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Impact of *Caesalpinia pulcherrima* (L.) Sw. (Fabaceae) Ethanol and Acetone Leaf Extracts on the *Aedes aegypti* (Diptera: Culicidae) Eggs

Krishnaveni G., Tamilarasi A., Mohanraj R.S.* and Saranya M.

PG and Research Department of Zoology, Government Arts College, Coimbatore 641018, Tamil Nadu, India

**Corresponding Author*

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Abstract: Mosquito control is essential to prevent mosquito borne diseases such as dengue, chikungunya, Zika and filariasis. Synthetic insecticides such as organophosphate and pyrethroids are commonly used for mosquito control program. Synthetic insecticides are effective, nonetheless cause adverse effects on the environment and human health. Due to their hazardous effects, alternatives are required for mosquito management. One such alternative approach is to explore the floral biodiversity. Insecticidal compounds from natural sources, notably from plants are promising for managing such vectors. Hence, the study aimed to analyze the ovicidal potential of ethanol and acetone leaf extracts of *Caesalpinia pulcherrima*. Effect of ethanol and acetone leaf extracts of *Caesalpinia pulcherrima* on the hatchability of *Aedes aegypti* eggs were determined adopting the standard procedure. Per cent hatch of eggs placed in control medium was 95% where as in 0.1, 0.3, 0.5, 0.7%. concentrations it was 75, 50, 25, 15 (ethanol) and 70, 55, 30, 20 (acetone), respectively. 0.9% completely arrested egg hatching in both extracts. From the above, the ethanol and acetone leaf extracts of *C. pulcherrima* can be recommended for the development of ovicides against *Ae. aegypti* eggs.

Keywords: *Aedes aegypti*, Ethanol, Acetone leaf extracts, *Caesalpinia pulcherrima,* Phytochemical, GC/MS analysis, Ovicidal activity

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Introduction

Mosquitoes belong to the Diptera order and the Culicidae family of insects. They are classified into 41 genera and 3500 species and about 404 of them are found in India (WHO. 2013; Selvan *et al.,* 2020). Among the 41 genera, *Anopheles, Culex* and *Aedes* are the most important as it transmit several deadly diseases; such as protozoan and viral diseases including Malaria, Lymphatic filariasis, Japanese encephalitis, Yellow fever, Zika fever and Dengue fever. These diseases continue

to be a major public health issue in developing countries (Katchhwaha *et al.,* 2017; Daniel *et al.,* 2020; Aparana *et al.,* 2021). Nearly 700 million people get a mosquito borne illness each year resulting in greater than one million deaths in world (Caraballo, 2014). *Aedes aegypti* is the main vector that transmits the viruses that cause dengue, chikungunya and Zika. According to the World Health Organization (WHO) guideline 1997 (WHO, 2009) dengue patients can be categorized into three categories including dengue hemorrhagic fever, dengue fever and dengue shock syndrome. As per WHO guideline 2009, dengue patients can be further categorized on the severity basis that includes severe dengue patients, dengue patients with few warning signs and dengue patients with no warning signs (Giang *et al.,* 2018). This fever is generally widespread in South-East Asia, Africa, Western Pacific and South American countries. In India, official records of the Union Health Ministry reveal a massive increase in dengue infections every year (NVBDCP, 2015). According to the NVBDCP (National Vector Borne Disease Control Programme) number of dengue cases from 2000 to 2022 in India was 10,43,951 and 1633 deaths and in Tamil Nadu it was 57,583 and 100 deaths. Chikungunya cases in India was 1,30,84,190 and no deaths and in Tamil Nadu it was 64, 877 and no deaths.

Till date, specific medications and vaccinations are not available commercially for treating dengue, chikungungya and Zika fever. The only approach followed to reduce the incidence of these diseases is by the control of its vector *Ae. aegypti*. However, in the past, the frequent and repeated use of chemical insecticides has resulted in the worldwide development of resistance, destabilization of the ecosystem and toxic effects on humans and non-target organisms (Nicolopoulou-Stamati *et al.,* 2016). Face to these realities, it is become essential to develop new alternatives for mosquito control.

