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Phytochemical profiling and larvicidal activity of *Ananas Comosus* peel extracts against the mosquito vectors

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Abstract
The Bromeliaceae family includes the monocot perennial plant *Ananas comosus* (L.). It is a common fruit in India and is called pineapple. One of the most important fruit crops grown in India is the pineapple (*A. comosus* L.). Peels continue to be the principal byproduct and account for the majority of the fruits' weight. Fruits of *A. comosus* are mostly utilized to extract juice in industrial processes, which results in massive residues. These residues turn into garbage and cause significant environmental pollution if they are not further treated. Using an aqueous, chloroform, hexane, and methanol solvent system, the current work sought to extract the metabolites from discarded peels. The extracts' larvicidal effectiveness against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* larvae in their fourth instar was assessed. The findings demonstrated strong larvicidal action against each of the three mosquito vectors under investigation. Qualitative phytochemical profiling, TLC, and GC-MS were used to identify the phytocompounds in the extract. The findings of the phytochemical profiling showed that there were a lot of phytocompounds, including terpenoids, flavonoids, carbohydrates, and tannins. Therefore, *A. comosus*peel extracts have a lot of potential as a biocontrol agent against mosquito vectors. The identified bioactive phytochemical may soon be utilized as a source of a potent insecticide.

Keywords: *A. cosmosus*, larvicidal activity, phytochemical profiling, GC-MS analysis

Introduction
Around 2100 million people worldwide are at danger due to mosquito-borne diseases, which are endemic in more than 100 countries and kill almost two million people annually, including at least one million children (Muhammad *et al.*, 2017) [1]. Due to favorable ecological conditions, diseases spread by mosquitoes are endemic in India (Sumodan Elumalai *et al.*, 2016) [2]. About 553 million people in 17 endemic states and six Union territories in India are at risk of contracting the disease (Joshi, 2018) [3]. A greater percentage of health issues in poor nations are caused by mosquito-borne illnesses like viral encephalitis, dengue, filariasis, and malaria (Anoopkumar and Aneesh, 2022) [4]. Synthetic insecticides have been used repeatedly to control mosquitoes, which has upset natural biological control systems and caused mosquito populations to rebound. According to Dahmana *et al*. (2020) [5, 9], it also led to the development of resistance, unfavorable impacts on nontarget organisms, and concerns about the environment and human health.

In India and other countries, *Anopheles stephensi* is the vector of malaria in both rural and urban lowlands (Subbarao *et al.*, 2019) [6]. Widespread across the tropics and subtropics is *Aedes aegypti*, the main vector for the viruses that cause dengue fever, dengue hemorrhagic fever, chikungunya fever, and yellow fever. Around 120 million people globally are infected with *Culex quinquefasciatus*, a vector of lymphatic filariasis (Rai *et al.*, 2019) [7], and 44 million of them have common chronic manifestations (Gordon *et al.*, 2018) [8].

Because of the frequent use of synthetic insecticides to suppress mosquitoes, natural biological control systems have been disrupted, leading to a comeback in mosquito populations. The emergence of resistance (Dahmana and Mediannikov, 2020) [5, 9], adverse effects on organisms that are not the target, and increased environmental and human health concerns (Khan and Ahmad, 2019) [10] led to a search for alternative control methods. Since plants are believed to have a wealth of bioactive compounds, they could be a different source of mosquito repellents. Recently, efforts have been undertaken to use natural plant-based chemicals with insecticidal properties to manage a variety of insect pests and vectors (CE *et al.*, 2019) [11]. Bromelain and tacorin were found in the stem of *A. comosus* in earlier investigations by Zaman *et al*. (2016) [12] and Rahayu *et al*. (2017) [13], respectively. The stem also contained protein, amino acids, alkaloids, glucose, cardiac glycoside, flavonoids, saponin, and phytosterol (Helen *et al.*, 2019) [14]. The current study's objectives were to assess the extracts from the peels of *A. comosus*against the mosquito vectors *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus*. Additionally, the extracts' secondary metabolites were examined.

Materials and Methods
Selection and procurement of fruit peels
Healthy and disease-free fruits belonging to the family *Bromeliaceae*, were procured from Koyambedu market, Chennai, Tamil Nadu India. The fruit peels and agro waste selected for the study were *Ananas comosus (L.).* The Fruits and agro waste were identified and authenticated by Plant Anatomy Research Centre (PARC), Tambaram, Chennai, Tamil Nadu, India.

