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Prediction of the Insecticidal Potency of Biduri Plants *Calotropis gigantea* (L.) W.T.Aiton using the PASS online web resource

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Abstract

Calotropis gigantea (L.) W.T.Aiton, a wild plant thriving in arid environments, has been traditionally used for medicinal purposes by communities near Baluran National Park (BNP). The latex of *C. gigantea* is used as a crab poison due to its ability to cause fatal damage and separation of body parts, possibly related to chitin disruption in insects. This study explores the potential insecticidal properties of secondary metabolites in *C. gigantea* using Prediction of Activity Spectra for Substance (PASS) Online. Out of 68 identified secondary metabolites, six show significant insecticidal potential, namely Profenophos, Ethion, Alpha-Citral, 1-Phenylethyl acetate, (E)-dec-3-en-2-one, and Benzaldehyde. Notably, Profenophos, Ethion, Alpha-Citral, and Benzaldehyde exhibit toxic properties effective against insects, with enzyme inhibitory activity affecting nerve signaling and immune systems, suggesting potential for bio-insecticide development.

Keywords: Biduri, *Calotropis gigantea*, PASS Online, Secondary Metabolite, Insecticide Potency

Introduction

Calotropis gigantea (L.) W.T.Aiton occupied xeric conditions and arid soil (Al Sulaibi et al., 2020; Kemala et al., 2022). The foliage of *C. gigantea* is characterized by ovate, robust, and stemless leaves, notable for their fine hairs covering the entire surface of the plant. Notably, all parts of *C. gigantea* have the capacity to produce latex (Lakshani et al., 2022). Its geographic distribution encompasses regions across India, South China, New Guinea, Hawaii, and Southeast Asia (Patil, 2020).

Globally, *C. gigantea* has been utilized for an extensive period. All components of the plant, including leaves, flowers, stems, roots, latex, and fibers, are widely utilized. The utilization of *C. gigantea* encompasses a wide array of applications in traditional medicine, where its leaves, flowers, stems, roots, and latex are employed to treat various ailments such as syphilis, leprosy, stomach tumors, tuberculosis, skin diseases, hemorrhoids, insect bites and wounds, dysentery, laxatives, toothaches, and diabetes (Lakshani et al., 2022). Furthermore, its fiber finds application as a primary material in the fabrication of carpets, ropes, fishing nets, and sewing thread due to its potential as a substitute for synthetic fibers (Ramesh et al., 2021).

Baluran National Park (BNP), located in East Java, is where C. gigantea, known as "Biduri" in Indonesia, is widely distributed in the wild. (Octavia et al., 2008). Indigenous communities residing around BNP utilize this plant for medicinal purposes, specifically to alleviate toothache and as a poison for freshwater crabs known as "yuyu" (the local name for freshwater crab). Information gathered from local communities indicates that Biduri's latex, when used as a poison for freshwater crabs, leads to their demise and the subsequent separation of body segments. This segment separation is closely associated with the damage inflicted upon the exoskeleton of freshwater crabs (Morsli et al., 2015), composed primarily of chitin—a substance present in all members of the Arthropod Filum, including insects (Vogan et al., 2008). Consequently, it is inferred that the toxic latex properties of *C. gigantea* cause mortality in insects.

Several studies have indicated that *C. gigantea* possesses numerous biological activities that can be harnessed (Kumar et al., 2013). Among its potential activities is its efficacy as an insecticide (Kumar et al., 2013; Habib and Muhammad, 2016; Khasanah et al., 2021; Takshila et al., 2022). The insecticidal potential of plants is often associated with their secondary metabolites. In their respective studies, Habib and Karim (2016), Singh et al. (2018), and Madhavan et al. (2020) reported several compounds found in *C. gigantea* that exhibit insecticidal activity against various insects, as determined through gas chromatography-mass spectrometer (GC-MS) analysis. Despite numerous secondary metabolites being identified for their insecticidal activity, there is currently no research report that has focused on identifying the most potent secondary metabolites for use as insecticides in C. gigantea.

The biological activity analysis of secondary metabolite compounds can be readily and cost-effectively accessed through international databases. These databases facilitate the prediction of a compound's potential before conducting additional laboratory research. Hence, this article conducted an analysis to forecast the potential of C. gigantea's secondary metabolites as insecticides using platforms like PubChem and Way2drug. The objective of this study was to investigate the insecticidal efficacy of *C. gigantea* based on the PASS online database.