Research could be directed towards natural chemicals from plants (Aouinty *et al.,*2018). In this context a number of plant components are used against several of mosquitoes species (Sukumar *et al.,* 1999). The phytochemicals provide better candidates for new classes of insecticides because they (i) consists of variable components with diverse mechanisms of action that diminish the odds of development of resistance in the mosquito to the phytochemicals (Jankowska *et al.,* 2018), (ii) generally have minimal acute toxicity to vertebrates, and (iii) ecofriendly (Salehzaden *et al.,*2002). Applications of phytochemicals in mosquito control were in use since the 1920's (Saleh *et al.,* 2013) but the discovery of synthetic insecticides such as DDT in 1939 side tracked the application of phytochemicals in mosquito control programme. Some of the plant leaves extracts are tested for their diverse insecticidal properties on the medically important mosquitoes: methanol and ethanol extracts of *Artemisia absinthium* (Sofi *et al.,* 2013); hot water extract *Swietenia mahagoni* (Hasan *et al.,* 2022); n-hexane extract of *Murraya paniculate* (Alafia *et al.,* 2022); ethanol extract of *Tridax procumbens* (Marin *et al.,* 2022).

To the best of our knowledge there exists no information available on the ovicidal effect of the ethanol and acetone leaf extracts of *C. pulcherrima*.

Caesalpinia pulcherrima (Fabaceae), popularly known as peacock flower or "Barbados" in English and as "Mayilkondrai" in tamil, it is an ever green shurb growing three meter tall. It is a striking ornamental plant, widely grown in the tropical and subtropical zones. The leaves are bipinnate, 20-40 cm long, bearing 3-10 pairs of pinnac, each with 6-10 pairs of leaflets 15-25 mm long and 10-15 mm broad with oblong to ovate shape. *C. pulcherrima*, is used for various purposes of herbal medicine. It is used as emmenagogue, purgative, stimulant and abortifacient and also in bronchitis, asthma, malarial fever and kidney stone. The different parts of this herb have been used in common remedies for treatment of a number of disorders including pyrexia, menoxenia, wheezing. The leaves, flower, bark and seeds of *C. pulcherrima* were also used by American Indians in traditional medicine as abortifacients and for suicide by enslaved peoples. Leaves used as

antibirutic, antimicrobial (Sudhakar *et al.,* 2003), antioxidant, cytotoxic activity (Pawer *et al.,* 2009), antitubercular activity (Promsawan *et al.,* 2003), gastric antiulcer activity (Kumar and Nirmala, 2004) and anticonvulsant activity (Dhinesh Kumar *et al.,* 2009).

The aim of the present study was—(i) Qualitative phytochemical analysis of ethanol and acetone leaf extracts of *C. pulcherrima*, (ii) GC/MS analysis of ethanol and acetone leaf extracts of *C. pulcherrima*, and (iii) Ovicidal activity of the ethanol and acetone leaf extracts of *C. pulcherrima* on *Ae. aegypti* eggs.

Materials and Methods

The eggs of *Ae. aegypti* were collected from National Institute for Communicable Disease (NICD), Mettupalayam, Coimbatore (Dt), Tamil Nadu, India. They were hatched, reared and have been still maintained for many generations in the laboratory. The larvae were reared in plastic cups (27±2°C, relative humidity at 70-80%) and provided with commercial fish food *ad libitum* (Lymio and Koella, 1992). The pupae were collected from culture trays and were transferred to glass beakers. The glass beaker containing pupae were kept inside mosquito cage for adult emergence. The adult female *Ae. aegypti* **were fed by human blood** (Judson, 1967; Briegel, 1990). Both females and males were provided with 10% glucose solution on cotton wicks (Villani *et al.,* 1983). Plastic cup (200 ml) (ovitraps) lined with filter paper containing water was kept in the cage.

Collection and preparation of plant extracts:

C. pulcherrima leaves were collected from Periyanaickanpalayam, Coimbatore, Tamil Nadu, Southern India. The plant was authentified at BSI Coimbatore (NO:BSI/SRC/5/23/2019/ Tech/135). The leaves washed with distilled water and then they were kept for drying under shade at room temperature (27± 2°C) for about 2 weeks till they dried completely. The dried leaves were finely powdered using electric grinder. Powdered plant materials (100 g) was soaked in ethanol and acetone (1000 ml) in airtight wide mouth bottle and kept separately for 4 days with periodic shaking. After that, the extract was filtered using Whatman No.1 filter paper and kept in Petri dishes for drying at room temperature (Kongkathip, 1994). Dried extracts were then used for the preparation of stock solution. This stock solution was used to prepare the desired concentrations of the extract for exposure to the mosquito larvae.