Fig 1: *A. comosus*fruit and its peels

Selection of mosquito species

The fourth instar larvae of *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* were the mosquito species chosen for this investigation (Fig). *Aedes aegypti* is widely distributed, extremely domesticated, and anthropophilic. The species Aedes is in charge of spreading the arbovirus that causes dengue and dengue hemorrhagic fever (Foster and Walker, 2019) [15]. Plasmodium falciparum, the causative agent of the most severe type of malaria, is one of the four parasites that can be spread by Anopheles species (Saif, 2017) [16]. Widely found in trophical locations, *C. quinquefasciatus* is a vector of Wuchereria species that cause lymphatic filariasis (Nchoutpouen *et al.*, 2019) [17].

Phytochemical screening

The sample underwent phytochemical screening in accordance with the guidelines provided by Senthil Kumar and Reetha (2009) and Nweze *et al*. (2004) [18]. Proteins, anthocyanins and beta cyanins, alkaloids, flavonoids, phytosterols, steroids, phenols, tannins, saponins, glycosides, and carbohydrates were all checked for in the peel extract samples.

TLC Plate preparation

After cutting the aluminum sheets covered with silica gel 60 F 254 to 1.5 x 5.5 cm, the produced methanol peel extract was placed on a silica plate and allowed to air dry.

Mobile phase preparation

The extracts displayed distinct bands and were standardized in the ratio of hexane to ethyl acetate to chloroform (2:1:1) (Luong *et al.*, 2021) [20].

GC-MS analysis

The GC-MS (Agilent 7890A-240 MS with Ion Trap) was used to analyze the FAME. A silica capillary column Agilent J&W, HP-5ms, measuring 30 m x 0.250 mm x 0.25 µm (Agilent Technologies), attached to MSD, was installed in the GC-MS. The mobile phase was nitrogen (35 ml/min), while the carrier gas was helium (1 ml/min). By comparing their MS with reference compounds from the NIST and Willey libraries, the individual constituents displayed by GC were identified.

Maintenance of larvae

Aedes ageypti, *Anopheles stephensi*, and *Culex quinquefasciatus* vector mosquitoes raised in a lab without exposure to pesticides or diseases were used in all experiments. Vector mosquito cyclic generations were kept in insectariums at 23–29°C. Larvae were fed a 3:1 mixture of larval diet, powdered dog biscuit, and yeast, while adult mosquitoes were fed a 10% glucose solution.

Larvicidal assay

For the larval susceptibility test, three trials against vector mosquitoes were conducted, each with five replicates. Separate tests for the crude extract's larvicidal properties were carried out using A. ageypti, *A. stephensi*, and *C. quinquefasciatus* larvae in their fourth instar. By dissolving 100 mg of crude extract in 1 ml of acetone and increasing the volume to 100 ml with distilled water, a stock solution (1000 ppm) of the extract was created. Twenty fourth-instar larvae were released from various dilutions of 25 ppm, 50 ppm, 75 ppm, 100 ppm, and 150 ppm made in 200 ml of deionized water, and mortality was measured after 24 hours. The beakers were maintained at 29°C ± 2°C in a temperature control room, and the larvae were exposed to 200 ml of water with 0.1 ml of acetone as a control. Five replications of each treatment were conducted (Tonk *et al.*, 2006).

Twenty larvae each were put in glass beakers with 200 milliliters of the peel extract solution. Parallel control tests were conducted without extract. After the larvae in each solution were exposed for 24 hours, the number of dead larvae was recorded. The percentage mortality was then computed using the average of five replicates, and Abbott's (1925) [22] calculation was used to account for mortality in the control.

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

Statistical analysis

The software created by Han *et al*. (2013) [25] was used to calculate chi-square values and perform probit analysis on the average larval mortality data in order to determine LC50, LC90 (Finney, 1971) [23], and other statistics at 95% confidence intervals of the upper and lower confidence limits (Busvine, 1971) [24]. Statistical significance was defined as results with $p < 0.05$ (SPSS, 11.5).

Results

Qualitative phytochemical analysis of *A. cosmosus*

Alkaloids, carbohydrates, saponins, glycosides, terpenoids, triterpenoids, and phenol were all found in high concentrations in the phytochemical properties of *A. comosus*preparations. The methanol extract contained sterods, cardiac glycosides, and tannin in modest amounts. However, the methanol extract lacked flavonoids, anthocyanins, quinone, and coumarins. Chloroform and hexane extracts likewise demonstrated a modest presence of the phytocompounds in comparison to the aqueous methanol extract (Table 1).

