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# GC-MS analysis and biopesticide properties of different crude extracts of *Annona squamosa* and *Annona muricata*

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**Abstract** Most common compounds identified by GC-MS analysis of ethanolic extracts from leaves of *Annona squamosa* (sweetsop) and *Annona muricata* (soursop) were neophytadiene, hexadecanoic acid, and trans-caryophyllene. Hexadecenoic acid and ethyl oleate were identified in seeds of *A. muricata*, while only methyl-p-tert-butyl phenylacetate was identified in seeds extract of *A. squamosa*. Crude extracts were tested for their antifungal activities against postharvest plant pathogens, insecticidal activities against brown planthopper, *Nilaparvata lugens* (Stål) and cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero. Results showed low inhibitory effect (less than 20%) for the pathogenic fungi insecticidal test against cassava mealybug of all crude brown planthopper treated with 5% seed extract of *A. squamosa*, indicating its potential as an alternative botanical insecticide. Further studies are required to improve yield and extract quality.

**Keywords:** secondary metabolite; soursop; sweetsop; insecticidal activity; antifungal activity

## Introduction

Botanical insecticides are alternatives to synthetic chemicals and can be safely used to control insect pest populations without harmful effects on the environment and users. Unlike synthetic insecticides, natural compounds are safe for natural enemies of insect pests (Isman, 2006). Annona is a genus of the tropical Annonaceae family, consisting of 119 species. *Annona squamosa* and *A. muricata*, commonly known as sweetsop and soursop, are cultivated for consumption and medical purposes. Crude extracts from seeds, leaves, and fruits of both species have been tested for their biological activities. Many studies have reported diverse biological activities against pest insects including

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feeding deterrence, toxicity, growth inhibition, repellency, and oviposition deterrence (Begum *et al*., 2010; Dhembare *et al*., 2011; Kamaraj *et al*., 2011; Mondal *et al*., 2018). Here, crude extracts of different plant parts of *A. squamosa* and *A. muricata* were screened for chemical composition, and insecticidal effects on brown planthopper and cassava mealybug were evaluated.

## Materials and Methods

### *Preparation of crude extracts*

Plant samples of *A. squamosa* and *A. muricata* were collected from Nakhon Ratchasima and Chumphon Provinces. Fresh leaves, air-dried leaves, and seeds of the two species were subjected to solid-liquid extraction using ethyl alcohol. The samples were soaked with 95% ethyl alcohol (1:10 w/v) for 3 days and then filtered using Whatman #4 filter paper. The extraction was repeated three times. These extracts were dried using a rotary evaporator and then vacuum freeze-dried. Dried samples were kept at -20 $^{o}$C until required for use.

### *Gas Chromatography-Mass Spectrometry (GC-MS) analysis*

Crude extracts from leaves and seeds were subjected to phytochemical screening by GC-MS performed using an Agilent GC model 6890N with mass detector Agilent model 5973. Bioactive compounds were identified by comparing mass spectra with the spectra of known compounds in the spectral database, Wiley7n.

### *Insect rearing*

Adult brown planthopper, *Nilaparvata lugens* (Stål) specimens were collected from a rice field in Suphan Buri Province. Brown planthopper population was cultured in a rearing cage with rice seedlings in the Laboratory of Entomology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok. Second and third generation adults were used to determine the insecticidal activity of plant crude extracts.

Cassava mealybugs (*Phenacoccus manihoti* Matile-Ferrero) caught in a cassava field in Ladkrabang district of Bangkok were identified and reared in the Laboratory of Entomology, Faculty of Agricultural Technology, KMITL. For mass rearing of cassava mealybugs, an acrylic rearing cage containing fresh

pumpkins and okras was used. Third nymphal stages were subjected to concentration-mortality bioassays.

### *Insecticidal assay*

To evaluate the insecticidal activity of crude extracts of *A. squamosa* and *A. muricata*, 25 adults brown planthopper were introduced to each Petri disc containing a Whatman #1 filter paper soaked with 1 mL of crude extracts at concentrations of 0, 1, 2, 3, 4, and 5%. The number of dead individuals was recorded at 6 and 12 hours post-treatment. For the mortality test, the 3rd instar nymphs were subjected to an oral toxicity test using the leaf dipping method. Fresh okra was dipped in 0, 1, 2, 3, 4, 5, and 6% crude extracts for 1 min and air-dried at room temperature. Each treated okra was transferred to a moist paper disc in a cylindrical screen cage and 20 nymphs were released into the cage. The number of dead nymphs was recorded 24 hours after treatment and mortality percentage was calculated.

The experiment was arranged based on a completely randomized design (CRD) with three replicates and diverse concentrations of plant extract.