Qualitative phytochemical analysis of ethanol and acetone leaf extracts of C. pulcherrima:

Qualitative phytochemical analyses of the plant extracts was carried out using the standard protocol (Harbone,1984; Trease and Evans, 1989).

Gas Chromatography- Mass Spectrometry (GC-MS) analysis of ethanol and acetone leaf extracts of C. pulcherrima:

The GC-MS analysis was conducted at SITRA, Coimbatore, Tamil Nadu.

Ovicidal assay:

Effect of ethanol and acetone leaf extracts of *C. pulcherrima* on the hatchability of *Ae. aegypti* eggs were determined, adopting the procedure of Su and Mulla (1998). Before treatment, the eggs of *Ae. aegypti* were counted individually with the help of hand lens. 20 freshly laid eggs were exposed to different concentrations (0.1, 0.3, 0.5, 0.7 and 0.9%) of ethanol and acetone extracts. The hatchability was recorded after 96 h from the initial time of the experiment. The time was fixed because it was demonstrated that the completion of embryogeny occurs within 4 days (Judson and Gojrati, 1967). Hatching rate was calculated on the basis of non-hatchability of eggs (Sahgal and Pillai, 1993). After treatment, the eggs from each concentrations were individually transferred to distilled water cups for hatching assessment and counted by using dissection microscope. Five replications were conducted at each concentrations of test compounds. Control (water), positive controls (ethanol and acetone) was maintained separately and egg hatchability was observed.

The data were statistically examined using Student's *t*-test.

Results and Discussion

Phytochemical analysis of the ethanol and acetone leaf extracts of C .pulcherrima:

The qualitative phytochemical analysis revealed the presence of different phytochemicals such as carbohydrate, alkaloids, coumarins, steroids, phytosteroids (ethanol) and carbohydrate, alkaloids, coumarins, steroids, phenols (acetone) (Table 1).

Gas Chromatography- Mass Spectrometry (GC-MS) analysis of the ethanol and acetone leaf extracts of C.pulcherrima:

Important compounds identified in the GC- MS analysis of ethanol and acetone leaf extracts of *C.pulcherrima.* The compounds are phytol, a-D-Glucopyranose, 3-O-Methyl-d-glucose, 1-Hepatariacotanol, Dodecane, Rhodopin, 2'-Hydroxy-5'-methylchalcone, Lidocaine, Decane, Tetradecane (ethanol) and phytol, a-D-Glucopyranose, 3-O-Methyl-d-glucose, 1-Hepatariacotanol, Dodecane, Pregnenolone, Ursodeooxycholic acid, Hydrocortisone, 17-Pentatriacontene, Heptadecane,9-hexyl (acetone) (Tables 2, 3).

Effect of ethanol and acetone leaf extracts of C.pulcherrima on hatching of Ae. aegypti eggs:

Freshly laid eggs obtained from the general stock of mosquitoes were tested for their hatching ability in relation to the different concentrations of ethanol and acetone leaf extracts of *C.pulcherrima*. Per cent hatch of eggs placed in control medium was 95 % where as in 0.1, 0.3, 0.5 and 0.7% concentrations it was 75, 50, 25, 15 (ethanol) and 70, 55, 30, 20 (acetone), respectively. 0.9 % dose completely arrested hatching eggs (Table 4). The decrease in hatchability was found to be dose dependent.

