Ethylacetate Extract Of Annona Squamosa Seeds Containing Anti-Head Lice Activity

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ABSTRACT

The present study focused on the separation and identification of the active compounds against head lice from the ethyl acetate extract of *Annona squamosa* L seed. Chromatographic and spectroscopic techniques revealed that two major compounds of the hexane seed extract were oleic acid and triglyceride with one oleate ester. The yields of these compounds were 13.88% and 7.70% dry weight, respectively. The compounds were tested *in vitro* against head lice, comparing to the crude ethyl acetate extract of the seed. The triglyceride with one oleate ester and the crude ethyl acetate extract diluted with coconut oil 1:1. These compounds were found to kill all tested head lice in 10 and 31 minutes, respectively. The triglyceride ester can be used as a marker for quantitative analysis of the active compound for quality control of the raw material *A. squamosa* seed and its extract. This finding will be useful for quality assessment and the chemical stability of the anti-head lice preparation from this plant.

Keywords: *Annona Squamosa*, anti-head lice activity, silver-ion column chromatography.

Editor: Mueen Ahmed KK, Phcog.Net

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INTRODUCTION

Annona squamosa L. (Custard apple) is a plant belonging to the family Annonaceae. It is found in all parts of India especially in southern parts of India, Sugar apple, Sweet sop. The human head louse (*Pediculus humanuscapitis*) is a small insect causing a public health problem, especially in poor sanitary conditions. Farmers in Vietnam use seed oil to control rice leafhoppers and plant hoppers (1) .In Thailand, research has shown the anti-head lice activity of *A. squamosa*. Puapatanakul (6) reported that the extract of custard apple seeds in coconut oil at the ratio of 1:2 can kill 98% of head lice within two hours, while the leaf extract shows less potency. Gritsanapan *et al* (4) found that the petroleum ether extract of the leaves and seeds dissolved in coconut oil at a ratio of 1:1, kill 90% of head lice *in vitro* by 53 and 26 minutes, respectively. A 20% cream (oil/water) preparation of petroleum ether extract of custard apple seeds can kill 93% of head lice. Within 3 hours however, it is easier to control the quality and stability of the preparation by quantitative analysis of the active chemical components. The present study, therefore, is focused on the isolation and identification of the components in the seeds of *A.squamosa* having anti-head lice effect present.

MATERIALS AND METHODS

Preparation of plant seed crude powder:

A.squamosa seeds were collected from a village of Guntur district, Andhrapradesh, India in December 2008.Seeds were removed manually from these fruits, washed in water and dried in a hot air oven at 55°C for 24 hours. The dried seeds were ground in an electric mill.

Extraction and separation of major compounds:

The powdered seeds of *A. squamosa* (1 kg) were placed in soxhlet extractor. The solvent ethyl acetate is used

for successive extraction. Extraction was done for 48 hours until the solvent extracted have no color. After extraction the mixture was filtered and distillation was carried out with rotary evaporator to remove the solvent and evaporated in a hot water bath until a constant weight (280.5g) was obtained. The extract (75 g) was separated using silver-ion column chromatography with dichloromethane/methanol (90/10, v/v) was used as an eluent. Fifty milliliter fractions were collected and the fractions with the same silver-ion TLC pattern (hexane/ diethyl ether (90/10, v/v) were combined. The fractions containing two major spots (*Rf* 0.20 and 0.72) were eluted in 100% CH_2Cl_2 fractions.

To isolate pure compounds, the fractions containing major compounds were combined and concentrated. The mixture was further fractionated using silver-ion column chromatography. Isocratic elution by hexane: diethyl ether (90/10, v/v) was performed (approximately 25ml per fraction). The fractions with the same TLC pattern were combined to yield five fractions. The second and fourth fractions gave compound AS1 (38.7 g) and compound AS2 (22.6 g), respectively. Compounds AS1 and AS2 were purified to give pure compounds.