## Results

### *Phytochemical screening*

Results of GC-MS analysis of ethanol extracts of *A. squamosa* are given in Tables 1- 3. A total of 10 and 18 compounds were identified from fresh and dry leaves, respectively. In fresh leaves, common compounds were phytol isomer with peak area 48.72%, followed by neophytadiene with peak area 9.31% and germacrene-D with peak area of 5.38%. Analysis of dry leaves recorded major compounds as 2-hexadecen-1-ol, 3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]] with peak area 25.74%, neophytadiene with peak area 21.09%, and germacrene-D with peak area of 6.84%. GC-MS analysis showed that in *A. squamosa* leaves, more compounds were present in dry leaves. Methyl p-tert-butylphenylacetate was the only compound detected in the ethanol extract of seeds of *A. squamosa* (Table 3).

GC-MS analysis of *A. muricata* identified 10, 13, and 2 components in ethanol extracts of fresh leaves, air-dried leaves, and seeds, respectively (Tables 4-6). Hexadecanoic acid ethyl ester and ethyl oleate were common in all extracts of *A. muricata*. Major chemical compounds in fresh leaf extracts were phytol, neophytadiene, and 9,12,15-octadecatrienoic acid with area peaks of 23.16%, 19.22%, and 9.79%, respectively. Important secondary metabolites in

air-dried leaves were hexadecenoic acid, trimethylsilyl ester (19.23%), silane(3,7,1[1,15-tetramethyl-2-hexadecenyl)oxy]trimethyl (18.35%), and phytol isomer (13.92%).

***Insecticidal activity of crude ethanolic extracts***

The filter paper contact technique was used to evaluate insecticide efficacy against brown planthopper. Our results showed that treatment with 5% seed crude extract of *A. squamosa* had a mortality rate of over 50% at 12 hours after treatment, while others demonstrated low or no insecticidal activities (Figures 1-2). Oral toxicity test of botanical extracts *of A. squamosa* and *A. muricata* against cassava mealybug demonstrated that percent mortality of cassava mealybug over a 24 hour period increased with the increment of the concentrations. However, all concentrations of all extracts showed activities < 50 % (Figures 3 and 4).

**Table 1.** GC-MS analysis of ethanol extract from fresh leaves of *Annona squamosa*

| Name of the compounds | Retention time (min) | Peak area (%) | % Match |
|---|---|---|---|
| TRANS(BETA)-CARYOPHYLLENE | 38.52 | 3.64 | 96 |
| GERMACRENE-D | 42.30 | 5.38 | 98 |
| bicyclogermacrene | 43.18 | 1.77 | 95 |
| Cycloheptane,4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl- | 43.85 | 1.04 | 91 |
| Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-,(1.alpha.,4a.alpha,8a.alpha.)- | 51.69 | 3.49 | 80 |
| NEOPHYTADIENE | 62.48 | 9.31 | 96 |
| Hexadecanoic acid, ethyl ester | 70.24 | 2.47 | 94 |
| 3,7,11,15-Tetramethylhexadeca-1,6,10,14-tetraen-3-ol | 71.32 | 3.28 | 83 |
| PHYTOL ISOMER | 75.36 | 48.72 | 95 |
| ETHYL LINLEOLATE | 77.87 | 3.79 | 95 |

**Table 2.** GC-MS analysis of ethanol extract from air-dried leaves of *Annona squamosa*

| Name of the compounds | Retention time (min) | Peak area (%) | % Match |
|---|---|---|---|
| trans-caryophyllene | 38.52 | 3.28 | 99 |
| GERMACRENE-D | 42.30 | 6.84 | 98 |
| bicyclogermacrene | 43.17 | 2.70 | 97 |
| Bicyclo[5.3.0]decane,2-methylene-5-(1-methylvinyl)-8-methyl- | 43.83 | 1.47 | 93 |
| Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-,(1.alpha.,4a.alpha,8a.alpha.)- | 44.28 | 1.21 | 90 |
| 1,3-Cyclohexadiene,w1-methyl-4-(1-methylethyl)- | 44.95 | 1.14 | 97 |
| Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-,(1.alpha.,4a.alpha,8a.alpha.)- | 51.69 | 4.48 | 86 |
| Heptadec-8-ene | 54.08 | 1.41 | 96 |
| NEOPHYTADIENE | 62.50 | 21.09 | 99 |
| 3,7,11,15-Tetramethyl-2-hexadacen-1-ol | 64.61 | 5.91 | 86 |
| Hexadecanoic acid | 68.74 | 2.59 | 98 |
| Hexadecanoic acid,ethyl ester | 70.23 | 2.30 | 99 |
| GERANYL LINALOOL ISOMER | 71.32 | 8.77 | 96 |
| 2-Hexadacen-1-ol,3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]- | 75.36 | 25.74 | 91 |
| 9,12-Octadecadienoic acid,ethyl ester | 77.63 | 1.03 | 87 |
| 9,12,15-Octadecadienoic acid,ethyl ester,(Z,Z,Z)- | 77.87 | 3.77 | 99 |
| Octadecadienoic acid,ethyl ester | 79.28 | 1.23 | 98 |
| 2-Propenoic acid,3-(4-methoxyphenyl)-,2-ethylhexyl ester | 84.04 | 1.20 | 96 |

