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Larvicidal and pupicidal activities of *Kedrostis Foetidissima plant* extract against Mosquito vectors

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Abstract
Larvicidal and pupicidal activities of *Kedrostis foetidissima* plant leaf and stem extract against *Culex quinquefasciatus* mosquito vectors. *Kedrostis foetidissima* was tested against the fourth instar larvae and pupae of *C. quinquefasciatus* and the data pertaining to the experiments are shown in the perusal of the data clearly indicated that maximum larval mortality recorded in *C. quinquefasciatus* mosquito at 24 hrs. larvicidal and pupicidal activities of different concentrations of 500ppm, 250ppm, 125ppm, and 62.5ppm. The maximum values of the percentage of larval mortality of the leaf extract in 500ppm the values of larval mortality petroleum ether extract in 93.66±7.08 at 24 hrs. the percentage of larval mortality of the stem extract in 500ppm the values of larval mortality chloroform extract in 70.16±7.36 at 24 hrs. the percentage of pupal mortality of the leaf extract in 500ppm the values of larval mortality petroleum ether in 78.88±8.82 at 24 hrs. the percentage of pupal mortality of the stem extract in 500ppm the values of larval mortality chloroform in 88.16±9.16 at 24 hrs respectively.

Keywords: *Culex quinquefasciatus*, Larvicidal activity, Pupicidal activity, *Kedrostis foetidissima*

Introduction

In this situation, green mosquito larvicides have a promising future as they are target-specific and environment-friendly. They are also economical to use, which is a great advantage for developing continents like Asia, Africa, etc. These are also well recognized as a significant nuisance to millions of people, animals, and other creatures worldwide. proportion of the human population in industrialized nations resides in urban areas, the presence of vector-borne illnesses in cities poses particular concern to epidemiologists and public health authorities. Mosquitoes provide significant risks to the environment as vector of several detrimental illnesses that impact both people and animals Appadurai, D. R 2013 [2].

There are 3000 species of mosquitos, but only three members are responsible for the fatal diseases. Only female mosquitos have the mouth parts necessary for sucking blood. So, they are responsible for the diseases like Malaria, Dengue, Yellow fever, Chikungunya etc. Some mosquitoes can be found 200 miles from their birthplace. One species of Anopheles frequently becomes frozen but after gradual thawing revives and is capable of laying eggs. Mosquitoes transmit diseases to more than 700,000,000 people annually and will be responsible for the deaths of 1 out of every 17 people currently alive Deepalakshmi, S and Jeyabalan, D. 2017 [3].

Dengue is a viral illness caused by the Flavivirus, which is largely spread by mosquitoes of the species *Ae. aegypti*, followed *Aedes albopictus* and other species of the Aedes genus. Dengue infection is a significant public health issue that has been documented in the Americas, Africa, Southeast Asia, Europe, Western Pacific, and Eastern Mediterranean areas, Jeyasankar A., and Chinnamani T. 2017 [12]. This arboviral illness is widespread in over 100 countries, with about 96 million infected persons experiencing symptoms of variable severity. Since the commencement of 2023, there has been continuous spread of dengue, along with an unforeseen increase in case lead to a nearly record-breaking total of over five million illnesses and over 5000 fatalities caused by dengue.

These cases and deaths have been documented in more than 80 countries/territories and throughout five regions as defined by the World Health Organization. Nevertheless, the extensive use of chemical pesticides has led to resistance in vectors, pollution, bioaccumulation, and several health and environmental concerns, Subhashini., K 2017. Mosquitoes are controlled by synthetic pesticides, which have substantial environmental consequences. Botanicals have historically been advocated as desirable alternatives to synthetic agrochemicals due to their alleged reduced environmental and human health impact in comparison to the latter Ravichandran, R 2013. Bioactive compounds, which have been utilized extensively in complementary and alternative medicine for decades, are prevalent in plants.

