International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2015; 2 (1): 01-08

Received: 22-09-2014
Accepted: 19-11-2014

K. Velu
Department of Zoology, Presidency College (Autonomous), Chennai 600 005, Tamil Nadu, India.

D. Elumalai
Department of Zoology, Presidency College (Autonomous), Chennai 600 005, Tamil Nadu, India.

P. Hemalatha
Department of Zoology, Presidency College (Autonomous), Chennai 600 005, Tamil Nadu, India.

M. Babu
Department of Zoology, Presidency College (Autonomous), Chennai 600 005, Tamil Nadu, India.

A. Janaki
Department of Zoology, Presidency College (Autonomous), Chennai 600 005, Tamil Nadu, India.

P. K. Kaleena
Associate Professor, Department of Zoology, Presidency College (Autonomous), Chennai-600 005, Tamilnadu, India

For Correspondence:
P. K. Kaleena
Associate Professor, Department of Zoology, Presidency-College (Autonomous), Chennai-600 005, Tamilnadu, India,

Phytochemical screening and larvicidal activity of peel extracts of *Arachis hypogaea* against chikungunya and malarial vectors

K. Velu, D. Elumalai, P. Hemalatha, M. Babu, A. Janaki, P. K. Kaleena

Abstract
Phytochemicals represent a rich resource for the discovery of novel pesticides that are effective, cheap and environmentally safe. The main targeted mosquito vectors *Aedes aegypti* and *Anopheles stephensi* cause serious human diseases. The chloroform, acetone, ethanol, methanol and aqueous peel extracts of *Arachis hypogaea* were studied for its larvicidal activity against the mosquito vectors. Phytochemical screening of the peel extracts showed the presence of bioactive compounds such as tannins, saponin, flavonoids, alkaloids, glycosides, beta cyanin, coumarins, quinones and steroids. GC-MS analysis of the peel extract of *A. hypogaea* was done to identify the major phyto compounds. Histopathological studies revealed damage in the tissues of the mid gut and cuticle. The results showed that the peel extract of *A. hypogaea* and its effective constituents can be considered as potent source for the production of natural larvicides.

Keywords: *Arachis hypogaea*, Phytochemicals, Larvicidal activity, Histopathology, Mosquito vectors.

1. Introduction
Development of resistance, cross-resistance, rising cost and possible toxicity hazards associated with synthetic insecticides are some of the reasons for revival of interest in plant based products in recent years [1].
Several mosquito species belonging to genera *Anopheles* and *Aedes* are vectors for the pathogens of various diseases like malaria, Japanese encephalitis, dengue, yellow fever and chikungunya [2].
Aedes aegypti is the vector that spreads yellow fever and dengue fever viruses and is endemic in tropical and subtropical regions around the world [3].
Anopheles mosquitoes transmit malarial parasites among humans. Approximately 91% of the 216 million cases estimated in 2010 were due to *Plasmodium falciparum*, transmitted by *Anopheles stephensi* [4]. The major problems associated with the use of chemicals for the control of pests is the development of resistance to the chemicals, and their undesirable side effects [5].
Extract from plants may serve as an alternative source of bioactive compounds that are biodegradable into nontoxic products and are potentially suitable for use to control mosquitoes. Plant extracts in general have best recognized as an important natural resource of insecticides [6].
Phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents, oviposition attractants and can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level [7, 8].
Considerable work has been reported on the effect of plant extracts against mosquito larvae. The crude hexane extract obtained from flower heads of *Spilanthes Acmella*, *Spilanthes calva*, *Spilanthes paniculata* [9], partially purified extracts of leaves of *Vitex negundo*, *Nerium oleander* and seeds of *Syzygium,* [10] leaves of *Artemisia annua* and *Azadirachta indica* [11] the acetone crude extracts of *Fagonia indica* and *Arachis hypogaea* [12], extracts of *Nerium indicum* and *Thuja orientalis* [13] were proved to have excellent toxic effects on the larval population of different mosquito species.
Arachis hypogaea (Fabaceae) Fig 1, is widely used for the treatment of various ailments in different countries, and is a rich source of resveratrol.

