



Identification of Compounds and Insecticidal Activity of the Root of Pride of Barbados (*Caesalpinia Pulcherrima* L)

*¹OSAYEMWENRE ERHARUYI; ¹VINCENT O. IMIEJE; ¹IRENE O. OSEGHAE; ²ABRAHAM E. UBHENIN; ¹ABIODUN FALODUN; ³M. IQBAL CHOUDHARY

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

²Department of Biochemistry, Faculty of Basic Health Science, Federal University Lafia, Nasarawa, Nigeria.

³HEJ Research Institute of Chemistry and Dr. Pajwani Centre for Molecular Medicine and Drug Research, International Centre for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan.

*Author for Correspondence: E-mail: osayemwenre.erharuyi@uniben.edu,

Mobile: +2348062355972

ABSTRACT: *Caesalpinia pulcherrima* (Caesalpinaceae) is an ornamental plant with several ethnomedicinal uses. The present study was designed to investigate the brine shrimp cytotoxicity and insecticidal activity of oil obtained from *C. pulcherrima* root. The powdered root was extracted with methanol and then defatted with petroleum ether (40-60°C) to obtain a viscous oil. The oil was investigated for its brine shrimp cytotoxicity and insecticidal activity *in vitro*. The chemical constituents were identified by Gas chromatography-Mass spectrometry. The oil showed significant lethal effect against *Artemia salina* (Brine shrimp) with LC₅₀ of 23.85 µg/mL and mild insecticidal activity against *Tribolium castaneum* and *Callosobruchus analis* with percentage mortality of 20% and 40% respectively at 1 mg/cm². GC-MS analysis identified 37 compounds mainly steroids, terpenoids and fatty acids. © JASEM

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The genus *Caesalpinia* consists of more than 500 species belonging to the family Caesalpinaceae. The species are mostly woody occurring in tropical and subtropical regions of the world. The genus has been found to contain numerous phytochemicals such as alkaloids, tannins, saponins, glycosides, phenolics, steroids and terpenoids. Diterpenoids of the cassane-type have been isolated from various species of the genus. Literature reports have shown the species to possess interesting pharmacological activities, such as, antidiabetic, antiulcer, anti-inflammatory, analgesic, adaptogenic, antimicrobial, antirheumatic, antipyretic, antioxidant and anticancer activities (Carvalho *et al.*, 1996; Sudhakar *et al.*, 2006; Kannur *et al.*, 2006; Srinivasan *et al.*, 2007; Saravanan *et al.*, 2008; Shukla *et al.*, 2010; Sgariglia *et al.*, 2011).

Caesalpinia pulcherrima L. Swartz is a well-known ornamental plant belonging to the family Caesalpinaceae. The plant is commonly known as Pride of Barbados. The plant is native to Central America but well distributed in tropical and subtropical regions of Africa, Asia and Australia (Zanin *et al.*, 2012). In traditional medicine, extracts from various parts of the plant have been used as stimulant, emenagogue and arbotifient (Srinivas *et al.*, 2003; Chiang *et al.*, 2003). Pharmacologically, the plant has been reported to possess antimicrobial,

analgesic, anti-inflammatory, anthelmintic, antimalarial, antiulcer, cytotoxic, and antioxidant activities (Roach *et al.*, 2003; Promsawan *et al.*, 2003; Sudhakar *et al.*, 2006; Pawar *et al.*, 2009; Patel *et al.*, 2010; Sharma and Rajani, 2011; Venkatesalu *et al.*, 2012; Kumbhare *et al.*, 2012). Phytochemicals including diterpenes, flavonoids, peltogynoids, steroids and glycosides have been isolated from various parts of the plant (McPherson *et al.*, 1986; Cheet *et al.*, 1986; Ragasa *et al.*, 2003; Maheswara *et al.*, 2006; Pranithanchai *et al.*, 2009; Das *et al.*, 2009; Das *et al.*, 2010; Yodsaoue *et al.*, 2011). In the present study, we report the brine shrimp cytotoxicity and insecticidal activity of the oil obtained from the root of *C. pulcherrima* and the identification of the constituents by GC-MS analysis.