Previous research has established the critical role of larvicides in mitigating mosquito populations at breeding sites. However, the emergence of resistance in mosquito vectors to chemical insecticide, larvicides remains an unresolved challenge, Kamaraj, C 2010 [7]. In light of the increasing interest in botanical insecticides as eco-friendly and sustainable alternatives to synthetic chemicals. Mosquito repellent is a substance or finishing process applied to skin, cloths, other surface which repels away mosquitos. Malaria results from infection with a protozoan carried by Mosquitoes and, according to reports from the World Health Organization, cause as many as 3,000,000 deaths annually, epidemic polyarthritis, several forms of encephalitis, Kadarkarai Murugan., 2012 [6].

Because of global warming the distribution of mosquitoes has expanded from topical region to northern latitudes, that leads to a spread in sources of viral infection from mosquitoes. By using this anyone can keep themselves far away from mosquitoes. Plant like Neem, Mint contained a great herbal characteristic. These natural products are abundantly available in nature and are widely distributed. Premalatha, S., 2007. They are cheap and not processed and can be used as raw materials for required applications. Natural repellents are identified by researchers to control the Mosquitoes. During the 20th Century the Biological control of Mosquito using larvivore's fishes was a very important

method to control the Spread of Malaria in and around the sub areas of Urban and Peri-Urban areas by Stav *et al*., (2000).

(WHO) states, "Malaria remains a significant health issue, contributing to an alarming figure of more than 200 million new cases and over 400 thousand annual deaths. The majority of these deaths occur in sub-Saharan Africa." Dengue fever has the alarming statistic of being the fastest growing infectious disease transmitted through mosquitoes with millions of diagnosed cases each year around the world, 2017 Subashini, K. The main vectors of these diseases are the Aedes and Anopheles mosquito species. Although *Kedrostis foetidissima* (Appakovay) is worldwide used in traditional medicine, toxicological data on the plant are scarce. In view of the above facts an attempt has been made to evaluate the controlling of mosquito vectors, *Cx. quinquefasciatus.*

Materials and method

Collection of plant materials

The plant *Kedrostis foetidissima* was identified as botanist, Department botany, Arignar anna government arts college, Namakkal District, Tamil Nadu, India. Fresh *Kedrostis foetidissima* was collected from in around our college, Arignar anna government arts college, Namakkal District, Tamil Nadu, India. Plant specimen was identified by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St' Joseph's College, Tiruchirapalli, Tamil Nadu, India. The voucher specimen of was prepared and deposited in PG and Research Department of Zoology, Government Arts College, Namakkal, Tamil Nadu, India.

Extraction method

The plant was shade dried under room temperature (27.0 ± 2ºC and 75 ± 5% RH). After drying the whole plant material was powdered by utilizing electric blender. 1000g of plant powder was separated by Soxhlet extraction methods with hexane, chloroform and ethyl acetate derivation, consecutively with expanding extremity of solvents and filtered through Whatman's No. 1 filter paper and it was condensed with Rotary Vacuum Evaporator. The concentrates were gathered in clean borosil vials and stored in the refrigerator for experiments against important mosquito vectors.

Mosquito rearing

The mosquito species *Culex quinquefasciatus* were selected for present investigation. Reared in laboratory of department of zoology, Arignar Anna Govt. Arts College, Namakkal.

Larvicidal activity

The extracts were prepared and tested against the freshly moulted (0-6 hrs) fourth instar larvae of selected mosquito species. For dissolving of plant extracts 2 drop tween 20 was added and then it was diluted with 100ml of dechlorinated water to obtain desired concentrations. The control was prepared using 2 drop Polysorbate 20 (Tween 20) in 100ml of dechlorinated water. 250-ml transparent cups were used for bioassay and five replications were maintained. The ten number of freshly moulted fourth instar larvae of mosquitoes were introduced in respective concentrations of plant extracts. The results were observed and recorded after the treatment of 12 and 24 hours. The LC_{50} value was calculated by using probit analysis (Finney, 1971) [4]. The LC_{50}, LC_{90} and other statistics chi-square values were calculated by using the software using statistical package of social science (SPSS) version 16.0 for windows, significance level was set at $p \leq 0.05$.