**Table 3.** GC-MS analysis of ethanol extract from seeds of *Annona squamosa*

| Name of compounds | Retention Time (min) | Peak area (%) | % Match |
|---|---|---|---|
| METHYL-P-TERT-BUTYL PHENYL ACETATE | 44.31 | 17.56 | 86 |

**Table 4.** GC-MS analysis of ethanol extract from fresh leaves of *Annona muricata*

| Name of the compounds | Retention time (min) | Peak area (%) | % Match |
|---|---|---|---|
| trans-Caryophyllene | 38.51 | 1.33 | 98 |
| Phenol,bis(1,1-dimethylethyl)- | 44.26 | 5.02 | 93 |
| Delta-Cadinene | 44.66 | 1.52 | 97 |
| (-)-Loliolide | 58.13 | 1.09 | 96 |
| NEOPHYTADIENE | 62.48 | 19.22 | 99 |
| Hexadecanoic acid, ethyl ester | 70.23 | 5.47 | 99 |
| PHYTOL ISOMER | 75.34 | 23.16 | 96 |
| Ethyl linoleate | 77.63 | 4.10 | 98 |
| 9,12,15-Octadecatrienoic acid,ethyl ester(z,z,z)- | 77.85 | 9.79 | 99 |
| Ethyl Oleate | 77.97 | 6.88 | 99 |

**Table 5.** GC-MS analysis of ethanol extract from air-dried leaves of *Annona muricata*

| Name of the compounds | Retention time (min) | Peak area (%) | % Match |
|---|---|---|---|
| TRANS(BETA)-CARYOPHYLLENE | 38.51 | 1.58 | 94 |
| Phenol,bis(1,1-dimethylethyl)- | 44.25 | 1.06 | 86 |
| Delta-Cadinene | 44.66 | 1.31 | 95 |
| NEOPHYTADIENE | 62.48 | 9.71 | 98 |
| Hexadecanoic acid, ethyl ester | 70.22 | 3.92 | 96 |
| Hexadecanoic acid, trimethylsilyl ester | 72.70 | 19.23 | 98 |
| PHYTOL ISOMER | 75.34 | 13.92 | 95 |
| Linoleic acid ethyl ester | 77.62 | 2.80 | 99 |
| 9,12,15-Octadecatrienoic acid,ethyl ester(z,z,z)- | 77.85 | 12.39 | 99 |
| Ethyl Oleate | 77.96 | 5.41 | 99 |
| Silane,(3,7,1[1,15-tetramethyl-2-hexadecenyl)oxy]trimethyl- | 78.37 | 18.35 | 91 |
| Octadecanoic acid,ethyl ester | 79.27 | 1.29 | 86 |
| Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester | 91.83 | 6.12 | 87 |

**Table 6.** GC-MS analysis of ethanol extract from seeds of *Annona muricata*

| Name of compounds | Retention Time (min) | Peak area (%) | % Match |
|---|---|---|---|
| Hexadecanoic acid,ethyl ester | 70.24 | 28.36 | 95 |
| Ethyl Oleate | 77.98 | 27.19 | 91 |

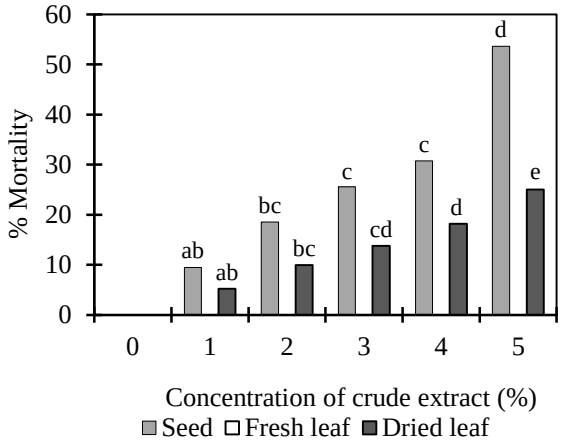


**Figure 1.** Insecticidal activity of *Annona squamosa* extracts against adults of brown planthopper, *Nilaparvata lugens* (Stål)