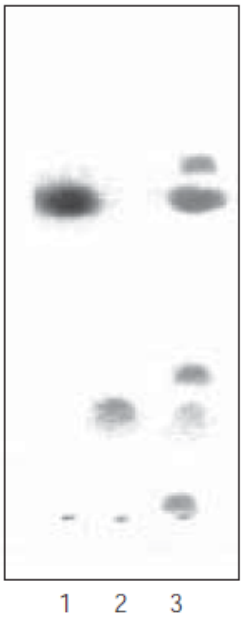
Testing for anti-head lice activity of pure compounds and crude extract:

The ethyl acetate extract and the two major pure compounds were tested for anti-head lice activity. The extract and pure compounds were separately dissolved in coconut oil at dilutions of 1:1to 1:8 (w:w). The same amount of each solution (0.05 ml) was put in a Petri dish and spread in a thin layer over a 2 cm^2 area. Seven equal sized head lice collected from school girls' hair were placed in the Petri dish containing solutions of the extract and the two major pure compounds. Non-moving head lice, which were determined as dead lice, were counted every 5 minutes until all the lice were dead. A commercial anti-head lice cream, Hexin, which is gamma benzene hexa chloride (1% w/w) and coconut oil were used as a positive and negative controls, respectively.

RESULTS

Compound AS1 was pale yellow oil, yielded 13.88% w/w of dried seeds. The silver-ion TLC hexane/diethyl ether (90/10, v/v) had an *Rf* value of 0.20 (Fig 1). The EI mass spectrum had a molecular ion peak at *m/z* 283.2 [M+1] and a prominent peak at *m/z* 264.3.

The IR spectrum of compound AS1 revealed absorption peaks at 3000-2930 (O-H stretch), 2850 (C-H stretch), and 1700 (C=O stretch, carboxylic) cm^{-1}.



1 = AS1, 2 = AS2, 3 = ethyl acetate crude extract.
Figure 1. Silver-Ion TLC chromatogram of AS1, AS2 and ethyl acetate crude extract

The ^1H NMR spectrum of compound AS1indicated the presence of one methyl proton at δ 0.90 (3H, t, H-18); a methylene proton group at δ 1.26 (20H, m, H-4-7 and H-12-17); two methylene proton groups at δ1.65 (2H, m, H-3);four methylene proton groups at δ 2.00 (4H, m,H-8, 11); two methylene proton groups at δ 2.35(2H, t, H-2); two olefinic methane proton group sat δ 5.35 (2H, m, H-9,10) and the broad peak of a hydroxyl proton at δ 10.15 (1H).

The ^{13}C NMR spectrum and Distortion less Enhancement by Polarization Transfer (DEPT) exhibited 16 carbon resonances, revealing the presence of thirteen methylene carbons, one methyl carbon, two olefinic methine carbons and one carbonyl carbon.

These spectral data suggested that compoundAS1 was a fatty acid. Comparing the NMR spectra of compound AS1 with Aldrich Library (1993) of 13C and 1H FT NMR spectra, confirmed the molecular structure of AS1 was an oleic acid (Fig 2).

Compound AS2 was also pale yellow oil, yielded 7.70% w/w of dried seeds. The *Rf* value(silver-ion TLC hexane/diethyl ether (90/10, v/v)) was 0.72 (Fig 1).The ^1H NMR spectrum looked similar to the AS1 spectrum, with additional signals at δ4.15 and 4.30.

The IR spectrum of compound AS2 showed bands at 2925 (C-H stretch) and 1746 (C=O stretch, ester) cm^{-1}.

After comparing the NMR spectra of compoundAS2 with Aldrich Library (1913) of ^{13}C and^1H FT NMR spectra, compound AS2 was felt to be a triglyceride with one oleate ester (Fig 2).

