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# Efficacy of karanjin and phorbol ester fraction against termites (*Odontotermes obesus*)

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ABSTRACT

The non-edible oil seeds of *Jatropha curcas* (physic nut) and *Pongamia pinnata* (karanja) contain some toxic components (phorbol esters in *J. curcas* and karanjin in *P. pinnata*), which may be used as biopesticides. In this study, the active components of *J. curcas* and *P. pinnata* oil were extracted and their efficacy against the termites *Odontotermes obesus* (Rambur), was tested. The phorbol ester fraction of *J. curcas* and karanjin of *P. pinnata* oil were found to be effective against termites. A mortality rate of 100% was achieved in 6 h with karanjin and in 12 h with phorbol ester fraction. The $LC_{50}$ levels of karanjin and phorbol esters fractions were 0.038 and 0.071 g $ml^{-1}$, respectively, after 24 h at a 95% (0.05) confidence limit.



## 1. Introduction

Termites are a serious menace to both plants and wooden structures. These are the problematic pests threatening agriculture and urban environment. They cause significant losses to annual and perennial crops and damage to wooden components in buildings, especially in the semi-arid and sub-humid tropics (Verma et al., 2009). Chemical control is the only method of termite control so far. The active component of botanicals having anti-termitic properties can be extracted to prepare potent biopesticidal formulations.

Among various non-edible oil seed crops, *Jatropha curcas* and *Pongamia pinnata* are widely used for biodiesel production. They can be cultivated on any type of soil and have a low moisture demand. *P. pinnata*, commonly known as karanja, is a forest tree belonging to the family Leguminosae. It grows abundantly along the coasts and riverbanks in Myanmar and in all parts of India, particularly in Tamil Nadu, Andhra Pradesh, and Karnataka. It possesses applications in agriculture and environmental management. The various parts of the *P. pinnata* tree have been used for the treatment of tumors, skin diseases, abscesses, painful rheumatic joints, wounds, ulcers, diarrhea, etc. (Shoba and Thomas, 2001; Meera et al., 2003). The seed contains 27–39% oil, 20–30% protein, and a group of furano-flavonoids that constitutes 5–6% by weight of the oil (Bringi, 1987). The major furano-flavonoids present in the seeds are karanjin (1.25%) and pongamol (<1%). Karanjin is 3-methoxy furanoflavone and pongamol is furanodiketone. The insecticidal, nematicidal, antifungal, antibacterial, and antiviral activities of *P. pinnata* have been widely tested (Elanchezhiyan et al., 1993; Baswa et al., 2001; Simin et al., 2002; Kesari et al., 2010). Karanja oil and karanjin have been reported to contain piscicidal (Rangaswamy and Seshadri, 1941) and insecticidal properties against insects such as cockroaches, aphids, houseflies, and nematodes (Osmani and Naidu, 1956; Singh, 1966; Parmar, 1969; Parmar and Gulati, 1969; Mishra and Prasad, 1973).

*J. curcas*, a tropical plant belonging to the family Euphorbiaceae, is cultivated mainly as a hedge plant in many Asian and African countries. It is a multipurpose, drought-resistant, perennial plant that is an important source for the production of biodiesel (Kumar and Sharma, 2008). The seed contains approximately 24.6% crude protein, 47.3% crude fat, and 5.54% moisture content (Akintayo, 2004). However, despite their advantages, the seeds are reported to have anti-nutritional factors such as phorbol esters, saponin, phytate, trypsin inhibitor, and cyanogenic glucosides (Makkar et al., 1997; Rakshit et al., 2008). Extracts from *J. curcas* seeds and leaves possess molluscicidal, insecticidal, and fungicidal properties (Nwosu and Okafor, 1995; Liu et al., 1997; Solsoloy and Solsoloy,

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1997). The high concentration of phorbol esters present in *J. curcas* seeds has been identified as the main component responsible for the toxicity (Adolf et al., 1984; Makkar et al., 1997).

The purpose of this study was to extract and identify the phorbol esters fraction from Jatropha and the karanjin from karanja and to test their efficacy against termites.

## 2. Materials and methods

### 2.1. Procurement of sample

The *J. curcas* and *P. pinnata* seeds were collected from Gujarat, India, and the IIT Delhi campus, India, respectively. The samples were cleaned manually to remove all foreign materials. The seeds were initially dried in the sun for two days and then in a hot-air oven at 80 °C. The samples were weighed after cooling in a dessicator repeatedly until constant weight was attained after 24 h.

### 2.2. Extraction of oil from seeds

Seed kernels (50 g) of both karanja and jatropha were ground separately for 1 min and sieved through a 2-mm sieve. For oil content determination (AOAC, 1984) the ground kernels were extracted in soxhlet apparatus using petroleum ether (boiling point 60–80 °C). The extract was concentrated by rotary evaporator and the oil was cooled and weighed.