Table 1: Phytochemical screening of *A. comosus*plant extracts

S. No	Secondary metabolites	Aqueous	Chloroform	Methanol	Hexane
1	Carbohydrate	+++	++	+++	++
2	Tannins	+	-	++	-
3	Saponins	+++	++	+++	+++
4	Flavonoids	+	-	-	-
5	Alkaloids	+	-	+++	++
6	Anthocyanin	++	++	-	+
7	Quinones	-	-	-	-
8	Glycosides	++	+	+++	+
9	Cardiac glycosides	+	-	++	-
10	Terpenoids	+	++	+++	++
11	Triterpenoids	++	-	+++	-
12	Phenols	+	++	+++	++
13	Coumarins	++	-	-	+
14	Fatty acids	-	-	+	-
15	Protein	+	-	+	-
16	Steroids	++	+	++	+++

+++ strong presence ++ moderate presence + trace amount-absent

Larvicidal activity *A. comosus*peel extracts

The findings are shown in Tables 2, 3, and 4 and are based on a probit analysis of the concentrations of plant extract against the fourth instar larvae of *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* following a 24-hour exposure. When compared to its toxicity against *A. aegypti*, which had LC50 values of 50.500 ppm and LC90 values of 88.340 ppm, respectively, the methanol peel extracts of *A. comosus*were found to be more toxic against *C. quinquefasciatus* and *A. stephensi*, with LC50 values of 36.089 ppm and 47.892 ppm and LC90 values of 61.133 ppm and 95.716 ppm, respectively.

Table 2: Mosquito larvicidal activity of *A. comosus*peel *extracts* against the fourth instar larvae of *A. aegypti*

Extracts	Concentration (ppm)	24hr % Mortality	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	Chi-Sq
Aqueous	25 50 75 100 150	17 35 59 81 100	63.327 59.515±78.216	128.635 113.702±150.796	19.185
Chloroform	25 50 75 100 150	14 27 48 72 94	79.619 61.706±83.950	139.512 128.722±190.452	20.062
Hexane	25 50 75 100 150	07 16 37 61 89	82.795 76.399±90.054	184.993 160.177±224.285	15.637
Methanol	25 50 75 100 150	25 49 78 100 100	50.500 38.678±46.208	88.340 79.612±100.609	21.867

Control- nil mortality, Significant at $p < 0.05$ level, LC_{50} - Lethal concentration that kills 50% of the exposed larvae
LC_{90} – Lethal concentration that kills 90% of the exposed larvae UCL- Upper confidence limit; LCL- Lower confidence limit

Table 3: Mosquito larvicidal activity of *A. comosus*peel *extracts* against the fourth instar larvae of *A. stephensi*

Extracts	Concentration (ppm)	24hr % Mortality	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	Chi-Sq
Aqueous	25 50 75 100 150	19 37 64 87 100	52.537 47.990±57.091	117.639 104.601±136.661	18.140
Chloroform	25 50 75 100 150	13 28 57 73 100	64.069 58.967±69.419	142.537 125.734±167.697	24.181
Hexane	25 50 75 100 150	10 24 39 64 93	81.400 69.245±82.240	135.004 154.169±218.530	16.693

Methanol	25 50 75 100 150	22 47 70 92 100	47.892 40.852±48.828	95.716 86.023±109.421	12.728

Control- nil mortality, Significant at $p < 0.05$ level, LC_{50} - Lethal concentration that kills 50% of the exposed larvae
LC_{90} – Lethal concentration that kills 90% of the exposed larvae UCL- Upper confidence limit; LCL- Lower confidence limit

Table 4: Mosquito larvicidal activity of *A. comosus*peel *extracts* against the fourth instar larvae of *C. quinquefasciatus*

Extracts	Concentration (ppm)	24hr % Mortality	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	Chi-Sq
Aqueous	25 50 75 100 150	16 33 61 84 100	56.163 51.548±60.846	122.667 109.245±142.200	18.520
Chloroform	25 50 75 100 150	14 29 54 78 100	61.257 56.361±66.329	134.661 119.331±157.316	22.733
Hexane	25 50 75 100 150	09 15 36 62 90	81.916 75.439±89.297	148.793 141.862±229.087	21.537
Methanol	25 50 75 100 150	28 62 97 100 100	36.089 32.930±39.124	67.133 61.048±75.561	16.399

Control- nil mortality, Significant at $p < 0.05$ level, LC_{50} - Lethal concentration that kills 50% of the exposed larvae
LC_{90} – Lethal concentration that kills 90% of the exposed larvae UCL- Upper confidence limit; LCL- Lower confidence limit

In comparison to *C. quinquefasciatus* (56.163 ppm and 122.667 ppm) and *A. aegypti* (63.327 ppm and 128.635 ppm), the aqueous extract of *A. comosus*demonstrated LC50 and LC90 values of 52.537 ppm and 117.639 ppm against *A. stephensi*, respectively. When compared to methanol and aqueous peel extracts of A. cosmosus, the other studied extracts (hexane and chloroform) also shown mosquito larvicidal action at a comparatively high concentration. The methanol extracts underwent additional phytochemical profiling since they demonstrated strong larvicidal efficacy in comparison to the other extracts.