Materials and Methods

The secondary metabolites in *C. gigantea* were identified through a review of scientific articles, focusing on those with potential as insecticides. These metabolites were then subjected to analysis to predict their potency.

The analysis involved comparing the Pa (active) and Pi (inactive) values of the secondary metabolites. Information regarding these values was gathered using Canonical Simplified Molecular Input Line Entry System (SMILES) data collected from the Pubchem online database at http://pubchem.ncbi.nlm.nih.gov/. The SMILES data were then analyzed to make predictions about the compounds' potential.

To predict the insecticidal potential of the compounds, the Prediction of Activity Spectra for Substance (PASS) tool available at http://www.way2drug.com/PASSOnline/predict.php was utilized. The results of the PASS analysis were evaluated based on the Pa (probability for active compounds) and Pi (probability for inactive compounds) values for each compound.

According to Matin et al. (2016), a Pa value greater than 0.7 indicates a high likelihood of the compound being highly active in experiments. A Pa value between 0.5 and 0.7 suggests moderate activity, while a Pa value less than 0.5 indicates a low activity likelihood in the experiment.

Results

Calotropis gigantea, a perennial herbaceous plant, has been identified as a source of secondary metabolites based on our literature review. We found a total of 68 secondary metabolites from *C. gigantea* with potential as insecticides (Suppl. 1). Among these, 6 compounds exhibited insecticidal potential with Pa values greater than 0.5 (Fig. 1). According to PASS online analysis, the Pa values of secondary metabolites from *C. gigantea* ranged from 0.1 to 0.8. This range indicates that the potential of *C. gigantea* secondary metabolites as insecticides ranges from 10% to 80%.

The highest potency of insecticidal activity (Pa > 0.7) was observed in Profenophos and Ethion compounds, suggesting a very active possibility as insecticides in experiments. On the other hand, compounds like Alpha-Citral, 1-Phenylethyl acetate, (E)-dec-3-en-2-one, and Benzaldehyde exhibited Pa values between 0.5 and 0.7, indicating a less active possibility as insecticides in experiments. Furthermore, 62 other secondary metabolites had Pa values below 0.5, suggesting a very small possibility of activity as insecticides in experiments.

Our literature review revealed that 4 of these secondary metabolites (Profenophos, Ethion, Alpha-Citral, and Benzaldehyde) have been reported to possess toxic properties effective in killing insects (Table 1). These secondary metabolites exhibit different characteristics, roles, and modes of action as insecticides.

Profenophos

Profenophos, belonging to the organophosphate group, stands as one of the most extensively utilized insecticides for pest management within agricultural ecosystems. Organophosphates constitute a class of aromatic derivative compounds originating from phosphoric acid, thiophosphoric acid, and other phosphoric acids (Kushwaha et al., 2016; El-bouhy et al., 2023). These compounds, known for their toxic properties towards organisms (Nugroho et al., 2015), serve as precursors for numerous insecticides, herbicides, and nerve agents (Kushwaha et al., 2016).

The potential of Profenophos as an insecticide stems from its toxic properties. The World Health Organization (WHO) has classified Profenophos as a class II hazardous toxin compound, signifying moderate toxic properties (Maharajan et al., 2013). Despite its toxicity, Profenophos finds widespread application in controlling insect pests on crops such as chilies, onions, corn, coffee, tomatoes, cotton, beans, potatoes, and various vegetables (Rodrigues et al., 2020; Kushwaha et al., 2016). Numerous studies have demonstrated the effectiveness of Profenophos against various insect pests including *Spodoptera litura* (Ahmed et al., 2019), sucking insects like *Aphis gossypii* and *Bemisia tabaci*, and plant lice (El-Sherbeni et al., 2019; Patil and Prakash, 2013).