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \text{X } 100$$

$$\text{Percentage mortality} = \frac{\text{Numbere of dead larvae}}{\text{No of larvea introduced}} \text{X100}$$

Pupicidal bioassay

The pupicidal activity of plant crude extract was assessed by using the standard method as prescribed by WHO (2005). Similar test concentrations as stated in the previous experiments was prepared and tested against the pupae of *Culex quinquefasciatus.* Polysorbate 20 (Tween 20) used as emulsifier and distilled water treated as control. Ten pupae of each mosquito species were introduced in respective concentrations of plant extracts. The pupal mortality was observed and recorded after 12 and 24 hours. of treatment. For each experiment, five replicates were maintained at a time. The percentage of mortality was calculated by using Abbott's formula (1925) [1].

$$\text{Percentage mortality Pupae} = \frac{\text{Numbere of dead pupae}}{\text{No of pupae introduced}} \text{X10}$$

Statistical Analysis

Data analysis was carried out using Microsoft Excel 2007. Least Significant Difference Lethal concentration. LC_{50} and LC_{90} was calculated along with their fiducial limits at 95% confidence level by probit analysis using SPSS software package 16.00 (Statistical Package of Social Sciences).

Results and disscussion

Larvicidal activity of *Kedrostis foetidissima plant leaf extract* against the freshly moulted (0-6hrs old) fourth instar larvae of *C. quinquefasciatus*

The use of biologically active plant-based products with insecticidal properties has attracted considerable interest of scientists in all over the world. Their easily biodegradable on nature and relatively. The result suggested that the ethyl acetate of *M. azedarach* leaf extract was an excellent larvicidal potential in controlling mosquito vectors. (Ravichandran and Kanayairam, 2014) [10]. T. Chinnamani *et al.*, 2020, With each extract at a concentration of 100ppm, the take of hatchability was very high and nil hatchability was recorded as the concentration of extract was better to 300ppm in the case of aqueous and ethanol extract (Roni *et al.*,2013). (Manis kumar., *et al*, 2022). Reported that (Sugauara *et al 2022*), larvisidal activity of *Brunfelsia uniflora* ectract on *Aedes aegypti* larvae.

The LC_{50} is4.89 and $LC_{99.9}$ is 11.14mg/ml and form flower extract were LC_{50}=3.82 and $LC_{99.9}$=11.03 mg/ml and the positive controle presented LC_{50}=0.04 and $LC_{99.9}$=1.14 mg/ml so this result shows *Brunfelsia uniflora* is an alternative source of *A. aegypti larvae.* In the present study *Kedrostis foetidissima* was tested against the fourth instar larvae of *C. quinquefasciatus* and the data pertaining to the experiments are shown in table 2. Perusal of the data clearly indicated that maximum larval mortality in ethyl acetate extract was recorded in *C. quinquefasciatus mosquito at* 24 hrs values was recorded in different concentrations of 500ppm, 250ppm, 125ppm, and 62.5ppm the values in the percentage of larval mortality 68.14%, 50.12%, 36.08% and 20.02% and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2

values for 272.94 (194.56-344.28), 865.97 (681.91-1320.13)0.597 respectively.

