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Efficacy of alkaloids as natural insecticides against Anopheles gambiae mosquitoes and their implications for malaria vector control

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Abstract

The increasing resistance of malaria vectors to synthetic insecticides necessitates the exploration of alternative, environmentally sustainable control strategies. This systematic review evaluates the efficacy and potential application of alkaloids as natural insecticides against Anopheles gambiae. Following PRISMA guidelines, literature published between 1990 and 2025 was retrieved from major databases, and 7 studies met strict inclusion criteria focusing on isolated alkaloids and their direct insecticidal effects. The findings show that alkaloids exhibit significant larvicidal and adulticidal activity, with strong dose- and time-dependent mortality across mosquito life stages. Mechanistically, these compounds act through multiple pathways, including neurotoxicity, enzyme inhibition, oxidative stress induction and disruption of hormonal and genetic regulatory systems. Evidence also suggests potential synergistic effects in crude plant extracts and interactions with mosquito detoxification pathways, which may influence resistance dynamics. However, variability in study design, limited mechanistic validation, and a lack of field-based evaluations constrain generalisation of the current findings. Additionally, sublethal effects on parasite transmission highlight complex ecological implications. Overall, alkaloids represent promising candidates for integration into vector control programs, but further research is required to optimise formulations, validate mechanisms and assess long-term efficacy and safety under field conditions.

Introduction

Human malaria is a global disease caused by parasites of the genus *Plasmodium*, which are transmitted by *Anopheles* mosquitoes. Despite substantial control progress in the last two decades, malaria remains a major cause of morbidity and infant mortality, with an estimated 282 million cases and 610,000 deaths in 2024; approximately 80% of the cases and 90% of the deaths occurred in sub-Saharan Africa [1]. Of the 70 *Anopheles* species that transmit malaria, only 40 are of major significance, with *Anopheles gambiae* s.l. being one of the most important vectors of *Plasmodium falciparum* malaria across Sub-Saharan Africa [2–5]. *Anopheles gambiae* s.l. mainly breeds in small, temporary, and sunlit water collections such as rain pools. However, it can also breed in a wide variety of other types of water bodies. It is usually an indoor resting species with an endophagic feeding habit. The prime choice vector control strategy remains the selective application of residual synthetic insecticides used in long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), such as organochlorines, organophosphates, carbamates, and pyrethroids, among other insecticides, to either control, reduce, or eradicate the breeding sites of malaria vectors [3, 6, 7]. Pyrethroids are among the prequalified active ingredients in the World Health Organisation (WHO) vector control products [8]. These synthetic insecticides not only affect the non-target population but can also constantly lead to toxicity to mammals, including livestock, fish, birds, and beneficial organisms; human poisoning; adverse effects on the environment resulting in contamination of soil, water, and air; emergence and spread of physiological and behavioural resistance to a wide range of insecticides [4, 5, 9]. However, the widespread emergence of resistance to synthetic insecticides such as pyrethroids, organochlorines, and organophosphates has increasingly compromised vector control efforts, and it is therefore important to develop new, environmentally friendly techniques and targeted insecticides that would enable insects to be controlled more effectively [4, 5, 10]. Between 2010 and 2019, 28 out of 82 malaria-endemic countries recorded resistance to the four major classes of insecticides, and 73 have reported resistance to at least one insecticide class [11]. Hence, insecticide resistance becomes a serious threat to mosquito control and the subsequent prevention of malaria transmission. Efforts to find new modes of action and develop active agents based on natural plant products have led to the isolation, screening and development of phytochemical compounds with insecticidal activity. Plants produce a diverse array of secondary metabolites that are used as natural defence chemicals against herbivores by various strategies, such as reducing their attractiveness to pests, repelling the pests, attracting natural enemies of pests, and even simply killing the pests by poisoning [12]. Natural compounds derived from plants, including alkaloids and related phytochemicals, have been reported as promising alternatives to synthetic insecticides due to their structural diversity, dynamic modes of action, biodegradability and reduced risk to non-target organisms [13]. Alkaloids are defined as “a cyclic organic compound containing nitrogen in a negative oxidation state which is of limited distribution among living organisms” [14]. Their insecticidal potential arises from a variety of mechanisms, including neurotoxicity, insect growth regulators, anti-feeding activity, and disruption of development, which makes them attractive candidates for mosquito control, such as *An. gambiae* [2, 11, 15]. A variety of different classes of alkaloids, including isoquinoline, quinoline, piperidine, pyridine, tropane, purine, steroidal, and acridone, among other alkaloids, have been reported to have interesting therapeutic effects against malaria and are efficient in controlling the mosquito vector *Anopheles gambiae* due to their chemical diversity and diverse modes of action. Hence, revealing their potential as alternatives to synthetic or chemical insecticides [16–18]. The effectiveness of plant-based compounds is multifaceted; some act as direct toxicants, others interfere with mosquito behaviour to enhance repellency, and, in some cases, these compounds can disrupt developmental processes, potentially reducing adult emergence rates from aquatic habitats [11, 19]. Beyond their intrinsic toxicological effects, plant alkaloids may also act synergistically by inhibiting cytochrome P450 detoxification and increasing susceptibility in resistant mosquitoes when used with synthetic insecticides [20]. These combined synergistic effects make alkaloids a promising insecticidal alternative against vector insecticide resistance [20]. With the rising burden of malaria and increasing resistance to synthetic insecticides, there is an urgent need for a review focusing specifically on alkaloids as natural insecticides against *Anopheles gambiae*. This review seeks to address the gap by building on previous and current knowledge on preclinical experimental

studies evaluating the insecticidal efficacy, mechanisms of action, and dose-response effects of alkaloids against *Anopheles gambiae* mosquitoes and their potential application in malaria vector control.