Thin Layer Chromatography analysis of methanol peel extract of *A. cosmosus*

With Rf values of 0.75, 0.71, 0.60, 0.52, 0.48, 0.46, 0.37, 0.32, 0.29, 0.24, and 0.19, the TLC of the methanolic extract of *A. comosus*revealed 11 major bands. These bands correspond to major compounds such as triterpenoids, steroids, phenolic compounds, glycosides, anthocyanin, saponin, alcohols, terpenoids, phenols, and alkaloids, respectively (Fig.2).

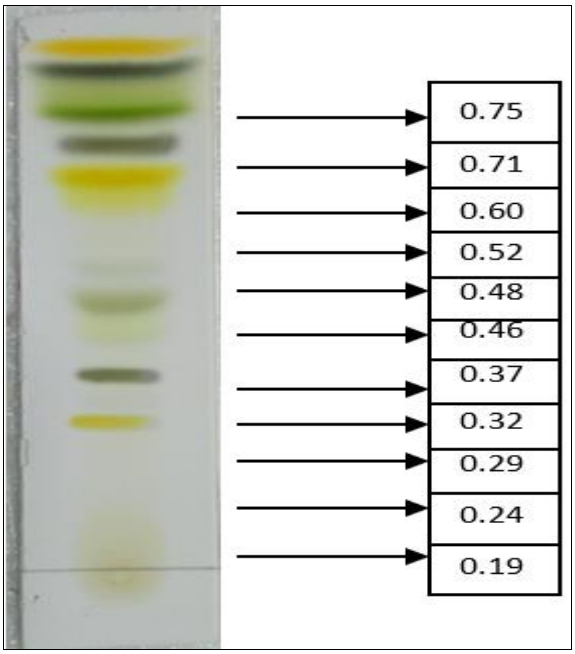

Fig 2: Thin layer chromatography analysis of methanol peel extract of *A. comosus*

GC-MS analysis of methanol peel extract of *A. cosmosus*

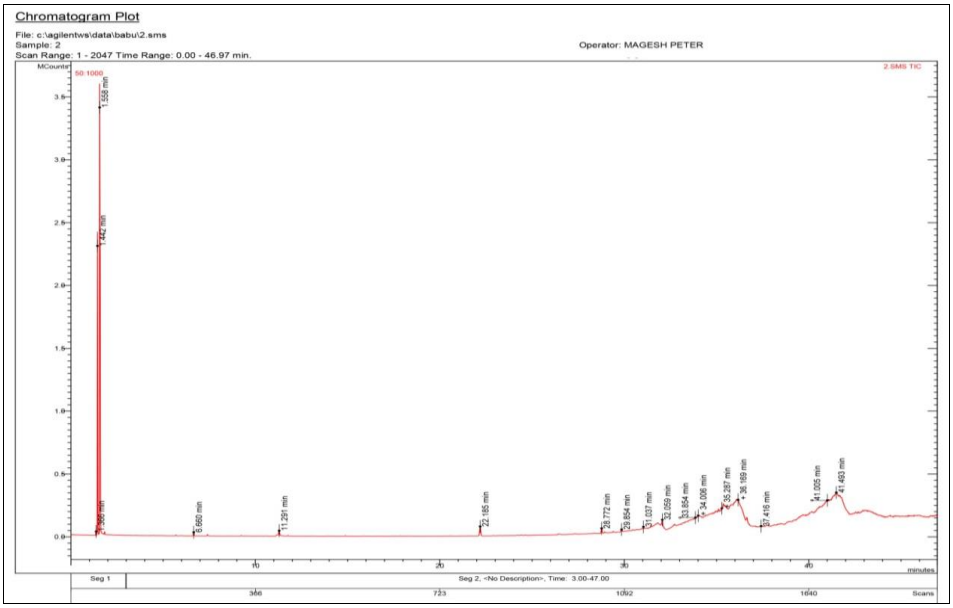

Fig 3: GC-MS analysis of methanol peel extract of *A. comosus*

The primary chemicals found in the methanol peel extracts of *A. comosus*are identified and their composition is displayed. By using GC MS, 17 chemicals were discovered. The primary substances were beta.The compound acetoxy-1',1'-dicar 3,7-Dimethyl-6,7-di(methylth, October 7th, October 2nd, 2,6-dimethyl- 5-Diethyl phthalate, hydroxymethylfurfural, neophytadiene, 3,7,11,15-Tetramethyl-2-hexa, 14-methyl, Oleic Acid, Hexadecanoic Acid, 18-Stearic anhydride, pentatriacontanone, 3,9-Epoxypregnane-11,14,18-t, 2-[(1-Hexadecylpyrrolidin-2-, 3,9.beta.:14,15-Diepoxypregnan, Octadecanoic acid, 1-[(tetra, n-Hexadecanoic acid, Tetracyclo[11.4.0.0(1,10).0 (Fig.3).