The obtained results with earlier reports. The investigation of the larvicidal efficacy of the crude leaf ethyl acetate extract of *T. procumbens* was tested against *Cx. tritaeniorhynchus* showed promising larvicidal activity (Kamaraj *et. al.*, 2011). (Manis kumar., *et al*, 2022). Reported that (Sugauara *et al 2022*), larvisidal activity of *Brunfelsia uniflora* extract on *Aedes aegypti* larvae. The LC_{50} is4.89 and $LC_{99.9}$ is 11.14mg/ml and form flower extract were $LC_{50=}$3.82 and $LC_{99.9=}$11.03 mg/ml and the positive controle presented LC_{50}=0.04 and $LC_{99.9}$=1.14 mg/ml so this result shows *Brunfelsia uniflora* is an alternative source of *A. aegypti larvae.* The Chloroform extract was recorded in *C. quinquefasciatus mosquito at* 24 hrs values was recorded in the percentage of larval mortality 78.20, 57.14, 37.14, 23.08 and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values for 239.14 (199.61-280.41), 624.62 (538.66-761.36) 0.218 respectively. Ethyl acetate extract was recorded in *C. quinquefasciatus* mosquito at 24 hrs values was recorded in the percentage of larval mortality in 93.66, 71.55, 58.56, and 39.10% and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values for 104.15(57.05-140.34), 427.96 (370.25-518.25)0.380 respectively.

Larvicidal activity of *Kedrostis foetidissima* plant stem extract against the freshly moulted (0-6hrs old) fourth instar larvae of *Cx. quinquefasciatus*

Kedrostis foetidissima plant stem was tested against the fourth instar larvae of *C. quinquefasciatus* and the data pertaining to the experiments are shown in table 2. Perusal of the data clearly indicated that maximum larval mortality in ethyl acetate extract was recorded in *Cx. quinquefasciatus* mosquito at 24 hrs values was recorded in different concentrations of 500ppm, 250ppm, 125ppm, and 62.5ppm the values in the percentage of larval mortality values in 68.14, 55.10, 38.08, 27.04 and then LC_{50} (LCL-UCL), LC $_{90}$ (LCL-UCL) X^2 values for values in 265.59 (211.54-327.33), 808.66 (659.81-1093.87)0.209 respectively.

larval mortality in Chloroform extract were recorded in *Cx. quinquefasciatus mosquito at* 24 hrs values was recorded in the percentage of larval mortality values in 70.16±7.36, 56.12±5.59, 36.08±5.58, and 26.02±9.97and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values for 260.15 (210.04-315.95), 761.17(630.61-798.66)0.214 respectively. In petroleum ether extract in result percentage of larval mortality in 66.14, 42.12, 30.04, and 21.02% and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values for 320.97(272.42-384.00), 795.70 (614.86-1026.28)0.117 respectively. Larvicidal activity of acetone, ethyl acetate, chloroform and butanol and butanol dried leaf extract of *Melia azedarach* tested against 3rd instar *Culex quinquefasciatus* and *Aedes aegypti*. The result suggested that the ethyl acetate of *M. azedarach* leaf extract was an excellent larvicidal potential in controlling mosquito vectors. T. Chinnamani, 2020. have reported that the ethyl acetate extract of *Phyllanthus Emblica* Linn. Exhibited more than 90% larval mortality at 250ppm on *C. quinquefaciatus.*

Pupicidal activity of *Kedrostis foetidissima plant leaf extract* against the freshly moulted (0-6hrs old) fourth instar larvae of *Cx. quinquefasciatus*

The obtained results with earlier reports. The investigation of the larvicidal efficacy of the crude leaf ethyl acetate extract of *T. procumbens* was tested against *Cx. tritaeniorhynchus* showed promising larvicidal activity (Kamaraj *et. al.*, 2011). In the present study *Kedrostis foetidissima* was tested against the pupae of *Cx. quinquefasciatus* and the data pertaining to the experiments are shown in table 2 and figar 1. Perusal of the data clearly indicated that maximum pupal mortality in ethyl acetate extract was recorded in *Cx. quinquefasciatus mosquito at* 24 hrs values was recorded in different concentrations of 500ppm, 250ppm, 125ppm, and 62.5ppm the values in the percentage of pupal mortality in 74.20, 56.20, 31.60, and 27.12% and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values for 256.36 (212.88-303.87), 687.67 (583.96-961.25) 0.852 respectively. Chloroform extract of pupal mortality result in 78.88, 60.80, 47.94, and 27.80 and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values in 161.45 (96.60-212.86), 678.82 (558.33-905.41) 0.737 respectively. Petroleum ether extracts pupal mortality in 98.66, 71.17, 56.66, and 38.84% and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values for 119.82 (82.44-143.42), 361.94 (316.84-430.41)0.285 respectively. The ethyl acetate extract of *Prosopis juliflora* showed remarkable pupicidal activity against the three mosquito species. The pupal death was observed in 48 hrs. Similar results of plant extracts on pupicidal activity was reported by Sivagnaname and Kalyanasundaram (2004), the methanolic extract of *Atlantia monophylla* were used for pupicidal assay with LC_{50} value of, 0.07, 0.07 and 0.05 (mg/l), respectively, against the pupae of *C. quinquefasciatus*, *A. aegypti* and *A. stephensi*. *M. Rajasekara Pandian, et al., 2023* [15].