Methods

Study design

For this systematic review, we employed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [21]. The review protocol was registered in PROSPERO (International Prospective Register of Systematic Reviews) with registration no. CRD420251251021 allocated to it.

Search strategy

To find keywords and synonyms, a preliminary search was done. Research publications from the PubMed, Scopus, Web of Science, and Google Scholar databases were systematically retrieved from January 1990 to December 2025. In order to find any recent items, this search was updated in February 2026 after being completed in December 2025. The article searching phase of this systematic review was conducted using a comprehensive search strategy. Terms including "alkaloids," "botanical insecticides," "*Anopheles gambiae*," and "insecticidal activity" were utilised in the search tactics. For every electronic database, combinations of various search phrases and strings were used to improve the sensitivity and specificity of the search (Table S1).

2.3 Inclusion and exclusion criteria

This review incorporated pre-clinical experimental studies that examined the control of malaria transmission by targeting *Anopheles gambiae*, which is the major mosquito that transmits *Plasmodium falciparum*. Quality studies included laboratory-reared or field-collected *Anopheles gambiae* at any life stage (larva, pupa, or adult), consisting of insecticide-susceptible and insecticide-resistant strains. The intervention of interest was exposure to alkaloids or alkaloid-containing extract of natural plant sources or synthetic compounds, being tested under laboratory or semi-field conditions under any of the application methods (e.g., contact, feeding, larvicidal or fumigation assays), dose, concentration or formulation. The studies that evaluated insecticidal effects like mortality, knockdown, developmental inhibition or biochemical toxicity indicators were included. Eligible designs included both comparative and non-comparative design which comprised the inclusion of proper controls (untreated, solvent, negative, or positive controls using standard insecticides) or well-defined baseline mortality corrections.

Research articles excluded were those that focused on non- *Anopheles gambiae* mosquito species, whether or not exposed to alkaloids; non-mosquito insects; or could not isolate and assess the insecticidal effects of alkaloids; did not include sufficient control conditions; or did not provide primary insecticidal endpoint measures. Further, field trials involving human subjects were excluded, as were epidemiological or modelling research, reviews, conference abstracts without complete data, and non-English publications.

2.4 Selection criteria

All articles which were retrieved were exported into Rayyan in order to automatically identify duplicates. These duplicates were manually screened and resolved using their titles and abstracts, and lastly, a full-text screening was carried out. Three reviewers were involved in the screening process and evaluated each article against preset inclusion criteria, including the study methods, the reported findings, and the methods of analysis. A fourth reviewer conducted another cross-check of the screening decisions to achieve consistency and minimize the impact of selection bias. Any differences of opinion between reviewers were resolved through discussion until unanimity was achieved. The multi-reviewer methodology guaranteed accuracy, transparency, and methodological rigour during the study selection process.

2.5 Risk of bias analysis

To ensure the internal validity of the synthesized findings, a structured risk of bias (RoB) assessment was conducted [22, 23]. A modified version of the SYRCLE risk of bias tool (Table 1) was used to evaluate the methodological quality of the included studies. The tool was adapted to reflect the experimental design of mosquito larvicidal bioassays and comprised ten items across six domains: selection bias, performance bias, detection bias, attrition bias, reporting bias, and other sources of bias [23, 22]. The signalling questions were tailored to assess key methodological aspects such as mosquito strain characterization, exposure conditions, bioassay procedures, and outcome reporting. Each item was judged such that a response of “yes” indicated a low risk of bias, whereas “no” indicated a high risk of bias. When the information provided in a study was insufficient to permit a clear judgment, the item was classified as “unclear risk,” suggesting either incomplete reporting or ambiguity in the study design [23]. To minimize subjectivity in the evaluation process, two reviewers independently assessed each included study, and any discrepancies in scoring were resolved through discussion and consensus.

Table 1
Modified SYRCLE 10-Item Risk of Bias tool

Bias Domain	Item	Signalling Question
Selection Bias	1. Mosquito strain characterization	Was the mosquito species/strain clearly identified and described (e.g., *Anopheles gambiae* strain or colony source)?
Selection Bias	2. Baseline experimental conditions	Were baseline experimental conditions standardized (life stage, environmental conditions, rearing protocol)?
Selection Bias	3. Random allocation of mosquitoes	Were mosquitoes randomly allocated to treatment and control groups?
Performance Bias	4. Standardized exposure conditions	Were exposure conditions (concentration range, exposure time, and bioassay protocol) consistently applied across groups?
Performance Bias	5. Use of appropriate controls	Were appropriate negative and/or positive controls (e.g., solvent control or synthetic insecticide comparator) included?
Detection Bias	6. Outcome measurement method	Were mortality or knockdown outcomes measured using standardized bioassay procedures (e.g., WHO larval bioassay guidelines)?
Detection Bias	7. Blinding of outcome assessment	Was outcome assessment conducted without knowledge of treatment allocation or using objective measurement criteria?
Attrition Bias	8. Completeness of outcome data	Were all experimental groups reported with complete outcome data (e.g., LC_{50}, LC_{90}, mortality rates)?
Reporting Bias	9. Mechanism of action reporting	Did the study clearly report or investigate the mechanism of action of the alkaloid compound (e.g., neurotoxicity, enzyme inhibition)?
Other Bias	10. Formulation and persistence description	Were the formulation of the compound (extract, purified alkaloid, nano formulation, etc.) and its persistence or residual activity adequately described?