MATERIALS AND METHOD

Collection and Preparation of Plant Material: Fresh *Caesalpinia pulcherrima* roots were collected in June, 2014 from the University of Benin, Ugbowo campus, Benin City. The plant material was identified and authenticated by Mr. Ugbogu O. A. and Shasanya O. S. of the Forestry Research Institute of Nigeria (FRIN), Ibadan where a herbarium specimen is preserved with voucher number FHI109969.

The roots were washed to remove earthy impurities,

* Correspondence: E-mail: osayemwenre.erharuyi@uniben.edu,

air dried and powdered. The crude powdered sample was stored in an air-tight glass jar until ready for use.

Extraction: The powdered plant material (2.9 kg) was macerated in methanol (7.5 L) at room temperature for 7 days. The extract was concentrated to dryness using a rotary evaporator at reduced pressure. The crude oily extract (240 g) was suspended in 250 mL of methanol-water (4:1) and extracted with petroleum ether (3 X 500 mL) to obtain a viscous oil.

Brine shrimp Lethality Assay: *Hatchine of brine shrimp: Artemia salina* (Brine shrimp) eggs were hatched in the hatching tray half-filled with brine solution incubated at 37°C.

Sample Preparation: Test sample (20 mg) was dissolved in 2 mL of DMSO to make a stock solution of 10 mg/mL. Concentrations of 10, 100 and 1000 µg/mL were prepared by transferring 5, 50 and 500 µL to vials and the solvent was allowed to evaporate overnight.

Treatment with test sample: After 2 days of hatching and maturation as nauplii, 5 mL of artificial seawater containing 10 larvae was transferred into each well containing different concentration of the test sample (final concentration 2, 20 and 200 µg/mL). The vials were incubated at 25-27°C for 24 h under illumination. Seawater and etoposide were used as negative and positive control respectively.

Each concentration was done in triplicate and the LC₅₀ determined.

Insecticidal Activity Screening (Impregnated filter paper test): **Test insects:** *Tribolium castaneum*, *Sitophilus oryzae*, *Rhyzopertha dominica*, *Trogoderma granarium* and *Callosobruchus analis* were reared in the laboratory at 25-27°C in 50% relative humidity in plastic bottles containing sterile breeding media. Insects of uniform age and size were used for the experiment.

Test procedure: Day-1: Filter papers (90 mm each) were put in petri plates and loaded with the test sample at 200 mg in 3 mL ethanol. The plates were left at room temperature for 24 h to evaporate the solvent completely.

Day-2: After the evaporation of solvent, 10 insects of each specie were placed in each plate using a clean brush. The plates were incubated at 27°C for 24 h at 50% relative humidity in the growth chamber. Permethrin was used as the standard insecticide while ethanol served as control.

Day-3: The number of surviving insects belonging to each specie were counted and the Percentage mortality was calculated using the formula:

$$\text{Percentage mortality} = 100 - \frac{\text{No. of insects alive in test}}{\text{No. of insects alive in control}} \times 100$$

Gas Chromatography/Mass Spectrometry (GC-MS):

The GC-MS analysis of the viscous oil was performed on a GC-MS-TQQQ instrument equipped with Agilent USB39375HHP-5MS column and capillary dimensions 30 m x 250 µm x 0.25 µm using helium as the carrier gas at a flow rate of 1.2 mL/min. The injection volume was 1 µL and pressure was 10.97 psi. The oven was equilibrated for 0.5 minutes and temperature was programmed at 70°C for 5 minutes, then 10°C/min to 180°C for 5 minutes, then 10°C/min to 280°C for 10 minutes, then 5°C/min to 290°C for 30 minutes. Total run time was 73 minutes. The MS transfer line was maintained at 325°C. The ionization mode used was electron ionization at 70 eV and source temperature of 250°C. Total Ion Count (TIC) was used to evaluate for compound identification at start mass of 20 amu and end mass of 650 amu for scan time of 200 ms. The Spectra of the separated compounds were compared with the database of the NIST Reference Spectra Library with Match Factor (MF) of ≥ 700 taken as satisfactory. The relative percentage composition of the identified compounds were estimated from the GC peak area.