Pupicidal activity of *Kedrostis foetidissima* plant stem extract against the freshly moulted (0-6hrs old) fourth instar larvae of *Cx. quinquefasciatus*

In the present study *Kedrostis foetidissima* plant stem was tested against the pupae of *Cx. quinquefasciatus* and the data pertaining to the experiments are shown in table 3. Perusal of the data clearly indicated that maximum pupae mortality in ethyl acetate extract was recorded in *Cx. quinquefasciatus mosquito at* 24 hrs. values was recorded in different concentrations of 500ppm, 250ppm, 125ppm, and 62.5ppm the values in the percentage of pupae mortality values in 69.18, 51.50, 37.58, and 26.64 and then LC_{50} (LCL-UCL), LC $_{90}$ (LCL-UCL) X^2 values for values in 242.54(173.84-315.65), 907.67(708.59-1355.30)0.677 respectively. Chloroform extracts in pupal mortality values in 88.16, 63.12, 45.48, 30.52 and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values for 182.19(146.04-215.79), 505.82 (442.65-601.22)0.284 respectively. Larvisidal activity of *melaleuca leucadendra* leaves extract with ethanol against *aedes aegypti* the mortality is observed after 24 hours the highest mortality (47.5%) reached by concentration 4%. The LOGIT test showed that the number of LC_{50} was 3.7% (37600 mg/l) with 95% significance the extract less effect to kill so it causes lethal effect of *A. aegypti M. Rajasekara Pandian, et al., 2023* [15]. The pupicidal, antifeedant, ovicidal and insect growth inhibitory activities of *Barleria longiflora* tested against *Helicoverpa armigera* and *Spodoptera litura*. Among, the lavicidal, antifeedant, pupicidal activities of *Atalantia monophylla* against Spodoptera litura. Larvicidal and ovicial activities of crude extracts, fractions and compounds A and B in plants leaf extract tested against *S. litura*. Larvicidal and ovicial activities of crude extracts petroleum ether, chloroform, and ethyl acetate tested in Spodoptera litura and Helicoverpa armigera

Kedrostis foetidissima was tested against the pupae of *Cx. quinquefasciatus* and the data pertaining to the experiments are shown in table 3. Perusal of the data clearly indicated that maximum pupae mortality in ethyl acetate extract was recorded in *Cx. quinquefasciatus* mosquito at 24 hrs values was recorded in different concentrations the values in the percentage of pupae mortality in 76.44, 60.52, 45.64, and 26.43 then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values for 197.36 (143.66-245.46) 681.73(567.82-886.08) 0.063 respectively.

Conclusion

In conclusion, based on the results, plant-based compounds have significant potential in mosquito vectors control programme. The diversified use of plant-based compound (*Kedrostis foetidissima*) properties and their active formulation in the mosquito control programme of human health importance across the world. Therefore, future research work should be directed towards the practical application of bioactive compounds

Table 1: larvicidal activity of *Kedrostis foetidissima* plant leaf different solvent extracts against *Culex quinquefasciatus* mosquitoes' vector in 24 hrs.