Results

Search results

Web of Science database, PubMed, and ScienceDirect database were searched systematically to identify 424 records. Having removed 38 records of duplication, 404 Titles and abstracts were filtered, from which 376 studies were excluded based on the already established eligibility criteria. Twenty-eight full-text articles were then assessed on the parameter of eligibility, with all of them retrieved successfully. Of them, 21 papers were eliminated, the majority of them due to the lack of explicitly extracted and experimentally quantified alkaloids, lack of complete or inadequately detailed methodology. In the end, 7 articles were found that had the inclusion criteria and were incorporated in the qualitative synthesis (Fig. 1). Interestingly, only those articles in which alkaloids had been isolated and directly assessed as insecticides were taken into account. This extreme method of inclusion enhanced internal validity of the review since it minimized confounding effect of non-alkaloid phytochemicals that prevailed in crude extracts. In addition, it enabled the bioactivity comparison of studies to be more dependable as the results that were obtained could be directly attributed to the particular alkaloid compounds rather than complex extracts. Consequently, the obtained dataset can be described as having a high level of specificity in the testing of alkaloid-based interventions as a malaria vector control measure.

Records identified from Web of Science, PubMed and Science Direct Databases

(n = 424)

Records removed *before screening*:

Duplicate records removed (n = 38)

Identification

Records screened

(n = 404)

Records excluded

(n = 376)

Reports sought for retrieval

(n = 28)

Reports not retrieved

(n = 0)

Screening

21 Full-text Reports excluded.

Did not isolate any alkaloids (n = 15)

Incomplete methodology (n = 6)

Reports assessed for eligibility

(n = 28)

Studies included in review

(n = 7)

Included

Figure 1

PRISMA flow diagram of search phases with numbers of studies included/excluded at each stage

1. **3.2 Overview and thematic structure of the evidence base**
2. **3.2.1. Conceptual structure of alkaloid-based mosquito control research**

A keyword co-occurrence network was developed using VOS viewer software in order to demonstrate the conceptual relationships between topics found within the literature retrieved initially concerning the use of alkaloids against mosquitoes (Fig. 2). This analysis indicates three distinct and clearly defined themes. The first theme (red) comprises topics associated with the concept of alkaloids and their insecticidal property as well as derivative activities like repellents. The second theme (green) is associated with larvicidal activity and its endpoints such as LC_{50} and LC_{90} values along with the concept of enzyme activity. The third theme (blue) encompasses the concepts of *Anopheles gambiae*, mortality rates, and insecticide resistance, indicating the biological and epidemiological aspect within which this compound is investigated. It is noteworthy that the term alkaloid acts as an important connector among all the three themes, highlighting its importance within the framework of compound characterization, efficacy testing, and application outcomes. Overall, this network demonstrates that the present research focuses on the integration of different aspects such as the chemical and biological activity of these compounds and their resistance profile, providing a justification for the synthesis provided herein.

3.2.2. Study characteristics

All the experiments within this review were done with standardized laboratory bioassay procedures [24] to assess insecticidal activity against *Anopheles gambiae*. These were the WHO-suggested larval and adult toxicity tests, feeding tests and probit-based mortality tests. The papers were published between 2007 and 2025 and conducted in various geographical locations, including Africa and Asia, as well as other malaria-endemic regions. Most of the studies incorporated controlled laboratory experimental designs, and the relationships in dose-response and toxicity as time progressed of alkaloid compounds were emphasized. Five studies (out of the seven studies) used crude plant extractions and then chromatographically isolated and structurally characterized the alkaloid compounds (e.g., LC-MS, HPLC), and two studies directly assessed previously identified or synthesized alkaloid compounds. Bioassays were performed on both third-instar larvae and adult female mosquitoes under standard insectary conditions, and mortality, knockdown, and survival were assessed using LC50 and LC90 estimates and, in some cases, Kaplan-Meier survival analysis. Moreover, some studies compared crude extracts and purified alkaloids to clarify the roles of individual compounds in total bioactivity (Table 2). On balance, the studied articles showed a consistent methodology in the form of an experiment but differed in the degree of chemical characterization and mechanistic research of insecticidal action through alkaloids.

Table 2
Characteristics of included studies

Author/Year	Country	Alkaloid source	Identified alkaloid	Class of alkaloid	Mosquito stage	Method of assay	Key outcomes
[13]	Kenya	Ricinus communis	Ricinine and its derivative (3-carboxy-4-methoxy-N-methyl-2-pyridone)	Pyridone	Adult and larvae	Feeding assay for adults; WHO guidelines for larval assay	Strong dose- and time-dependent mortality; reduced survival
[25]	Cameroon / Republic of Congo region	Bark of *Zanthoxylum heitzii*	Pellitorine, dihydronitidine	Amides- Alkaloid	Adult and larvae	WHO guidelines used for both adult and larvae assays	Pellitorine was identified as the active compound toxic to adults and larvae
[26]	Burkina Faso	*R. communis*	Ricinine	Pyridone	Adult	Feeding assay for adults	Reduced survival of mosquitoes despite difference in species and infection to parasite
[27]	Kenya	Leaf extracts of *Murraya koenigii*	Neplanocin A	Carbocyclic nucleoside	Larvae	WHO guidelines for larval assay	significant larval mortality linked to specific compounds
[28]	Nigeria	Synthetic / identified alkaloid derivatives	6-nitro-2-[4-(trifluoromethyl)phenyl]-4-phenylquinoline 5-bromo-1-furoyl-7-azabenzimidazole 5-bromo-1-(phenylpropan-1-one)-7-azabenzimidazole	Quinoline Azabenzimidazole Azabenzimidazole	Larvae or adults	WHO guidelines used for both adult and larvae assays	Shows significant activity greater than crude extracts
[18]	Cameroon/ Kenya	*Zanthoxylum leprieurii* (Rutaceae)	1-hydroxy-3-methoxy-10-methyl-9-acridone 1-hydroxy-3-methoxy-9-acridone 1-hydroxy-3,4-dimethoxy-10-methyl-9-acridone	acridone	Larvae	WHO guidelines for larval assay	Demonstrates that a specific plant-derived acridone alkaloid has potent larvicidal activity against the primary malaria vector,
[29]	Tanzania	*Friesodielsia obovata* bark extract	N-formyl-7-hydroxyglaucine	aporphine	Larvae	WHO guidelines for larval assay	Exhibited larvicidal activity at LC_{50} = 8.6 mg/mL