Heavy and rampant use of synthetic chemi - mosquitocides has ill effects on human health and also results in issues related to environmental pollution. One of the best remedies to cope with such situation is to promote the use of plant extracts which will surely decrease overuse of stockpiled chemicals. The richness of botanical extracts can be traced back to last four decades for its use as ovicidal, larvicidal and repellent. The result of the present study is in agreement with the earlier findings on the ovicidal effect of different plant origin. Ovicidal activity (egg mortality) in *Murraya koenigii* leaf extracts against *Ae. aegypti, An.stephensi* and *Cx. quinquefasciatus* at 500 and 1000 ppm was 60.4, 79.4, 62.4% and 73.4, 51.8 and 67.8%, respectively, the per cent mortality of eggs in control medium was 98, 92 and 88%, respectively. The increase in egg mortality was found to be dose dependent. Among the three species *An.stephensi* was most susceptible followed by *Ae. aegypti* and *Cx. quinquefasciatus* (Arivoli and Tennyson, 2011). The ovicidal activity was studied at 0.25%, 0.50%, 1.00% and 2.00%. At the lowest concentrations, among the extracts, no absolute mortality was obtained. The hexane leaf extracts of *Cassia occidentalis* cause maximum egg mortality in both *An.stephensi*, (74.5%) and *Cx. quinquefasciatus* (66.5%) and methanol extract in *Ae. aegypti* (92.5%) indicating poor ovicidal activity (Raja *et al.,* 2016). The morality of *Cx. quinquefasciatus* eggs was observed 100% at 250 ppm of the acetone extract followed by benzene, ethyl acetate, petroleum ether and aqueous leaf extracts of *Kalanchoe pinnata* exerting 100% morality at the concentration of 300 ppm. The control eggs showed 100% hatchability (Rajesh and Shamsudin, 2017). Petroleum ether, chloroform and ethanol extract of *Catharanthus roseus* leaves was examined for its ovicidal activity against the eggs of *Cx. quinquefasciatus* mosquito. The maximum ovicidal activity was found in ethanol extract in which egg hatchability was totally inhibited at concentration ranging from 150 – 300 ppm. At 100 ppm zero percentage egg hatchability was recorded at 72 h and 96 h. Moderate ovicidal activity was recorded in chloroform extract of *Catharanthus roseus* leaf. Egg hatchability was found to be totally inhibited at concentrations ranging from 200 – 300 ppm. At 100 and 150 ppm zero percentage egg hatchability was recorded at

Table 1: Qualitative phytochemical analysis of *C. pulcherrima* ethanol and acetone leaf extracts

Phytochemical constituents	Ethanol	Acetone
Carbohydrates	+	+
Tannins	_	–
Flavonoids	_	–
Alkaloids	+	+
Quinones	_	–
Glycosides	_	–
Cardiac Glycosides	_	–
Terpenoides	_	–
Triterpenoides	_	+
Phenols	_	–
Coumarins	+	–
Steroides	+	–
Phytosteroides	+	–
Phlobatannins	_	–
Anthraquiones	_	–
Saponins	_	–

+ : Present; - : Absent

Table 2: Important compounds identified in the GC-MS analysis of ethanol leaf extract of *C. pulcherrima*

S. No.	Retention Time	Area (%)	Compound Name
1.	21.781	23.80	Phytol
2.	13.328	5.895	a-D-Glucopyranose
3.	13.258	3.937	3-O-Methyl-d-glucose
4.	28.479	2.410	1-Heptatriacotanol
5.	5.965	2.274	Dodecane
6.	28.944	2.083	Rhodopin
7.	28.163	1.975	2'-Hydroxy-5'-methylchalcone
8.	17.439	1.966	Lidocaine
9.	3.108	1.842	Decane
10.	8.701	1.771	Tetradecane

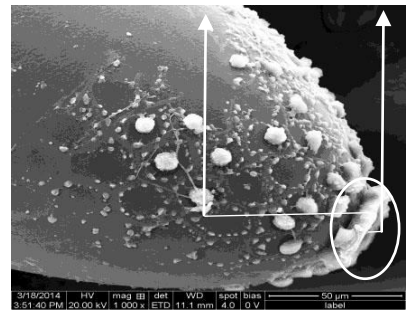
Table 3: Important compounds identified in the GC-MS analysis of acetone leaf extract of *C. pulcherrima*