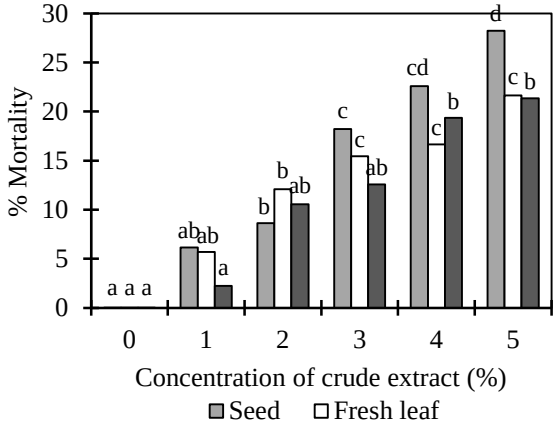


**Figure 2.** Insecticidal activity of *Annona muricata* extracts against adults of brown planthopper, *Nilaparvata lugens* (Stål)

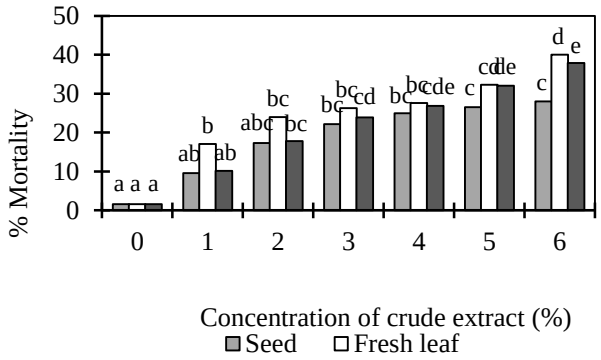


**Figure 3.** Insecticidal activity of *Annona squamosa* extracts against 3rd instar nymphs of cassava mealybugs (*Phenacoccus manihoti*)

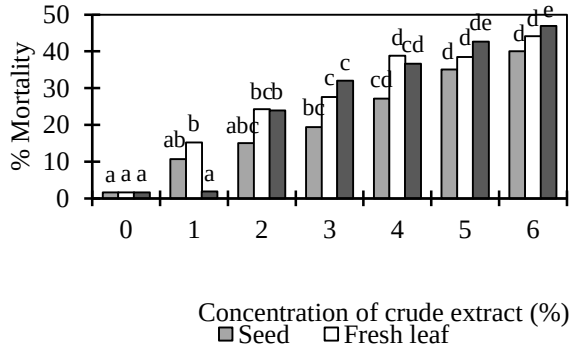


**Figure 4.** Insecticidal activity of *Annona muricata* extracts against 3rd instar nymphs of cassava mealybugs (*Phenacoccus manihoti*)

## Discussion

Phytochemical assays showed that fresh leaf extract contents of the two species were dominated by phytol, with common compounds in fresh and air-dried leaves as neophytadiene, hexadecanoic acid, and trans-caryophyllene. Neophytadiene, an enzyme inhibitor in the sequiterpene class, is one of the major compounds in marigold that contributes to repellent activity against insect pests (Laosinwattana *et al.*, 2018). Hexadecenoic acid, a monounsaturated fatty acid detected in many botanical extracts, has been reported to have insect attraction and oviposition enhancing activities (Tewari *et al.*, 2015; Ong and Jaal, 2015), while trans-caryophyllene, a sequiterpene compound found in essential oils of many medicinal plants, has been reported to have many pharmacological effects (Astani *et al*., 2011; Chavan *et al*., 2010)

along with mosquito repellent activity (de Paula *et al*., 2003). Results of GC-MS analysis indicated that chemical compositions of ethanol crude extracts of different plant parts from the same species showed diverse chemical patterns. Isolation and biological activity analysis of an individual phytochemical compound can identify the responsible bioactive ingredients; however, isolation of a single component from a mixture of complex plant crude extracts is a difficult task and many components may show synergy in the efficacy.

Our results of biological insecticidal activities revealed the possibility of using the seed extract of *A. squamosa* as a biological insecticide. Findings concurred with a previous study showing that seed oil of *A. squamosa* had insecticidal properties against the leaf hopper, *Nephotettix virescens* (Hemiptera: Cicadellidae) and reduced transmission of rice tungro virus (Mariapan *et al*., 1988). However, biological insecticidal properties could be improved by methanol extraction, as methanol extracts tend to achieve higher yields and show greater biological activities (Ghasemzadeh and Jaafar, 2011; Kulkarmi, 2017). Previous investigations also reported that seed extracts of *A. squamosa* and *A. muricata* showed promising insecticidal activities including larval toxicity and growth inhibitor properties against mosquitos (Harivelo *et al*., 2014) and houseflies (Begum *et al*., 2011). In our study, only a mortality test was performed. Previous investigations reported other bioactivities of plant crude extracts from the Annonaceae including repellent activities against pests of cereal products (Ukeh *et al*., 2012) and growth inhibition of bruchid beetle (Konkala *et al*., 2012).

From our results, we conclude that the seed ethanol extract of *A. squamosa* is a promising candidate as a botanical compound to control brown planthopper. However, extraction method modification for a better yield and other biological properties including ovicidal, oviposition-deterrent and repellent activities require further investigation.

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