Risk of bias

Using a modified SYRCLE Risk of Bias (RoB) tool on biases Q1-Q10, we see an average of moderate to high risk of bias within the studies (Fig. 3). For example, studies on the generation of allocation sequences (Q1) are rated as unclear, which is the case for all studies that fail to mention formal randomization. Reporting a random framework (Q4) is also rated as unclear for the same reason. Compared with other fields, laboratory-based studies such as those by Wachira [13], Moussavi [25], and Talontsi [18] are rated as low risk for baseline comparability (Q2) because they used controlled mosquito strains. Meanwhile, Oluwafemi [28] is rated as high risk for the same question because they used heterogeneous larvae that were field collected. Most studies were rated as high risk for Q3 allocation concealment and Q5 blinding of

caretakers/investigators, due to the open-label design of the experimental bioassays. High and unclear risk of blinding the outcome assessor (Q7) is the norm, particularly for studies with vague endpoints like the ones by Hien [26] and Mang'era [27] that did not quantify morphologies; however, studies that aimed to assess binary mortality outcomes were less likely to be impacted by detection bias. Sampling Random selection to assess outcome (Q6) is nearly always rated as unclear for a reason, even in full-census mortality counting. Most studies recorded high integrity in the outcome data (Q8); however, Hien [26] noted a significant risk due to the confounding of alkaloid concentration and mosquito species, which was a significant impediment to interpretability. Selective reporting of outcomes (Q9) was generally unclear, with the exception of Joseph [29], who was assessed to be at high risk due to very low degrees of methodological opacity and partial reporting of dose-response data. Lastly, high risk was the predominant allocation for the other sources of bias (Q10), which was very high due to the presence of DMSO and ethanol solvent effects, a small sample size, lack of statistical standardisation (e.g., non-probit LC_{50}), and environmental variability. Overall, these studies, while providing significant contributions to the understanding of alkaloid bioactivity, are compromised by a number of methodological flaws that compromise the internal validity, and the studies reflect a need for a greater number of studies that comply with standardised reporting and experimental design.

Mechanism of action

All investigated studies demonstrate that alkaloids exhibit a diverse range of neurotoxicity and interfere with metabolic, hormonal, and enzymatic pathways in mosquito vectors. Experiments performed by Wachira [13] and Talontsi [18] showed that alkaloids, such as ricinine, nitidine, and chelerythrine, cause progressive mortality rather than rapid, acute toxicity. This further indicates the likelihood of disruption of an energy metabolism and/or neural pathways. Moussavi [25] showed that plant alkaloids may cause toxicity through inhibition of an enzyme and oxidative stress, which supports systemic action. In a similar manner, computational results showed that alkaloids are highly attracted to important mosquito enzymes, including regulators of hormonal growth and development proteins. Furthermore, Mang'era [27] showed that the phytochemical extracts affect the transcriptome of the detoxification and stress response genes, which leads to a cell defense pathway that contributes to homeostasis imbalance and metabolic stress. Likewise, Joseph [29] defined a set of diverse structures of alkaloids with notable bioactivities, which demonstrated a high relevance of toxicological activity. The results of Hien [26] indicated that ricinine, which reduces the mosquito lifespan, also affects the development of the mosquitos' parasites, under a specific scenario, increasing *Plasmodium falciparum*. All the studies demonstrate the certainty of multiple actions of alkaloids, including enzyme inhibition, hormonal interference, and modulation of gene expression.

Formulation and preparation

The studies used combinations of crude plant extracts, semi-purified fractions, and fully isolated alkaloids, permitting some evaluative comparisons between formulation-dependent activity. Solvent-based extraction, primarily of methanol and other polar solvents, subsequent to some chromatographic separation and LC-MS characterization to determine active alkaloids, was used by Wachira [13] and Moussavi [25]. Likewise, Talontsi [18] and Joseph [29] utilized elaborate purification strategies, including column chromatography, to isolate certain alkaloid structures, specifically some of the benzophenanthridines and isoquinoline derivatives. Generally, crude extracts demonstrated greater larvicidal activity than isolated compounds across most studies, implying synergism among some of the phytochemicals. Moreover, these synergistic interactions among certain phytochemicals having broader biological activity than isolated compounds were exemplified by the studies of Mang'era [27] on whole extracts. Contrastingly, Oluwafemi [28] used in silico-driven compound optimisation and suggested that the refined alkaloids would be more effective in a structure-based enzyme inhibition. Hien [26] also developed a more environmentally friendly formulation by providing the alkaloids in a sugar feeding system as a mimic of the natural nectar uptake of the plant. These studies emphasized that the crude formulations were alkaloid synergistic, and the refined phytochemicals offered mechanistic specificity.