S. No.	Retention Time	Area (%)	Compound Name
1.	27.791	17.803	Phytol
2.	28.804	3.567	Drostanolone
3.	27.088	3.484	Norethindrone
4.	24.607	3.308	Retinal,9-cis
5.	29.284	2.483	Rhodopin
6.	27.808	2.428	Pregnenolone
7.	29.619	2.072	Ursodeooxycholic acid
8.	27.398	1.983	Hydrocortisone
9.	26.123	1.843	17-Pentatriacontene
10.	28.734	1.725	Heptadecane,9-hexyl

Table 4: Alteration in the hatchability of *Ae. aegypti* eggs exposed to different concentrations of ethanol and acetone leaf extracts of *C. pulcherrima* and control

Parameters	Control	Concentrations (%)				
		0.1	0.3	0.5	0.7	0.9
Number of eggs introduced	20	20	20	20	20	20
Mean number of eggs hatched #	19(e) 19(a)	15(e) 15(a)	10(e) 14(a)	5(e) 6(a)	3(e) 4(a)	0(e) 0(a)
S.D	±0.012(e) ±0.212(a)	±0.014(e) ±0.001(a)	±0.001(e) ±0.003(a)	±0.003(e) ±0.113(a)	±0.002(e) ±0.002(a)	±0.001(e) ±0.001(a)
Percent hatchability	95(e) 95(a)	75*(e) 70*(a)	50*(e) 55*(a)	25*(e) 30*(a)	15*(e) 20*(a)	0*(e) 0*(a)
Percent reduction over control		21.05(e) 21.05(a)	47.36(e) 42.10(a)	78.94(e) 68.42(a)	84.21(e) 78.94(a)	100(e) 100(a)

-Mean ±SD of 5 replicates; *Significantly different from control (P<0.001); e - ethanol leaf extract; a - acetone leaf extract



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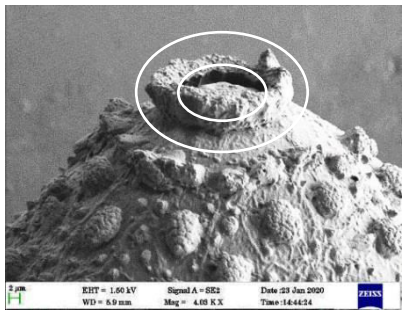

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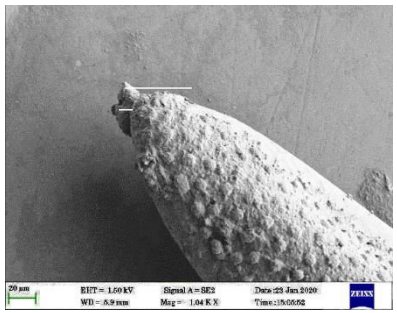

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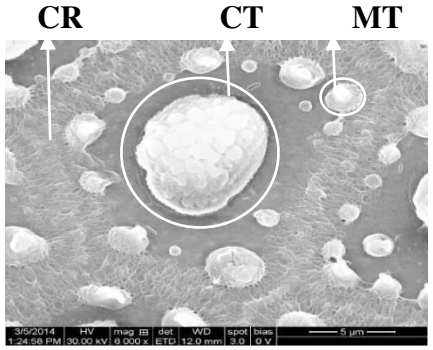

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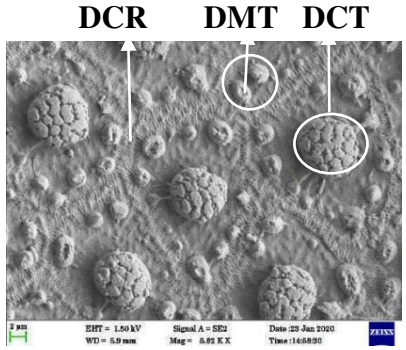

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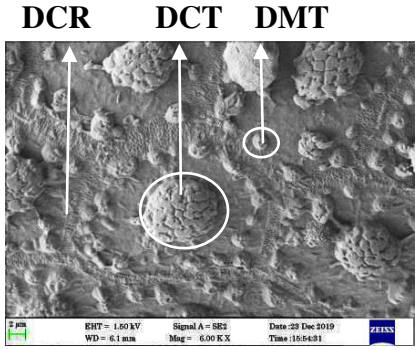

(1e)

(Scanning electron microscope images)

Fig. 1: Microphylar apparatus (MPA) and microphylar pore (MPP) of the control *Ae. aegypti* egg

