silico research into the molecular mechanisms underlying the bioactivity of *Cassia alata* compounds is needed to provide deeper insights and identify potential targets as a foundation for drug development. Employing in silico techniques to predict the activities of *Cassia alata*, such as its effects on constipation, diarrhea, and as an expectorant, is important because they are frequently used in society based on ethnopharmacology practices but lack scientific research, which highlights the importance of studying this plant. Another recommendation given for future research based on the research findings is the need to continue research on *Cassia alata* leaf extracts or fractions until we obtain isolated compounds that play a role in an activity. Then, the in silico research that has been carried out can be continued to the next stage such as in vitro/in vivo testing or drug preformulation. Future perspectives will offer valuable insights for drug discovery and development, mechanistic understanding, and future research directions. It may greatly advance our knowledge of these medicinal plants from a scientific perspective as well as its practical uses.

Author Contributions

All authors contributed significantly to the work reported, whether it was through conception, study design, execution, data acquisition, analysis, and interpretation, or in all of these areas; helped draft, revise, or critically review the article; approved the final version that was published; agreed on the journal to which the article was submitted; reviewed and agreed on all versions of the article before submission, during revision, the final version accepted for publication, and any significant changes introduced at the proofing stage; and agreed to take responsibility for the work in its entirety.

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