Method of assay

Several similar procedures, including bioassays utilising larvicidal activity, feed assays for adults, molecular assays, and computational analyses, were used within the studies to evaluate the activity of alkaloids. Larvicidal assays done by Wachira [13], Talontsi [18] and Moussavi [25] consist of exposing third or fourth-instar mosquito larvae to various concentrations of either alkaloids or extracts from plants and then measuring the deaths that occurred over the course of 24 to 72 hours to derive LC_{50}, which is then calculated by means of probit analysis. The adult toxicity of the mosquitoes that were used in the feeding assays, as is the case in Wachira [13] and Hien [26], was determined by means of an alkaloid sugar solution, and then the survival rate was studied and determined to be controlled by means of the Kaplan-Meier analysis. Mang'era [27] was the first of the authors to extend the range of methods to include transcriptomic analysis, enabling an understanding of changes in gene expression caused by the presence of alkaloids. In the same way, Oluwafemi [28] used approaches from the aforementioned classes to demonstrate strong interactions between the alkaloids and mosquito target proteins, employing molecular docking, molecular dynamics, simulations, and binding free energy calculations. Standardized controls allowed for the same experimental conditions to be utilized across all studies, including temperature, time of exposure, and appropriate controls. Mortality rates, LC_{50} values, time to survival, and biochemical binding were analysed to compare the studies.

Impact and biological effects

Alkaloids elicited strong and consistent biological effects across all life stages of mosquitoes, including larvicidal activity, reduced adult longevity, and alterations in vector-parasite transmission dynamics. Wachira [13] reported highly potent larvicidal effects of Ricinus communis extracts, with an LC_{50} of 0.18 mg/mL after 72 hours, whereas Talontsi [18] reported mortality rates approaching 100% at higher concentrations of isolated alkaloids. Similarly, Moussavi [25] reported a significant, dose-dependent increase in larval mortality, demonstrating the toxicity of alkaloids. Adult mosquitoes died 4–5 days after exposure to the alkaloid, indicating that the effects are substantial, both sublethal and lethal. Mang'era [27] demonstrated that, in addition to mortality, alkaloids trigger the stress response and alter the expression of stress-related genes, indicating diminished overall mosquito fitness, metabolic function, and stress resistance. There is also the dual effect. In Hien's [26] study, a dual effect is observed: 14% higher *Plasmodium falciparum* infections in mosquitoes at higher concentrations of alkaloids and accelerated parasite development. Also, the potential of alkaloids to disrupt pivotal regulatory mechanisms (bioinsecticidal properties) was demonstrated by the computational study done by Oluwafemi [28]. In all studies, the response to treatment, whether isolated or synergistic effects, was evident in relation to treatment concentration, duration, and drug. Overall, alkaloids showed a broad range of bioactive properties, evidenced by high potency in larval stages, reduced adult mosquito survival, and increased interactions between mosquitoes and increased parasite transmission.

DISCUSSION

This systematic review shows that the bioactive effects of plant-derived alkaloids on mosquito vectors are larvicidal and affect adult survival, as well as the complexity of their interactions in the transmission of parasites. In comparison with Wachira [13] and Talontsi [18], we showed that the alkaloids ricinine, nitidine, and chelerythrine cause dose- and time-dependent mortality of Anopheles larvae and adults, with the LC_{50} of these alkaloids being similar to other botanical insecticides, such as neem limonoids, and pyrethrin-type compounds [30, 31]. As such, these studies, and many others, add to the understanding of the immense bioefficacy of alkaloids and their potential to be a valuable resource in integrated vector management (IVM) efforts.

Looking at the mode of action, the multi-target toxicity is consistent with the documented modes of action of various insecticides classified as alkaloids and similar phytochemicals. Alkaloids have been documented to disrupt nervous system activities by inhibiting acetylcholinesterase activity, disrupting octopaminergic activity, and affecting GABA receptor activity [32]. Moussavi [25] reported that alkaloids induced oxidative stress and inhibited metabolic enzymes, consistent with biochemical results showing increased lipid peroxidation and inhibition of ATPase activity in exposed larvae [33]. The gene expression results obtained in Mang'era [27] showed upregulation of detoxification genes, including CYP450 and GST, as has been documented in response to rotenone and limonoids [34]. This shows that alkaloids induce a systemic stress response in larvae, leading to metabolic stress and larval mortality.

This review also shows the combined effects of crude extracts compared to those of pure compounds. This is similar to the effects observed in other phytochemical studies, which show that different phytochemicals, such as alkaloids, terpenoids, phenolics, and flavonoids, act together to exhibit greater potency than their pure compounds [35]. For example, the effect of neem seed extract with high azadirachtin and non-limonoid content shows greater larvicidal activity than that of pure azadirachtin [36].

The effects of alkaloids on parasite transmission are also noteworthy. Hien [26] showed that sublethal ricinine exposure resulted in greater rates of *Plasmodium falciparum* infections. Similar effects were observed in other studies, in which suboptimal insecticide concentrations led to greater vector competence [37, 38]. Studies with sublethal concentrations of pyrethroids and organophosphates show similar effects in *Anopheles spp.*, leading to higher *Plasmodium spp*., transmission rates because of the disruption in the immune system caused by the sublethal concentrations [39].

Formulation strategies can play a great role in determining expected bioefficacy and ecological effects and impacts. Some lab assays, as discussed in this review, use solvent-based extracts or sugar feeding solutions. These do not account for environmentally induced stability and field-specific delivery issues. Advancements are being made in nanoencapsulation and controlled-release systems. These include alkaloid emulsions in capsules and larvicides in polymer-coated capsules. These have been shown to have greater persistence, target specificity, and lower non-target toxicity [40, 41]. Nano-encapsulated analogues of nicotine, for example, were more active as larvicides than the non-encapsulated analogues [41]. It is clear that improvements in formulations can enhance their practical usefulness.