Because of significant pharmacological activity exhibited by the resveratrol, several researchers have focused on the development of various analytical methods to determine resveratrol in different matrices such as plant extracts, wine and serum. Peanut peels are a waste from blanched processing of peanut kernels. The peanut peel are sometimes used to feed cattle, however their value could be increased if other more valuable uses could be found [14]. The objective of this work was to evaluate the phytochemical compounds from peanut peels using different solvents and to determine the larvicidal activity of the extracts against the vectors of *chikungunya* and *malaria.*

2. Material and Methods

2.1 Preparation of plant Extract

Arachis hypogaea peels were collected and dried under shade at room temperature for about 20 days. The dried peels were powdered and sieved to get fine powder using an electric blender. 70 g of the powder was filled in the thimble and extracted successively with chloroform, acetone, ethanol, methanol and aqueous using soxhlet extractor for 10 h. All the extracts were concentrated using rotary flash evaporator and preserved at 5 ºC in an airtight bottle until further use.

2.2 Phytochemicals screening

The phytochemical screening was carried out using standard procedure [15]. By this analysis, the presences of several phytochemicals listed in (Table 1) were tested.

2.3 Separation of bioactive compounds using TLC

a) Preparation of extract

10 mg / ml of the extract in methanol solvent was used for TLC examination. The same procedure was followed for methanol and chloroform extract preparation [16].

b) TLC Plate preparation

The silica gel 60 F_{254} coated aluminum sheets were cut in size 1.5X5.5cm. Prepared methanol extract was loaded in silica plate and air dried.

c) Mobile phase preparation

The extracts were standardized in ethyl acetate with acetone and finally chloroform: ethyl acetate: methanol (3; 1.5; 0.5) ratio showed separated bands.

2.4 GC–MS analysis

GC–MS analysis were carried out an SHIMADZU QP 2010T which comprised of an auto sampler and gas chromatography interfaced to a mass spectrometer (GC–MS) instrument employing the following condition: capillary column −624 ms (30 m × 0.32 mm × 1.8 m) operating in an electron mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1.491 ml/min and injection volume of 1.0 ml, injector temperature was 140 ˚C; ion source temperature of 200 ˚C. The oven temperature was programmed from 45 ˚C. Mass spectra were taken at 70 eV.

2.5 GC-MS Identification of compounds

Interpretation of mass spectrum GC–MS was conducted using database of National institute standard and technology having more than 62,000 patterns. The spectrum of the unknown compounds are stored in the NIST library. The compound prediction was based on Dr. Duke's Phytochemical and Ethnobotanical Database by Dr. Jim Duke of the agricultural research service/USDA.

The names of the components of the test material were ascertained.

2.6 Selection of Mosquito species

The mosquito species selected for the present study were, *A. stephensi* and *A. aegypti. A. stephensi* is a vector of malaria in India and larvae of these species are generally found in distinctly different habitat. These are nocturnal and crepuscular in nature and also transmit the filarial worm causing filariasis [17] *Aedes aegypti* (L) mosquito spreads yellow fever, dengue fever and chikungunya. *A. aegypti* is a vector for transmitting several tropical fevers and the female bites for blood which she needs to mature her eggs [18].

2.7 Mosquito culture

All tests were carried out against laboratory reared *A. stephensi* and *A. aegypti* free of exposure to insecticides and pathogens. Cyclic generation of vector mosquitoes was maintained at 25-29 ºC in the insectariums. Larvae were fed on larval food (powdered dog biscuit and yeast in the ratio 3:1) and adult mosquitoes on 10 per cent glucose solution. Adult female mosquitoes were periodically blood-fed on restrained albino mice for egg production [18].

2.8 Larvicidal Bioassay

A total of three trials were carried out with five replicates per trial against vector mosquitoes for the following bioassays. Toxicity assays of the crude peel extract was conducted separately using the fourth instar larvae of *A. stephensi* and *A. aegypti.* Stock solution (1000 ppm) was prepared by dissolving 100 mg of crude extract in 1 ml acetone and volume raised to 100 ml with distilled water. From this different dilution of 25 ppm, 50 ppm, 75 ppm, 100 ppm and 150 ppm were prepared in 200 ml de-ionised water in 250 ml beaker and 25 fourth instar larvae were released in it and mortality was scored after 24 h. The beakers were kept in a temperature control room at 28 ºC ± 2 ºC and the larvae were exposed to 200 ml water containing 0.1ml of acetone served as control. Each treatment was replicated five times [19].

2.9 Larval susceptibility tests

The larval susceptibility tests were carried according to standard WHO procedure [20]. The extract solutions of different concentrations were prepared and the fourth instar larvae of *A. stephensi* and *A. aegypti* were placed in each test solution to observe the larvicidal property as per the following procedure.