RESULTS AND DISCUSSION

Prior to the discovery of the organochlorine and organophosphate insecticides in the late 1930s and early 1940s, botanical insecticides have remained an important weapon in managing insect pests disease of farm produce (Forim *et al.*, 2012). Botanical source insecticides may serve as alternatives to popularly used synthetic chemical insecticides due to its biodegradability, and non-toxicity to none target organisms. Previous Studies on insecticidal activities of *Caesalpinia pulcherrima* have focused particularly on the whole plant extract. For example, the crude extract of *C. pulcherrima* exerted zero hatchability (100% mortality) at 375, 300 and 225 ppm for house mosquitoes (*Culex quinquefasciatus*), yellow fever mosquitoes (*Aedes aegypti*) and malaria mosquitoes (*Anopheles stephensi*) respectively and showed LD₅₀ of 99.52 and 110.886 µg/mL in brine shrimp cytotoxicity for aqueous and methanolic extract respectively (Govindarajan *et al.*, 2011, Pawar *et al.*, 2009, Govindarajan *et al.*, 2013). Govindarajan *et al.* evaluated the larvicidal activity of crude benzene and ethyl acetate extracts of leaves of *Caesalpinia pulcherrima* for toxicity against three important vector mosquitoes, namely, *Culex tritaeniorhynchus*,

Aedes albopictus, and *Anopheles subpictus*. All extracts showed moderate larvicidal effects, with the benzene extract showing the highest larval mortality (Govindarajan *et al.*, 2013).

Defatted methanol extract of *Caesalpinia pulcherrima* root bark yielded 9.23% w/w of the fixed oil. The oil was evaluated for its *in vitro* cytotoxicity using the brine shrimp (*Artemia salina*) lethality assay and its potential insecticidal activity was evaluated by the impregnated filter paper test.

In the brine shrimp lethality assay the oil showed significant lethality compared to control. The percentage mortality were 36.67%, 66.67% and 90% at 0.01, 0.1 and 1 mg/mL respectively. The half-maximal lethal concentration (LC₅₀) was 23.85 µg/mL (Table 1).

The insecticidal activity was investigated against four common stored grain pests including red flour beetle (*Tribolium castaneum*), rice weevil (*Sitophilus oryzae*), lesser grain borer (*Rhyzopertha dominica*) and pulse beetle (*Callosobruchus analis*). The oil

showed 20% mortality against *Tribolium castaneum* and 40% mortality against *Callosobruchus analis* at 1019.10 µg/cm² concentration. Compared to the standard insecticide (permethrin), the oil was significantly less active against the tested grain pests (Table 2).

The LD₅₀ values recorded in this present study is lower than those reported previously, indicating higher cytotoxic activities of the oil extract. Compared to the standard insecticide (permethrin), the LD₅₀ values for the oil were higher. The lower LD₅₀ for permethrin was as a result of their highly purified state as against complex composition of the oil extract. Considering the effect of synthetic insecticides especially on environment and food, LD₅₀ values for botanical insecticides do not need to be low before it is accepted.

The GC-MS analysis of the oil identified 37 compounds (Figures 1 - 3). The relative percentage composition, retention time, chemical names and molecular formulas of the identified compounds are shown in table 3.

