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

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ABSTRACT: Caesalpinia pulcherrima (Caesalpiniaceae) 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 Callosbruchus 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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Keywords: Caesalpinia pulcherrima, chemical constituents, cytotoxicity, insecticidal activity.

The genus Caesalpinia consists of more than 500 species belonging to the family Caesalpiniaceae. 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, antihemorrhagic, antipyretic, antioxidant and anticancer activities (Carvalho et al., 1996; Sudhakar et al., 2006; Kannur et al., 2006; Srinivasan et al., 2007; Saravananan 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 Caesalpiniaceae. 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 arboticient (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; Mahewswara et al., 2006; Pranithanchai et al., 2009; Das et al., 2009; Das et al., 2010; Yodsaoe 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. Ugbo 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,
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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 obtained 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.

**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:

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

**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, Culex*
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.

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

| Conc. (µg/mL) | No. of shrimps | No. of survivors | Percentage mortality | LC₅₀ (µg/mL) |
|---------------|----------------|------------------|----------------------|--------------|
|               | sample         | Std. drug        | sample               | Std. drug    | sample            | Std. drug |
| 10            | 30             | 19               | 3                    | 36.67        | 90                | 7.46      |
| 100           | 30             | 10               | 1                    | 66.67        | 96.67             | 23.85     |
| 1000          | 30             | 3                | 0                    | 90           | 100               |           |

Table 2: Insecticidal activity of C. pulcherrima root oil

| Name of insect     | Percentage mortality |
|--------------------|----------------------|
| Tribolium castaneum| Positive control 100  |
| Negative control   | 0                    |
| C. pulcherrima oil | 20                   |
| Sitophilus oryzae  | 100                  |
| Rhyzopertha dominica| 100                 |
| Callosobruchus analis| 100                 |
|                   | 0                    |
|                   | 0                    |
|                   | 40                   |
| 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     | 296 | 24.60    | 913      |
| Heptadecanoic acid, 16-methyl-, methyl ester     | C_{19}H_{38}O     | 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     | 282 | 25.40    | 879      |
| Retinal, 9-cis-                                   | C_{20}H_{32}O     | 284 | 25.69    | 764      |
| 9-Hexadecenoic acid                              | C_{18}H_{32}O     | 254 | 26.98    | 785      |
| Mibolerone                                        | C_{20}H_{32}O     | 302 | 28.44    | 724      |
| 2-[(4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-tri-enyl)cyclohex-1-en-1-carboxaldehyde] | C_{23}H_{32}O | 324 | 29.09    | 772      |
| Retinoic acid                                     | C_{20}H_{32}O     | 300 | 29.56    | 771      |
| 1-Heptatriacotanol                                | C_{25}H_{32}O     | 536 | 29.70    | 847      |
| Oxymesterone                                      | C_{20}H_{36}O     | 318 | 30.10    | 712      |
| 6β-Hydroxymethandienone                          | C_{20}H_{36}O     | 316 | 30.33    | 714      |
| Fenretinide                                       | C_{26}H_{32}NO_{2} | 391 | 30.35    | 779      |
| 2,6,10,14,18,22-Tetracosahexaen-2,6,10,15,19,23-hexamethyly-(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-hydroxycosapentaenyl)-(all-E)-| C_{23}H_{32}O_{3} | 426 | 32.69    | 875      |
| 1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethylly-(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_{22}H_{32}O_{3} | 468 | 34.35    | 871      |
| Geranylgeraniol                                   | C_{20}H_{34}O     | 290 | 34.45    | 856      |
| Tetracosa-2,6,10,14,18-pentadeca-22-o1, 2,6,10,15,19,23-hexamethyl-23-methoxyly-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-cyclopent[a]phenanthren-5-yl ester | C_{23}H_{32}O_{3} | 358 | 37.58    | 723      |
| β-Sitosterol                                       | C_{25}H_{34}O     | 414 | 38.32    | 900      |
| Dihydrotestosterone 3-formate-17-benzoate        | C_{27}H_{34}O_{4} | 422 | 38.56    | 730      |
| Fluoxymesterone                                   | C_{20}H_{28}O_{3} | 316 | 41.16    | 750      |
| 6β-Hydroxymethandienone                           | C_{20}H_{36}O     | 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-(acetoxy)-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-dihydroxyyl-(8S)- | C_{15}H_{24}O_{3} | 252 | 48.72    | 752      |
| Pregnenolone                                       | C_{21}H_{32}O_{2} | 316 | 51.96    | 725      |
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Fig 1: Steroids identified from *C. pulcherrima* root oil: Mibolerone(1); 6β-Hydroxymethandienone(2); Oxymesterone(3); 6β-Hydroxymethandienone(4); 3,17-dihydroxy-,(3β,5β)-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-diepopy-(3β,5α,6α,16α)-(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, (3β,4α,5α)-(20); Geranylgeraniol(21); Spiro[tricyclo[4.4.0.0(5,9)]decane-1,4,6,7,9,10,12,13-hexadecahexaene, 2,6,10,15,19,23-hexamethyl-23-methoxy-, alltrans(23); 2,6,10,14,18,22-Tetracosa-3,7,11,15,19,23-hexamethyl-23-methoxy-, alltrans(23); 2,6,10,14,18,22-Tetracosa-3,7,11,15,19,23-hexamethyl-23-methoxy-, alltrans(23); 2,6,10,14,18,22-Tetracosa-3,7,11,15,19,23-hexamethyl-23-methoxy-, alltrans(23); 2,6,10,14,18,22-Tetracosa-3,7,11,15,19,23-hexamethyl-23-methoxy-, alltrans(23); 2,6,10,14,18,22-Tetracosa-3,7,11,15,19,23-hexamethyl-23-methoxy-, alltrans(23).
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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-hexamethyldocosahexaen-3-ol, (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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