Groups of 25 larvae were placed in 200 ml of the extract solution. Control experiments without extract were run in parallel. The larvae in each solution were then left for 24 h, the numbers of dead larvae were counted after 24 h of exposure, and the percentage mortality was reported from the average of five replicates. Mortality was recorded when control mortality ranged from 5 – 20 percent, and was corrected by Abbott's [21] formula.

2.10 Histopathological studies

The morbid larvae in the treatment and control were fixed in 10% formalin. The tissues were dehydrated with ethyl alcohol for 5 hours, after which they were placed in xylene for tissue clearing. They were then embedded and blocked by paraplast and sectioned with a microtome. Sections were stained using hematoxylin and eosin according to routine staining methods. Untreated larvae were also investigated in the same manner [22]

2.11 Statistical analysis
The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% confidence limits of upper confidence limit lower confidence limit and chi-square values were calculated using the SPSS 11.5 (Statistical Package of Social Sciences) software. Results with $P<0.05$ were considered to be statistically significant.

3. Result
3.1 Phytochemical screening
The preliminary phytochemical screening is a means of evaluating the potential phyto compounds in the peel extract of *Arachis hypogaea*. Phytochemical characterizations of methanol extracts of *A. hypogaea* are presented in Table 1. The phytochemical screening revealed the strong presence of carbohydrates, flavonoids, β- cyanin, phenols, coumarins and steroids. The other phytocompounds present were tannin, saponin, alkaloids, glycosides and quinine. Triterpenoids and terpenoids were present in trace amounts. The phytocompounds present in the peel extracts was further analyzed by TLC. Methanol peel extract of *A. hypogaea* showed five distinct bands under UV visualization. The compounds detected in Band 1, 2, 3, 4, 5, 6, 7 with R_f values of 0.93, 0.86, 0.81, 0.77, 0.70, 0.59, 0.46 showed the presence of Triterpenoids, alkaloids, phenolic compound, tannins, unknown compound, anthraquinones and quercetin (Fig 2).

3.2 GC MS analysis
The composition and identification of the compounds present in the methanol peel extracts of *A. hypogaea* by GC-MS analysis shown in Table 2. The active principles with their retention time (RT), molecular formula, molecular weight (MW) are presented in Fig 3. The main constituents were hexadecanoic acid, benzene dicarboxylic acid, oleic acid, octadecenoic acid, ethyl ester, eicosanoic acid and methyl [Z].

3.3 Larvicidal activity of *A. hypogaea*
Based on the probit analysis between the concentration of peel extract against fourth instar larvae of *A. aegypti* and *A. stephenesi* after 24 h exposure are represented Table 3, 4 and Fig 4. The results clearly indicate that peel extracts of *A. hypogaea* at very low concentrations was toxic against fourth instar larvae of *A. aegypti* and *A. stephenesi*. The methanolic peel extract was found to be potent against fourth instar larvae of *A. aegypti* and *A. stephensi* with LC_{50} and LC_{90} value of 45.75 ppm and 137.14 ppm and 45.98 ppm and 137.94 ppm respectively. Methanol extract of *A. hypogaea* showed 100 % mortality at 150 ppm against both the fourth instar larvae *A. aegypti* and *A. stephenesi.*
Ethanol peel extract was also found to be effective against the fourth instar larvae of *A. stephensi* with LC_{50} and LC_{90} value of 47.49 ppm and 143.77 ppm and *A. aegypti* with LC_{50} and LC_{90} 52.95 and 158.85 ppm respectively. All the other tested extracts also showed mosquito larvicidal activity at a relatively high concentration when compared to methanol and ethanol peel extracts.

3.4 Histopathalagical effects
The histopathological effects of *A. hypogaea* in gastric caeca, stomach regions were studies. The choice of these regions is justified by the fact that they are directly in contact with biocompounds of the extract. The untreated larvae of *A. aegypti* (control) midgut and gastric caecum showed a well preserved layer of epithelial cells. Regular microvilli border in the midgut and gastric caeca was observed in the control mosquitoes (Fig 5 A). The treated larvae of *Aedes aegypti*, the signs of intoxication began at the level of gastric caecum. The histology of *A. aegypti* larvae showed morphological and serious damage of the epithelial columnar cells (Fig 5 B). The gastric caeca and brush border cells were totally damaged. The second sign of intoxication was perturbation of alimentary flow in the alimentary canal and a few cells appear slightly hypertrophied with a perceptible beginning of vacuolization at the apical level. The pleiotropic membrane surrounding the lumen is ruptured and oozing of midgut content was observed. The third signs of intoxication are perceptible in the posterior part of the gut of *A. aegypti* larvae. Epithelial cells of the intestine start to burst and we noted a cytoplasmic rejection of cells material mixed with food was observed.