Table 1: Brine shrimp cytotoxicity of *C. pulcherrima* root oil

Conc. (µg/mL)	No. of shrimps	No. of survivors		Percentage mortality		LC ₅₀ (µg/mL)	
		sample	Std. drug	sample	Std. drug	sample	Std. drug
10	30	19	3	36.67	90		
100	30	10	1	66.67	96.67	23.85	7.46
1000	30	3	0	90	100		

Table 2: Insecticidal activity of *C. pulcherrima* root oil

Name of insect	Percentage mortality		
	Positive control	Negative control	<i>C. pulcherrima</i> oil
<i>Tribolium castaneum</i>	100	0	20
<i>Sitophilus oryzae</i>	100	0	0
<i>Rhyzopertha dominica</i>	100	0	0
<i>Callosobruchus analis</i>	100	0	40

Table 3: compounds identified from *C. pulcherrima* root oil

Compound name	Molecular formula	MW	RT (min)	RMF (DB)
Caryophyllene	C ₁₅ H ₂₄	204	13.47	935
Caryophyllene oxide	C ₁₅ H ₂₄ O	220	15.60	934
Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	21.36	926
n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	22.40	889
Trachylobane	C ₂₀ H ₃₂	272	22.78	847
n-Heptadecanol-1	C ₁₇ H ₃₆ O	256	24.37	920
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294	24.51	931
9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	296	24.60	913
Heptadecanoic acid, 16-methyl-, methyl ester	C ₁₉ H ₃₈ O ₂	298	24.97	827
9,12-Octadecadienoic acid	C ₁₈ H ₃₂ O ₂	280	25.32	863
cis-Vaccenic acid	C ₁₈ H ₃₄ O ₂	282	25.40	879
Retinal, 9-cis-	C ₂₀ H ₂₈ O	284	25.69	764
9-Hexadecenoic acid	C ₁₆ H ₃₀ O ₂	254	26.98	785
Mibolerone	C ₂₀ H ₃₀ O ₂	302	28.44	724
2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	C ₂₃ H ₃₂ O	324	29.09	772
Retinoic acid	C ₂₀ H ₂₈ O ₂	300	29.56	771
1-Heptatriacotanol	C ₃₇ H ₇₆ O	536	29.70	847
Oxymesterone	C ₂₀ H ₃₀ O ₃	318	30.10	712
6β-Hydroxymethandienone	C ₂₀ H ₂₈ O ₃	316	30.33	714
Fenretinide	C ₂₆ H ₃₃ NO ₂	391	30.35	779
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	C ₃₀ H ₅₀	410	31.73	944
Pregnan-20-one, 3,17-dihydroxy-, (3β,5β)-	C ₂₁ H ₃₄ O ₃	334	32.30	763
Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)-	C ₃₀ H ₅₀ O	426	32.69	875
1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	C ₃₀ H ₅₀ O	426	33.60	911
9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3β,4α,5α)-	C ₃₂ H ₅₂ O ₂	468	34.35	871
Geranylgeraniol	C ₂₀ H ₃₄ O	290	34.45	856
Tetracos-2,6,10,14,18-pentaen-22-ol, 2,6,10,15,19,23-hexamethyl-23-methoxy-, alltrans	C ₃₁ H ₅₄ O ₂	458	35.53	870
Stigmasterol	C ₂₉ H ₄₈ O	412	37.21	895
Acetic acid, 17-(1-hydroxy-ethyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-yl ester	C ₂₃ H ₃₄ O ₃	358	37.58	723
β-Sitosterol	C ₂₉ H ₅₀ O	414	38.32	900
Dihydrotestosterone 3-formate-17-benzoate	C ₂₇ H ₃₄ O ₄	422	38.56	730
Fluoxymesterone	C ₂₀ H ₂₉ FO ₃	336	41.16	750
6β-Hydroxymethandienone	C ₂₀ H ₂₈ O ₃	316	43.33	703
2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-	C ₁₀ H ₁₀ O ₄	194	44.92	733
Pregnan-20-one, 3-(acetyloxy)-5,6:16,17-diepoxy-, (3β,5α,6α,16α)-	C ₂₃ H ₃₂ O ₅	388	46.28	730
Spiro[tricyclo[4.4.0.0(5,9)]decane-10,2'-oxirane], 1-methyl-4-isopropyl-7,8-dihydroxy-, (8S)-	C ₁₅ H ₂₄ O ₃	252	48.72	752
Pregnenolone	C ₂₁ H ₃₂ O ₂	316	51.96	725