The continuous challenge with developing plant-based insecticides is overcoming insecticide resistance. It is less well understood how resistance mechanisms occur with plant-based formulations, especially compared with synthetic insecticides. It is documented, however, that repeated exposures to phytochemicals can select for metabolic forms of resistance, especially if a detoxification pathway is up-regulated [31, 42]. If a vector is exposed to alkaloids, then transcriptomic activation of the CYP450s and GSTs can occur, suggesting an adaptive response to

the synthetic material. This warrants the recommendations to apply integrated control measures and to also use insecticides in a rotational manner to manage insecticide resistance.

The significance of these findings can be enhanced by the supporting literature. Research conducted in both Western and Eastern Africa shows that the local species *Azadirachta indica, Ocimum gratissimum*, and *Ricinus communis* have established a species- specific, and site-specific collection of plant species with proven larvicidal activity [43]. Traditionally, in ethnobotanical surveys conducted in Cameroon and the Sahel, the same plants were identified as those associated with larval mortality observed in laboratory bioassays [44]. Likewise, in South America, the larvicidal activity of the alkaloid extracts of *Chrysobalanus icaco* and *Erythrina velutina* against *Aedes aegypti* larvae [45, 46] has expanded the geographical scope of alkaloid activity. All these studies, with examples from specific geographical regions, show the importance of the broader alkaloid activity and the need to incorporate regionally specific plants in the development of biopesticides.

Despite these promising avenues, several limitations in the current body of work warrant attention. Variation in study design, including differences in larval instar stage, exposure duration, solvent types, and concentration ranges, presents challenges to direct comparability. There is also a paucity of field-based evaluations, with most evidence limited to laboratory or semi-field settings. Additionally, while computational docking and transcriptomic profiling provide valuable insights into potential targets and pathways, direct biochemical validation (e.g., enzyme inhibition kinetics, receptor binding assays) remains limited.

Study limitations

This systematic review had limitations across all studies reviewed. First, the variability in the design of experiments in the seven reviewed studies [13, 18, 25–29] indicated differences in larval instars, time of exposure, solvents, and concentration ranges directly affecting the comparability of outcomes, including LC_{50} and mortality. Also, studies lacked validation in semi-field or field settings, thereby limiting their applicability and ecological relevance. Furthermore, the studies lacked biochemical validation of receptor binding and the mechanism of action for crude extracts or enzyme inhibition, which were investigated by several researchers using LC-MS and molecular docking. Also, there was variability in the phytochemical composition of crude extracts from plants of the same species due to geographical location, climate, and extraction techniques; however, most researchers did not consider these factors in their studies. Finally, a majority of studies, other than a few Hien [26]; Mang'era [27], which studied sublethal or molecular effects, focused on mortality, therefore limiting the understanding of the impact.

Research gaps and future directions

As demonstrated in the studies provided, the bioactivity of these alkaloids is promising, but significant research gaps remain. Some studies fail to address molecular mechanisms of action, and this is a significant gap in most studies, making them less conclusive. Additionally, it is common to see studies lacking enzyme kinetics, receptor-binding studies, and/or gene knockdowns that support the specific study's arguments. Studies lacking in these areas can often make it overly difficult to make a clear comparison amongst studies. Another common research gap in these studies is the lack of clear, referenced approaches to larvicidal and adulticidal activities. Hien [26] was the first to illustrate a dual effect of alkaloids in relation to the survival of the mosquitoes and the transmission of the parasites. As a result, it is clear that the influence of alkaloids is under scrutiny in relation to other vector competence studies. Another study gap is the under-research of formulation optimisation and the creation of nano-formulations and other field-applicable delivery systems that are stable and can be utilised in the field. The creation of stable, in-field delivery systems that include nanoformulations and slow-release systems is also an area of needed study. A field study gap is research focusing on environmental safety and the evaluation of resistance developed under naturally occurring conditions, which are critically needed. The studies focus on identifying and isolating powerful alkaloids. However, these studies have only lightly addressed the idea of identifying these alkaloids as specific, less toxic lead compounds. These studies have also made a small investment in research focused on developing a highly desired SAR (structure-activity relationship). These research gaps must be addressed to implement effective field practices and achieve sustainable goals through effective vector control.

CONCLUSION

This review affirms that alkaloids are a potent class of bioinsecticidal compounds with significant potential for mosquito control in IVM frameworks. Their multi-target mechanisms, coupled with strong larvicidal and adulticidal effects, position them as promising alternatives or complements to existing synthetic insecticides. However, their broader impacts on parasite transmission, potential for resistance development, and formulation challenges underscore the need for further research. Future work should prioritize mechanistic validation, formulation advances, and rigorous field trials to fully translate the promising laboratory bioactivity of alkaloids into effective, sustainable vector control tools.

Declarations

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All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Clinical trial number

Not applicable.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

Conflicts of interest

The authors declare that they have no competing interests, financial or otherwise, that could have influenced the work reported in this manuscript.

Ethical approval

Not applicable.

Data availability

Not applicable.