Fig 1: *Arachis hypogaea* plant
A-*Arachis hypogaea* **B - Peel powder**

Table 1: Phytochemical screening of peel extracts of *Arachis hypogea*

S. No	Secondary metabolites	Chloroform	Acetone	Ethanol	Methanol	Aqueous
1	Carbohydrates	+++	+++	+++	+++	+++
2	Tannins	–	+++	+++	+++	–
3	Saponins	–	–	+++	+++	–
4	Flavonoids	–	+++	+++	+++	+++
5	Alkaloids	–	+++	+++	+++	–
6	Betacyanin	–	+++	+++	+++	+
7	Quinones	–	+	+++	+	–
8	Glycosides	–	++	+++	+++	–
9	Cardiac Glycosides	–	–	–	–	–
10	Terpenoids	–	+	+	++	–
11	Triterpenoids	–	–	–	+	+
12	Phenols	+	+++	+++	+	+
13	Coumarins	–	+++	+++	+++	+
14	Acids	–	+	+	+	–
15	Protein	–	–	–	–	+++
16	Steroids	+	++	+++	+++	–

+++ Strongly positive ++ Positive + Trace - Not detected

Table 2: Gas Chromatography Mass Spectrometry of methanol peel extracts of *A. hypogaea*

S. No	Retention Time	Compounds	Molecular Formula	Molecular Weight
1	17.38	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45
2	17.87	1,2 – Benzenedicarboxylic acid , butyl octyl ester	$C_{20}H_{30}O_4$	334.44
3	18.07	Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	284.47
4	18.33	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
5	19.15	10- Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.48
6	19.37	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
7	19.77	Oleic acid	$C_{18}H_{34}O_2$	282.46
8	20.05	9- Octadecenoic acid, [E]	$C_{18}H_{34}O_2$	282.46
9	20.58	Oleic acid	$C_{18}H_{34}O_2$	282.46
10	22.27	9- Octadecenoic acid [z]-, 2,3-dihydroxybropyl ester	$C_{21}H_{40}O_4$	356.53
11	23.03	Methyl [Z]-5,11,14,17-eicosatetraenoate	$C_{21}H_{34}O_2$	318.49
12	23.48	Hexadecanoic acid, 2-hydroxy-1-[hydroxymethyl] ethyl ester	$C_{19}H_{38}O_4$	330.50
13	26.35	9- Octadecenoic acid [Z]-, 2,3- dihydroxypropyl ester	$C_{21}H_{40}O_4$	356.53
14	24.03	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368.64
15	21.85	Eicosanoic acid, ethyl ester	$C_{22}H_{44}O_2$	340.58