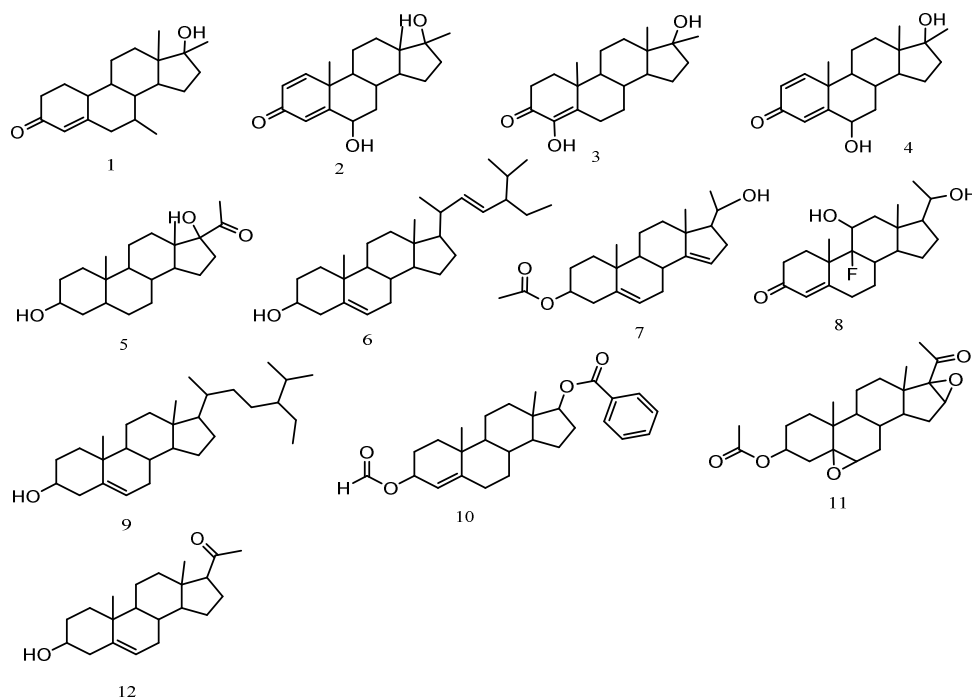


Fig 1: Steroids identified from *C. pulcherrima* root oil: Mibolerone(1); 6 β -Hydroxymethandienone(2); Oxymesterone(3); 6 β -Hydroxymethandienone(4); 3,17-dihydroxy-, (3 β ,5 β)-Pregnan-20-one(5); Stigmasterol(6); Acetic acid(7), 17-(1-hydroxy-ethyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-yl ester; Fluoxymesterone(8); β -Sitosterol(9); Dihydrotestosterone 3-formate-17-benzoate(10); Pregnan-20-one, 3-(acetyloxy)-5,6:16,17-diepoxy-(3 β ,5 α ,6 α ,16 α)-(11); Pregnenolone(12).