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Figures

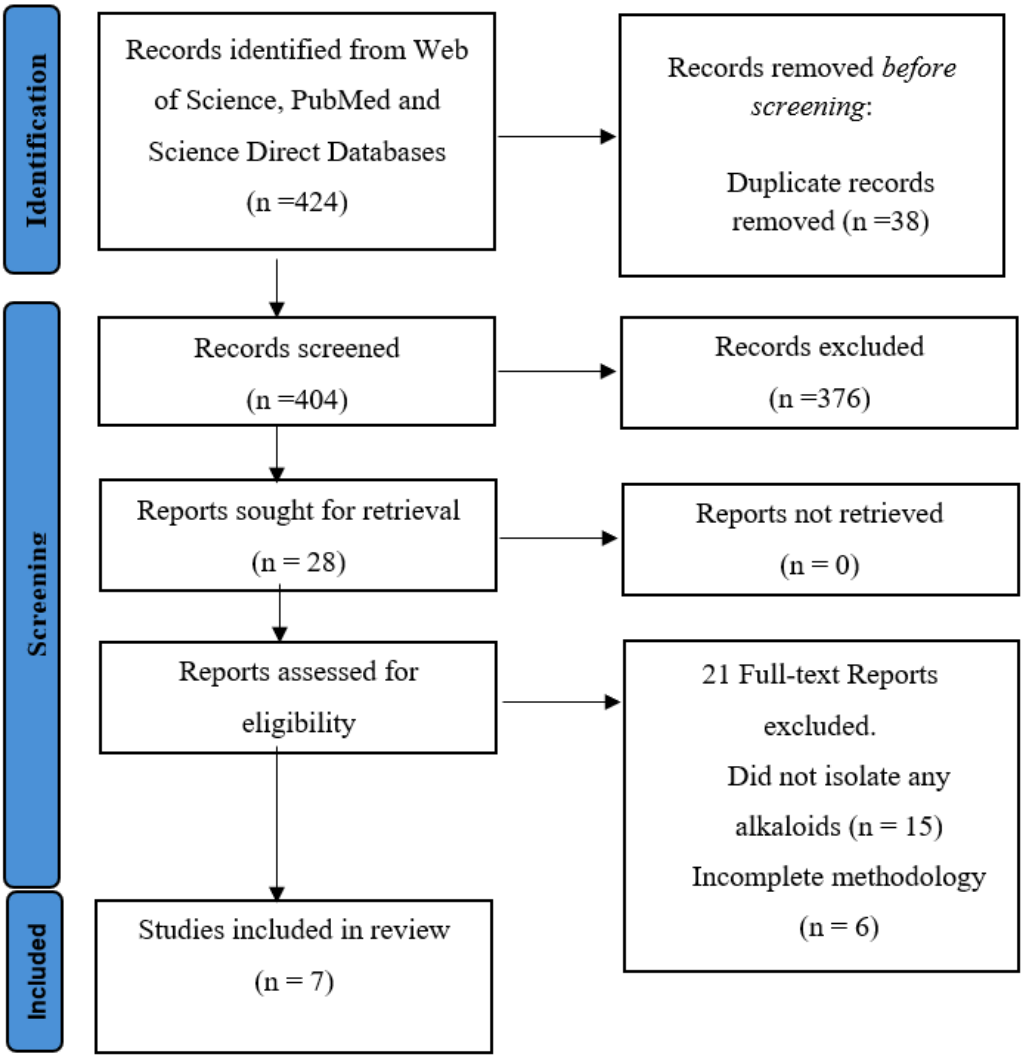


Figure 1

PRISMA flow diagram of search phases with numbers of studies included/excluded at each stage