### 2.3. Extraction of toxic fraction and estimation of phorbol ester by HPLC

Extraction of the phorbol esters fraction (Gandhi et al., 1995) from *J. curcas* oil was done by the solvent–solvent extraction method. The seed oil was extracted in a methanol–water (9:1) mixture using a separating funnel. The combined methanol–water layer was evaporated in a rotary evaporator. A viscous oily fraction was separated from the aqueous solution after concentration. It was again extracted with diethyl ether. The combined ether layer was washed with water and evaporated. A brown viscous mass was obtained and thin layer chromatography (TLC) of that fraction was done for qualitative determination of toxic constituents. The phorbol esters fraction of oil was dissolved in tetrahydrofuran (THF) for determination of phorbol esters by HPLC.

The phorbol esters fraction of *J. curcas* seed oil was analyzed with a high-performance liquid chromatography (Waters 600 HPLC) system equipped with a reverse phase $C_{18}$ column (Waters Spherisorb, 5 μm, 250 mm × 4 mm i.d.), a Waters 2998 photodiode array detector, a Waters 600 HPLC quaternary pump, a Waters inline degasser, and Empower software. The column temperature was controlled at 25 °C and the flow rate was 1.3 ml $min^{-1}$. The solvents used were 1.75 ml *O*-phosphoric acid (85%) in 1 L distilled water (A) and acetonitrile (B). All solvents were filtered and degassed by a Waters inline degasser. The gradient used was as follows: 0–10 min, 60% A and 40% B; 10–40 min, 50% A and 50% B; 40–55 min, 25% A and 75% B; 55–60 min, 100% B and then the column was adjusted to the starting condition (60% A and 40% B). The peaks were integrated at 280 nm and the results were expressed as equivalent to phorbol-12-myristate 13-acetate (Sigma Chemicals), which appeared at 51 min. Each analysis was conducted in triplicate.

### 2.4. Extraction of karanjin from P. pinnata

The crude karanjin fraction was extracted from *P. pinnata* oil by the solvent–solvent extraction method as described by Gandhi and Cherian (2000). One hundred grams of the oil was dissolved in 100 ml of petroleum ether (40–60 °C) and extracted repeatedly with 100 ml of aqueous methanol (methanol–water 9:1 v/v) until the alcoholic layer was colorless. The combined methanol–water layer was concentrated in a rotary evaporator and a viscous paste was obtained. The aqueous methanolic extract of *P. pinnata* oil was purified by column chromatography. The adsorbent silica gel of 100–200 mesh was packed in the glass column by preparing the slurry in hexane. The slurry of extract mixed with silica gel was placed above the adsorbent and the column was initially eluted in n-hexane and the polarity of the solvent was increased by adding ethyl acetate. The eluents were collected at frequent intervals and the purity was checked by TLC. The fraction obtained from column chromatography containing karanjin was crystallized in acetone and pure crystals were obtained by vacuum filtration and repeated washing with cold acetone (stored at 4 °C). The compound was identified by physical characteristics and spectral data. The $^1$H NMR (nuclear magnetic resonance) spectra of the compound were carried out on a Brucker 300-MHz instrument (Brucker DPX 300, Rheinstetten, Germany).

### 2.5. Toxicological studies on termites

#### 2.5.1. Test insect

Adult workers of the subterranean termite *Odontotermes obesus* were collected from the Indian Institute of Technology, Delhi campus,

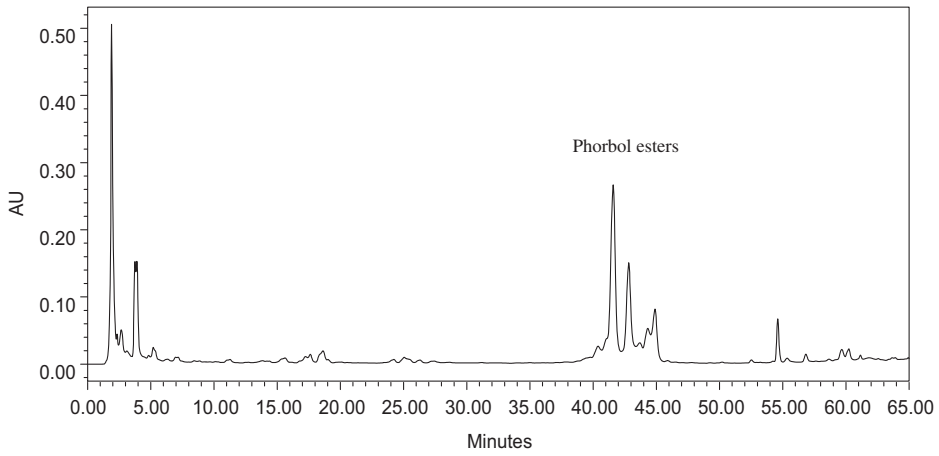


**Fig. 1.** The figure represents the HPLC chromatogram of phorbol esters. The phorbol esters (Four peaks) appeared between retention time ($t_R$) 40–46 min. The estimation of phorbol ester content was done by using Waters 600 HPLC system equipped with a reverse phase $C_{18}$ column (Waters Spherisorb, 5 μm, 250 mm × 4 mm i.d.), a photodiode array detector. The column temperature was kept at 25 °C and the flow rate was 1.3 ml $min^{-1}$. The solvents used were 1.75 ml *O*-phosphoric acid (85% v/v) in 1 L distilled water (A) and acetonitrile (B).