Fig. 1a, b: Damaged microphylar apparatus (DMPA) and Damaged microphylar pore (DMPP) of *Ae. aegypti* egg treated with ethanol and acetone leaf extracts of *C. pulcherrima*

Fig. 1c: Control *Ae.aegypti* egg with Central tubercle (CT), Minute tubercle (MT) and Chorionic reticulum (CR)

Fig. 1d, e: Damaged Central tubercle (DCT), Damaged Minute tubercle (DMT) and Damaged Chorionic reticulum (DCR) of *Ae. aegypti* egg treated with ethanol and acetone leaf extracts of *C. pulcherrima*

72 h and 96 h (Philosia and Dhivya, 2017). The ovicidal assay of *Ae. aegypti* eggs demonstrated that *Artemisia vulgaris* in the concentration of 1000 ppm resulted 82.67 % unhatched eggs. Ovicidal activity is 79.33 % at a concentration of 750 ppm. In the concentration of 500 ppm, ovicidal activity is 44 % (Vika *et al.,* 2020). The ethanolic extract of *Pometia pinnata* leaves at 0.05, 0.1, 0.15, 0.2 and 0.25 concentration have ovicidal activity of 11, 28, 87, 94 and 98% against *Ae. aegypti* eggs, respectively (Luthfi *et al.,* 2020). The highest concentration of *Pulicaria jaubertii* ethanolic leaf extract at 150 ppm produced complete ovicidal activity in the *Ae. aegypti* eggs (Shehata *et al.,* 2020). Ethanol leaf extracts of *Cyathocline purpurea, Blumea lacera, Neanotis lancifolia* and *Neanotis montholoni* have ovicidal activity on *Ae. aegypti* eggs. Extract of all the plants caused 70 to 90% mortality at higher concentrations (Torawane *et al.,* 2021). n-hexane leaf extract of *Murraya paniculata* showed ovicidal activity on *Ae. aegypti* eggs at all concentrations (20, 40, 60 and 80 ppm) (Alafia *et al.,* 2022). In ovicidal experiments, egg hatchability of *Ae. aegypti* was reduced after treatment of *Malvastrum coromandelianum* and *Mimusops elengi* methanol leaf extract which exerted 100% mortality post-treatment with 400 ppm, while control eggs showed the 100% hatchability (Irudayaraj and Mary Fabiola, 2022).

In the case of ovicidal activity, exposure of freshly laid eggs was more effective than that of the older eggs (Miura *et al.,* 1976). The ethanol and acetone leaf extracts of *C. pulcherrima* treated eggs exhibited an allayed hatchability and this may be due to the action of phytochemicals present in the extracts. The extracts may inhibit the hatchability of the eggs by interfering with their chorion (Rajkumar *et al.,* 2011). Any compound that can cause permeability or a disruption to the chorionic layers in order to effectively deliver compounds that can terminate embryogenesis can be considered for development of effective ovicides. Our compound disrupted chorionic layers (Figs. 1d, e) evidenced from SEM images so it can be considered for development of effective ovicides. The mechanism through which the embryos died may not be known as this was not investigated by the present study but it is speculated it could have been due to blockage of the micropylar apparatus thereby interfering with gases exchange or by interfering with purity of the gases within the operculum preventing the embryo from accessing air and thus ultimately dying. The ethanol and acetone leaf extracts of *C. pulcherrima* caused damages and block in the microphylar pore an orifice through which the sperm penetrates to fertilize the oocyte (Figs. 1a, b).

Conclusion

Diseases caused by mosquito vector populations are major human and animal health problems in all countries. A large number of various plant species from different geographical regions of the world have been identified to possess phytochemicals that are capable of controlling mosquito populations. Phytochemicals, obtained from plants are readily available in many parts of the world, relatively safe, inexpensive and can be used as ovicides or larvicides for killing mosquitoes. In conclusion, this study documented the potential of ethanol and acetone leaf extracts of *C. pulcherrima* as a ovicidal agent against *Ae. aegypti*. However, there is need for further studies to determine and isolate the active constituents of ethanol and acetone leaf extracts of *C. pulcherrima* responsible for ovicidal properties.

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