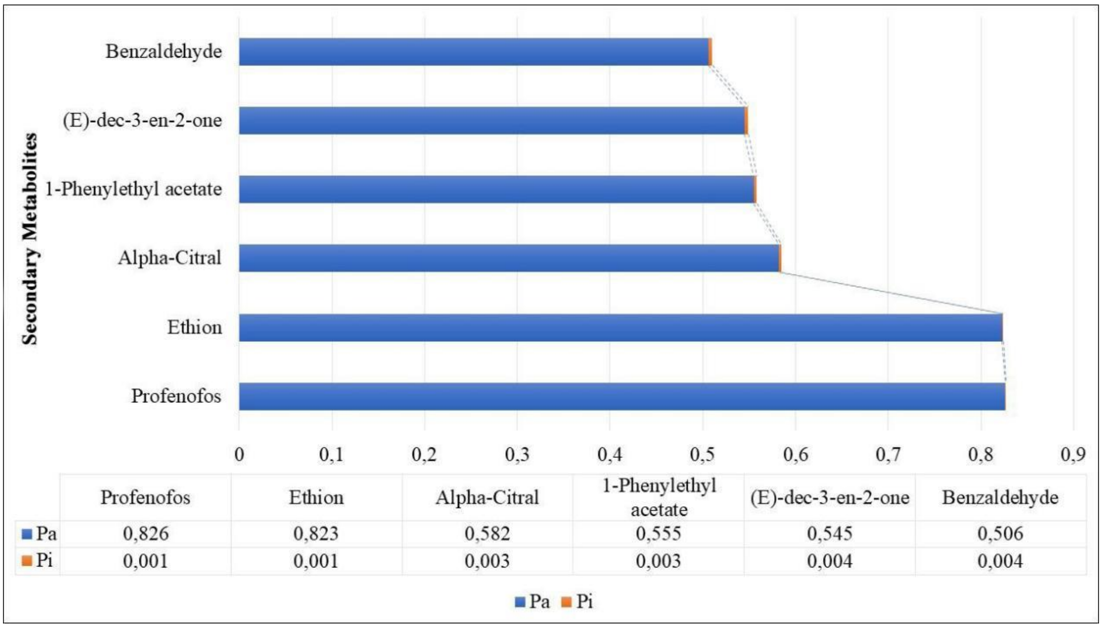

Figure 1. The Pa value (Potential activity) of several *C. gigantea* secondary metabolites that have the potential as insecticides.

Table 1. The Role of Secondary Metabolites of *C. gigantea* as Insecticides

No	Metabolite	Group	Role	Target Insects	References
1.	Profenophos	Organophosphates	√	*Spodoptera litura*, *Aphis gossypii*, *Bemisia tabaci*, plant lice	Ahmed et al., 2019; El-Sherbeni, et al., 2019; Patil and Prakash, 2013
2.	Ethion	Aromatic	√	*Anopheles culicifacies*	Marwaha, 2015
3.	Alpha-Citral	Aromatic	√	*Musca domestica*, *Anopheles stephensi*, *Aedes aegypti*	Aungtikun et al., 2021; Soonwera and Sirawut, 2020
4.	Benzaldehyde	Aromatic	√	*Galleria melonella*, *Drosophila melanogaster*	Kumar et al., 2022; Neto et al., 2021
5.	1-Phenylethyl Acetate	Aromatic	-	-	Liang et al., 2016; Bitterling et al., 2020
6.	(E)-dec-3-en-2-one	Aromatic	-	-	Api et al., 2021; Knowles and Knowles, 2012

Note: (-) there is no experimental evidence that this compound acts as an insecticide; (√) there is ex

Profenophos, also known as O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate, has been developed to combat pest strains resistant to other organophosphate insecticides (Gotoh et al., 2001). It functions by inhibiting the hydrolysis of Acetylcholinesterase (AChE), an enzyme crucial for neurotransmission in vertebrates and invertebrates. AChE regulates nerve impulses by breaking down acetylcholine (ACh) into acetic acid and choline, thus facilitating nerve impulse transmission (Dhamayanti and Fitri, 2018; Kushwaha et al., 2016).

However, Profenophos poses significant environmental hazards due to its persistence and non-degradability. It can contaminate the environment, including surface waters, soil, and groundwater (Subsanguan et al., 2020; Kushwaha et al., 2016). Nonetheless, efforts to mitigate Profenophos contamination have been explored through bacterial applications, as documented in studies by Ghani et al. (2021) and Jabeen et al. (2015).