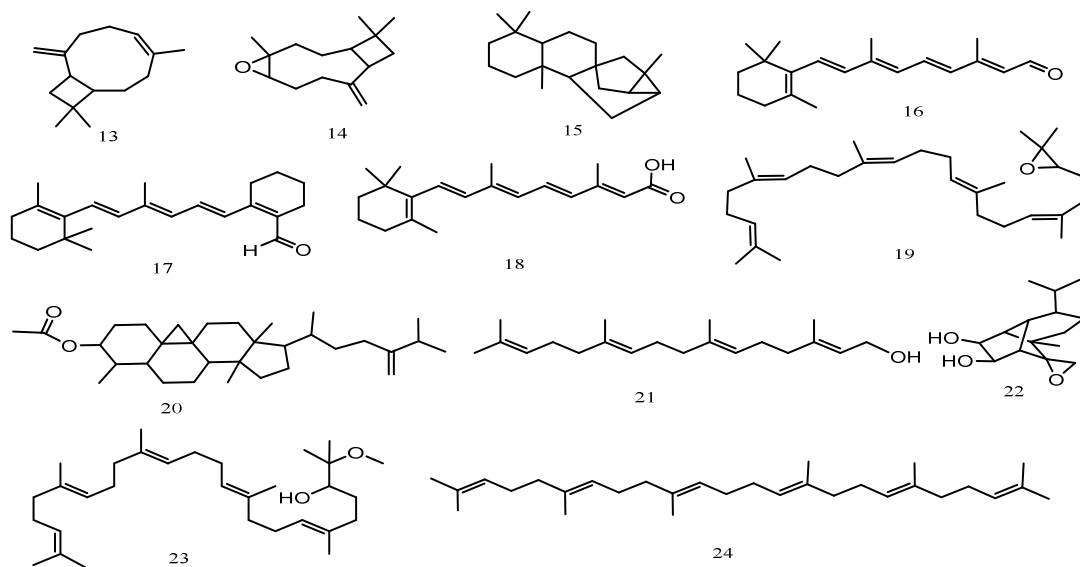


Fig 2: Terpenoids from *C. pulcherrima* root oil: Caryophyllene(13); caryophyllene oxide(14); Trachylobane(15); Retinal, 9-cis-(16); 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde(17); Retinoic acid(18); Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)-(19); 9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3 β ,4 α ,5 α)-(20); Geranylgeraniol(21); Spiro[tricyclo[4.4.0.0(5,9)]decane-10,2'-oxirane], 1-methyl-4-isopropyl-7,8-dihydroxy-, (8S)-(22); Tetracos-2,6,10,14,18-pentaen-22-ol, 2,6,10,15,19,23-hexamethyl-23-methoxy-, alltrans(23); 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-(24).

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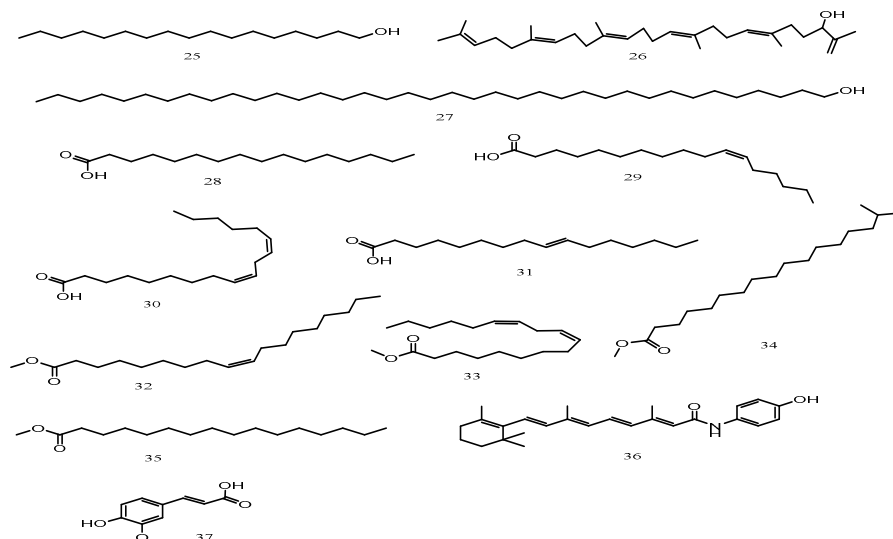


Fig 3: Fatty acids, alcohols and esters from *C. pulcherrima* root oil: n-Heptadecanol-1(**25**);1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)- (**26**);1-Heptatriacotanol (**27**); n-Hexadecanoic acid(**28**); cis-Vaccenic acid (**29**); 9,12-Octadecadienoic acid (**30**);9-Hexadecenoic acid (**31**);9-Octadecenoic acid (Z)-, methyl ester (**32**); 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (**33**); Heptadecanoic acid, 16-methyl-, methyl ester (**34**);Hexadecanoic acid, methyl ester(**35**); Fenretinide (**36**); 2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)- (**37**).

Conclusion: The present investigation has shown the cytotoxic effect of *C. pulcherrima* root oil. The identified compounds may serve as potential cytotoxic agents and may find usefulness in the control of insect pests if properly harnessed.

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