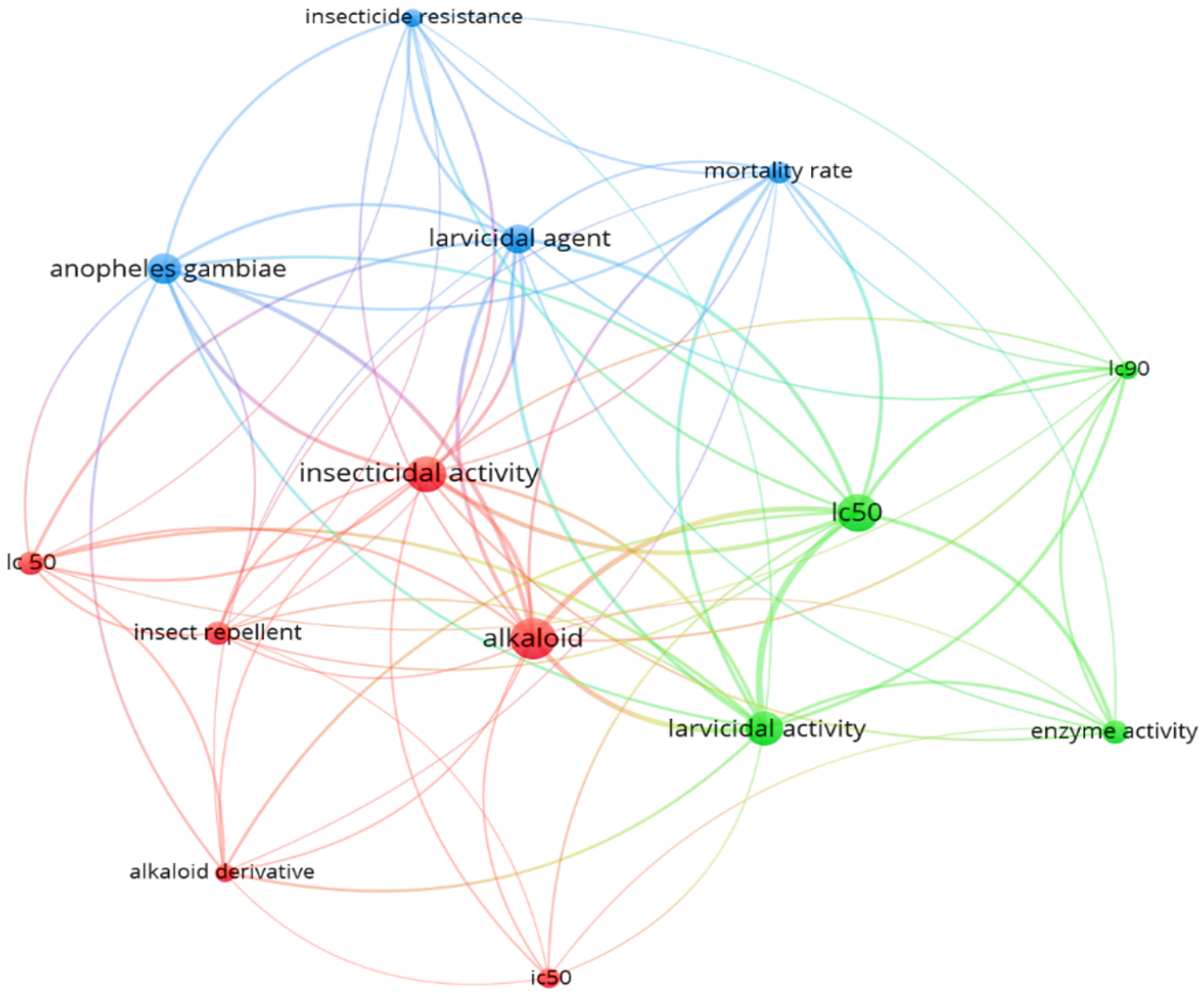


Figure 2

Network visualization of keyword co-occurrence generated using VOSviewer from the retrieved literature on alkaloid-based mosquito control. Node size represents keyword frequency, and link thickness indicates the strength of co-occurrence between terms. Colours denote clusters of related themes.

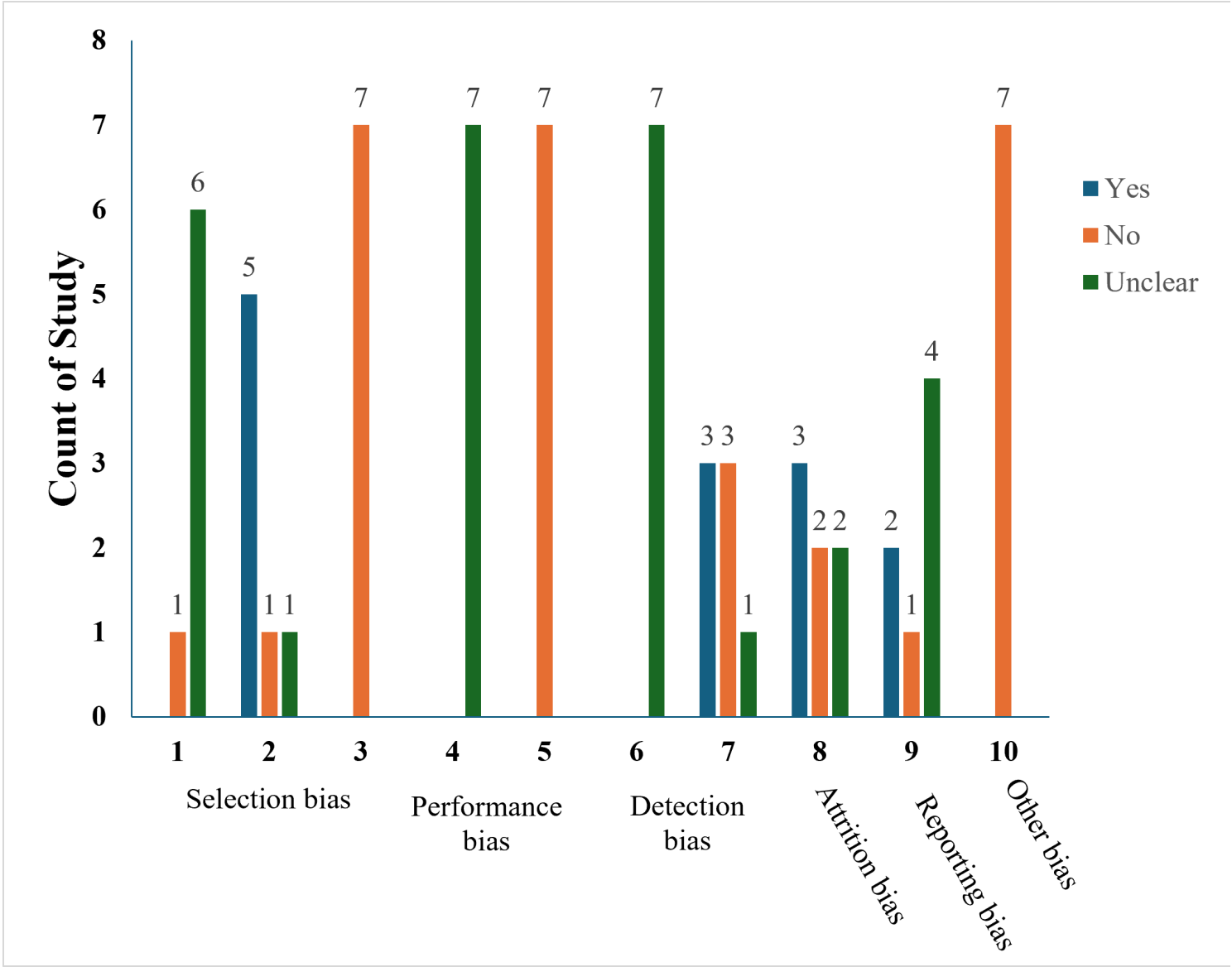


Figure 3

Summary of findings of risk of bias assessment

Supplementary Files

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- TableS1.docx