Ethion

Ethion, also known as O,O,O',O'-Tetraethyl S,S'-methylene bis(phosphorodithioate), is categorized as an organophosphate insecticide. Widely used in plantations and agriculture, ethion serves as a non-systemic insecticide and acaricide (mite killer), as documented by Abdel-Gawat et al. (2021) and Verma et al. (2018). Moreover, it finds application in controlling skin parasites and insects in livestock (Verma et al., 2018).

Similar to other organophosphate compounds, ethion exhibits insecticidal potential by inhibiting Acetylcholinesterase (AChE), an enzyme crucial for neurotransmitter breakdown. AChE catalyzes the conversion of acetylcholine into choline and acetate. Inhibition of AChE leads to the accumulation of acetylcholine at synaptic junctions, resulting in continuous nerve impulses and hyperexcitation that ultimately leads to involuntary muscle twitching and insect mortality (Marwaha, 2015).

Ethion is also known to induce oxidative stress by elevating stress markers and disrupting oxidative balance, leading to an excessive accumulation of reactive oxygen species (ROS) (Abdel-Gawat et al., 2021). Studies by Marwaha (2015) suggest that ethion induces genotoxicity in *Anopheles culicifacies* mosquitoes. Additionally, Verma et al. (2018) highlight its impact on various organisms, including mammals, chicks, fish, and freshwater invertebrates, underscoring its potential broader environmental effects beyond its insecticidal properties.

Alpha-Citral

Alpha-Citral, also known as geraniol or $C_{10}H_{18}O$, belongs to the class of monoterpene alcohols and aromatic compounds. Widely utilized as a fragrance ingredient in cosmetic and household products, Alpha-Citral emits a sweet, floral, and citrusy scent. It is naturally found in approximately 250 essential oils derived from various plant species such as *Cymbopogon citratus, Monarda fistulosa, Aeollanthus myrianthus*, and rose (Aungtikun et al., 2021; Maczka et al., 2020).

Alpha-Citral exhibits significant biological activity as an effective insecticide against *Musca domestica*, *Anopheles stephensi*, and *Aedes aegypti* (Soonwera and Sirawut, 2020). This efficacy is corroborated by research conducted by Aungtikun et al. (2021), which highlighted Alpha-Citral's bio-insecticidal effectiveness against *M. domestica*.

Moreover, Alpha-Citral possesses toxic properties (Setlur et al., 2023; Aungtikun et al., 2021; Soonwera and Sirawut, 2020) and acts as an active fumigant (Jang et al., 2016). Jang et al. (2016) investigated the insecticidal mechanism of Alpha-Citral and found that it inhibits Glutathione S-transferase (GST), a group of enzymes crucial for detoxification and elimination of toxic contaminants, as well as in insecticide resistance mechanisms. Alpha-Citral, being an α,β-unsaturated carbonyl compound, can inhibit GST in insects and induce nervous disorders. Notably, Alpha-Citral exhibits a higher GST inhibition compared to other compounds found in essential oils (Jang et al., 2016).

Benzaldehyde

Benzaldehyde, also known by various synonyms such as Artificial Almond Oil, Benzenecarbonal, Benzoic Aldehyde, Benzenecarboxaldehyde, Benzenemethylal, Benzene Carboxaldehyde, Bitter Almond Oil (synthetic), Phenylformaldehyde, and Phenylmethanol Aldehyde, is a clear to yellowish liquid oil with a bitter almond scent (Andersen, 2006). This aromatic aldehyde finds extensive use as an additive in cosmetic and food products, serving roles as a denaturant, flavoring agent, fragrance, and a crucial natural fruit flavoring (Kumar et al., 2022).

The insecticidal toxicity of benzaldehyde has been well-documented. Ullah et al. (2015) reported on benzaldehyde derived from secondary metabolites of the bacterium Photorhabdus temperata, showcasing its ability to inhibit the phenol oxidase immune response mechanism in *Galleria melonella*. Benzaldehyde exhibited 100% effectiveness against *G. melonella* larvae at a pure concentration of 8 mM, 108 hours after injection. Moreover, benzaldehyde's toxicity to insects has been observed in *Drosophila melanogaster* as well (Kumar et al., 2022; Neto et al., 2021).

Apart from its toxic properties, the aroma of benzaldehyde also serves as an attractant for insects, making it useful in insect monitoring. It has been reported as an attractant for *Sitona humeralis* (Lohonyai et al., 2019) and *Xyleborinus saxesenii* (Yang et al., 2018.

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