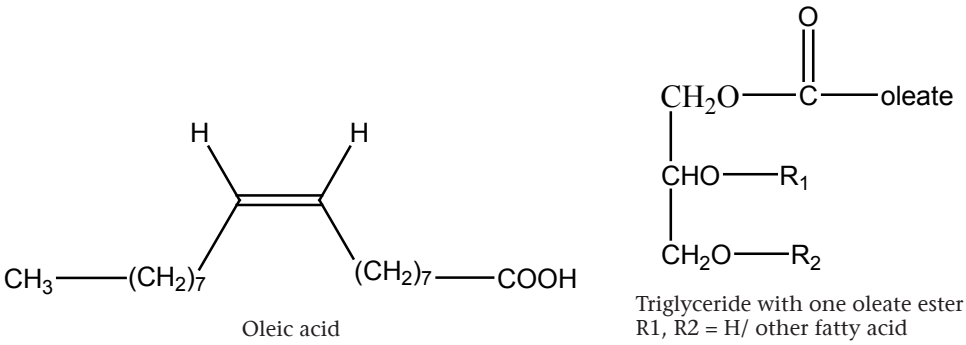


Figure 2. Structures of separated compounds from *Annona squamosa* seed

Test sample	Dilution (w:w)	Killing time (min)
Ethyl acetate crude	1:1	31.67±4.04
extract	1:2	34.33±4.04
	1:4	41.00±3.61
	1:8	55.00±5.00
leic acid (AS1)	1:1	47.33±3.06
	1:2	54.67±5.51
	1:4	59.00±6.56
	1:8	61.33±4.16
Triglyceride with one		
oleate ester (AS2)	1:1	10.00±1.00
	1:2	12.00±2.00
	1:4	16.00±1.00
	1:8	22.33±2.52
Coconut oil (-ve control)	Not diluted	>180
Hexin (+ve control)	Not diluted	>180

The results of anti-lice activity of ethyl acetate extract of seed of *Annona squamosa* is given

DISCUSSION

The purified form of ethyl acetate which contains Triglyceride with one oleate ester (AS2) shows maximum killing effect of lice at 1:8 (w:w) dilution with coconut oil with in less time , 22.33±2.52.The purified form of ethyl acetate crude extract in coconut oil (1:1) was significantly more active against head lice than gamma benzene hexachloride 1% cream and the hexane crude extract. These data are supported by previous reports of Gritsanapan *et al* (4). This result is useful for the standardization of *Annona squamosa* seed and its extract. The active compound may be used for the qualitative assessment of the chemical stability of the custard apple cream preparation.

ACKNOWLEDGEMENTS

We are thankful to my parents for providing financial support, to my management and HOD of pharmaceutical chemistry, Hindu college of pharmacy for providing necessary facilities for this project work.

REFERENCES

1. Brady N.C., Khush G.S. and Heinrichs E.A. (1978). Visit of the IRRI team to the Socialist Republic of Vietnam April 13-May 19. *IRRI Annual Report*: **49**.
2. Aldrich Chemical Co, Inc. *The Aldrich Library of 13Cand 1H FT NMR Spectra*. 1st ed, 1993.
3. Boonyaprapasara N., Chokchaicharorenporn O. and eds.Maipuenbaan Samunprai. Vol. **2**. Bangkok: Faculty of Pharmacy, Mahidol University, 1998: 454(in Thai).
4. Gritsanapan W., Somanabandhu A., Titirungruang C. and Lertchaiporn M.. A study on the anti-parasitic activities and chemical constituents of extracts from the leaves and seeds of custard apple (*Annona squamosa* Linn.). *Proceedings of Third NRCTJSPS Joint Seminar*, 1996: 209–15.
5. McCage C.M., Ward S.M., Paling C.A., Fisher D.A., Flynn P.J. and McLaughlin. Development of a paw paws herbal shampoo for the removal of head lice. *Phytomedicine* 2002; **9**: 743–8.
6. Puapatanakul O.. *Clinical studies of Annona squamosa seeds and leaves for the treatment of head lice*.1980: **37** pp (in Thai).
7. Tiangda C., Gritsanapan W., Sookvanichsilp N. and Limchalearn A.. Anti head-lice activity of *Annona squamosa* seed extract. *Southeast Asian Journal of Tropical Medicine and Public Health* 2000; **31**(suppl 1): 174–7.