Different Solvents extract	Concentration (PPM)	Percentage of Mortality	LC $_{50}$ and LC$_{90}$		X^2
			LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	
Ethyl acetate	500	68.14±5.61	272.94 (194.56-344.28)	865.97 (681.91-1320.13)	0.597
	250	50.12±8.51			
	125	36.08±10.97			
	62.5	20.02±7.03			
Chloroform	500	78.20±4.32	239.14 (199.61-280.41)	624.62 (538.66-761.36)	0.218
	250	57.14±10.77			
	125	37.14±8.23			
	62.5	23.08±7.07			
Petroleum ether	500	93.66±7.08	104.15 (57.05-140.34)	427.96 (370.25-518.25)	0.380
	250	71.55±8.22			
	125	58.56±8.29			
	62.5	39.10±7.07			

LC_{50}=Lethal Concentration brings out 50% Mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit;

Table 2: larvicidal activity of *Kedrostis foetidissima* plant stem different solvent extracts against *Culex quinquefasciatus* mosquitoes' vector in 24 hrs.

Different Solvents extract	Concentration (PPM)	Percentage of Mortality	LC $_{50}$ and LC$_{90}$		X^2
			LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	
Ethyl acetate	500	68.14±4.56	265.59 (211.54-327.33)	808.66 (659.81-1093.87)	0.209
	250	55.10±8.86			
	125	38.08±7.07			
	62.5	27.04±8.31			
Chloroform	500	70.16±7.36	260.15 (210.04-315.95)	761.17 (630.61-798.66)	0.214
	250	56.12±5.59			
	125	36.08±5.58			
	62.5	26.02±9.97			
Petroleum ether	500	66.14±5.61	320.97 (272.42-384.00)	795.70 (614.86-1026.28)	0.117
	250	42.12±9.04			
	125	30.04±9.94			
	62.5	21.02±8.92			

LC_{50}=Lethal Concentration brings out 50% Mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit;

Table 3: Pupicidal activity of *Kedrostis foetidissima* plant leaf different solvent extracts against *Culex quinquefasciatus* mosquitoes' vector in 24 hrs.

Different Solvents extract	Concentration (PPM)	Percentage of Mortality	LC $_{50}$ and LC$_{90}$		X^2
			LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	
Ethyl acetate	500	74.20±2.61	256.36 (212.88-303.87)	687.67 (583.96-961.25)	0.852
	250	56.20±5.41			
	125	31.60±5.57			
	62.5	27.12±5.53			
Chloroform	500	78.88±8.82	161.45 (96.60-212.86)	678.82 (558.33-905.41)	0.737
	250	60.80±1.67			
	125	47.94±9.53			
	62.5	27.80±8.87			
Petroleum ether	500	98.66±7.44	119.82 (82.44-143.42)	361.94 (316.84-430.41)	0.285
	250	71.17±5.27			
	125	56.66±4.69			
	62.5	38.84±4.87			

LC_{50}=Lethal Concentration brings out 50% Mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table 4: Pupicidal activity of *Kedrostis foetidissima* plant stem different solvent extracts against *Culex quinquefasciatus* mosquitoes' vector in 24 hrs

Different Solvents extract	Concentration (PPM)	Percentage of Mortality	LC 50 and LC90		X^2
			LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	
Ethyl acetate	500	69.18±5.65	242.54 (173.84-315.65)	907.67 (708.59-1355.30)	0.677
	250	51.50±1.88			
	125	37.58±6.04			
	62.5	26.64±5.34			
Chloroform	500	88.16±9.16	182.19 (146.04-215.79)	505.82 (442.65-601.22)	0.284
	250	63.12±1.41			
	125	45.48±4.58			
	62.5	30.52±5.57			
Petroleum ether	500	76.44±4.54	197.36 (143.66-245.46)	681.73 (567.82-886.08)	0.063
	250	60.52±5.55			
	125	45.64±4.17			
	62.5	26.43±2.98			

LC_{50}=Lethal Concentration brings out 50% Mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence

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