Table 5: GC-MS analysis of methanol peel extract of *A. comosus*

S. No		RT	Name of the compound	Peak Area (%)
1.	1.366	17.beta. -Acetoxy-1',1'-dicar	67657	
2.	1.558	3,7-Dimethyl-6,7-di (methylth	8.79	
3.	6.660	7-Octen-2-ol, 2,6-dimethyl-	74448	
4.	11.291	5-Hydroxymethylfurfural	125761	
5.	22.185	Diethyl Phthalate	292023	
6.	28.772	Neophytadiene	142701	
7.	29.854	3,7,11,15-Tetramethyl-2-hexa	69349	
8.	31.037	Hexadecanoic acid, 14-methyl	45415	
9	32.059	Oleic Acid	220268	
10	33.854	18-Pentatriacontanone	9264	
11	34.006	Stearic anhydride	39151	
12	34.394	3,9-Epoxypregnane-11,14,18-t	6152	
13	34.990	2- [(1-Hexadecylpyrrolidin-2-	27613	
14	35.155	3,9. beta.:14,15-Diepoxypregn	13304	
15	35.287	Octadecanoic acid, 1- [(tetra	52772	
16	36.641	n-Hexadecanoic acid	134800	
17	37.416	Tetracyclo [11.4.0.0(1,10).0(	4321	

Discussion

According to our tests, *A. comosus*methanol, hexane chloroform, and aqueous peel extracts shown increased larvicidal effectiveness against each of the three mosquito vectors under investigation. For the first time, the larvicidal effectiveness of *A. comosus*peel extracts has been investigated. The outcomes were consistent with Rodrigues's (2020) [26] observation. Marin *et al*. (2020) [27] tested the effectiveness of C. sinensis ethanolic peel extract against *A. aegypti* and *C. quinquefasciatus* and found that it was more effective against *A. aegypti*, with an LC50 value of 92.27 ppm, while the value reported against *C. quinquefasciatus* was 244.70 ppm. Our results are consistent with their findings.

The current study's findings are consistent with Mahalakshmi *et al*. (2018) [30]'s earlier studies. GC MS analysis of the *A. comosus*methanol extracts supports the findings of the phytochemical screening. The primary substances found were beta. -Acetoxy-1',1'-dicar, 3,7-Dimethyl-6,7-di (methylth, 7-Octen-2-ol, 2,6-dimethyl-, 5-Hydroxymethylfurfural, Diethyl Phthalate, Neophytadiene, 3,7,11,15-Tetramethyl-2-hexa, Hexadecanoic acid, 14-methyl, Oleic Acid. Kuppusamy *et al*. (2016) [31] used Parkia biglobosa seed extracts and reported similar findings. TLC and GC MS analysis verified the high concentration of tannins, saponins, phenols, quinones, triterpenoids, cardiac glycosides, alkaloids, and terpenoids found in the initial phytochemical examination of the methanol peel extracts of A. cosmosus. Major active components, including carbohydrates, tannins, glycosides, cardiac glycosides, terpenoids, etc., were identified through qualitative analysis of the aqueous extract of *A. comosus*peel.

Against the mosquito species under investigation, the fruit peels of *A. comosus*examined in this study had a low, moderate, or very strong powerful larvicidal action. According to Veni *et al*. (2017) [32], the extraction solvent may have an impact on the effectiveness of botanical extracts against mosquito developmental stages, and the polarity of the solvents may also have an impact on the extract's efficacy (Raveen *et al.,* 2017) [33]. When compared to other plant extracts, it was discovered that the methanol peel extracts of *A. comosus*had extremely strong larvicidal action at very low concentrations. This has made it possible to look into their effectiveness further.

Conclusion

Our research has unequivocally shown that *A. comosus*waste peels may be used as efficient mosquito control agents. In addition to helping to manage unmanageable waste outflow, turning wastes into useful products will also lessen pollutants and enhance the environmental profile of the fruit juice-processing sector.

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