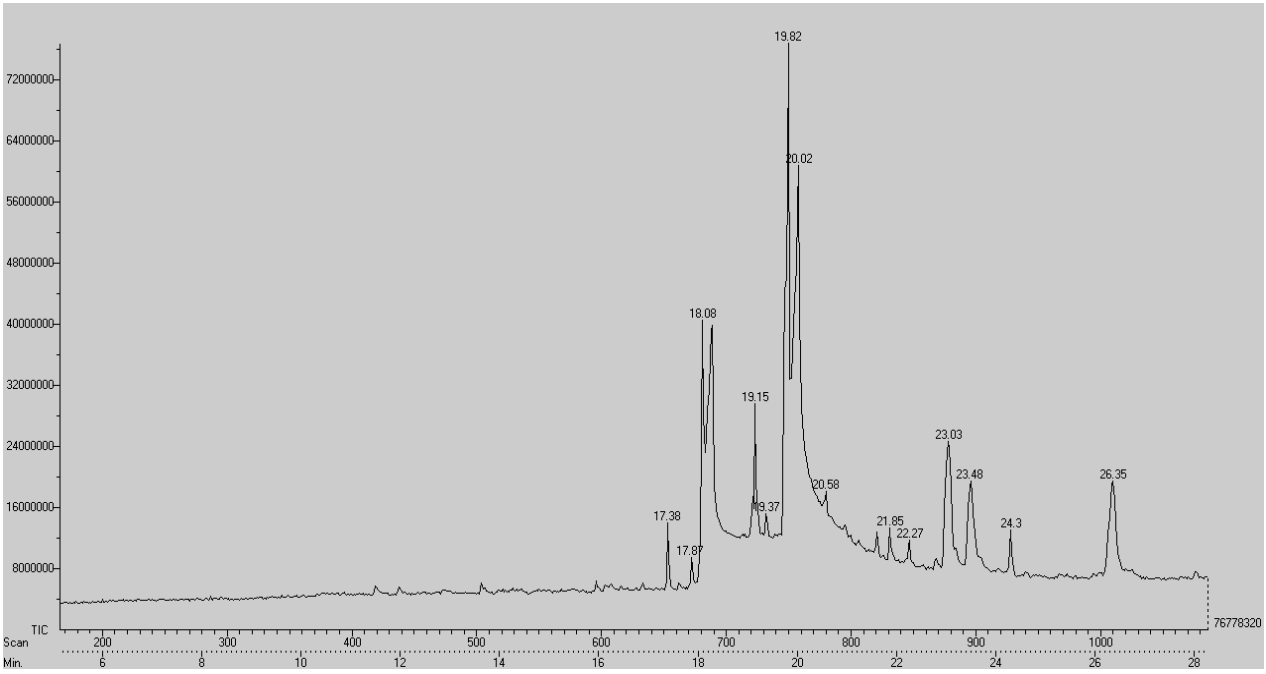


Fig 1: GC-MS analysis of methanol peel extract of *Arachis hypogaea*

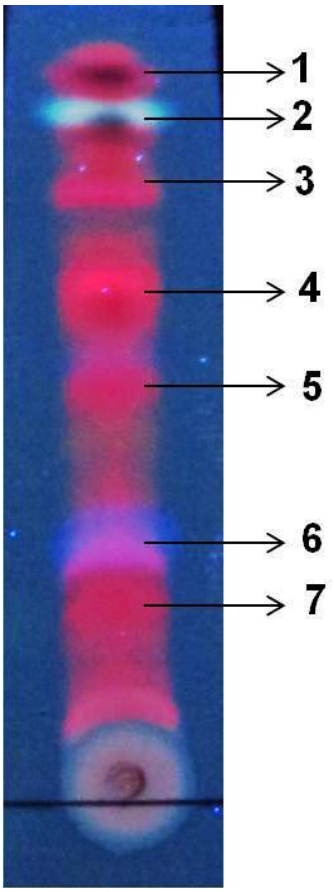


Fig 2: Separation of bioactive fraction of methanol peel extract of *A. hypogaea* by TLC.

Table 3: Larvicidal activity of peel extracts of *A. hypogaea* against fourth instar larvae of *Aedes aegypti*.

Extract / Species	Concentration (ppm)	24hr % Mortality	LC_{50} (UCL–LCL) (ppm)	LC_{90} (UCL–LCL) (ppm)	r^2
Aqueous	150 100 75 50 25	87 74 58 37 22	60.94 (68.88-53.92)	182.84 (203.55-167.14)	0.944
Acetone	150 100 75 50 25	90 74 56 41 35	59.58 (75.37-47.10)	178.74 (194.09-159.63)	0.974
Chloroform	150 100 75 50 25	94 82 63 44 26	53.21 (61.54-46.01)	160.72 (183.22-143.07)	0.946
Ethanol	150 100 75 50 25	99 86 60 52 37	52.95 (64.31-43.68)	158.85 (168.77-139.41)	0.956
Methanol	150 100 75 50 25	100 90 75 55 41	45.75 (59.48-35.19)	137.14 (152.98-121.09)	0.955

Table 4: Larvicidal activity of peel extracts of *A. hypogaea* against fourth instar larvae of *Anopheles stephensi*

Extract / Species	Concentration (ppm)	24hr % Mortality	LC_{50} (UCL–LCL) (ppm)	LC_{90} (UCL–LCL) (ppm)	r^2
Aqueous	150 100 75 50 25	75 65 52 33 20	71.57 (61.91-82.74)	214.62 (196.77-231.19)	0.935
Acetone	150 100 75 50 25	88 72 65 52 29	59.02 (66.53-52.36)	177.06 (197.33-153.14)	0.969
Chloroform	150 100 75 50 25	78 66 53 37 24	66.08 (76.76-56.89)	198.25 (218.54-177.42)	0.959
Ethanol	150 100 75 50 25	93 82 67 50 39	47.49 56.35-40.02	143.77 (169.01-123.88)	0.931
Methanol	150 100 75 50 25	100 85 65 50 37	45.98 (57.21-36.95)	137.94 (148.55-121.67)	0.947

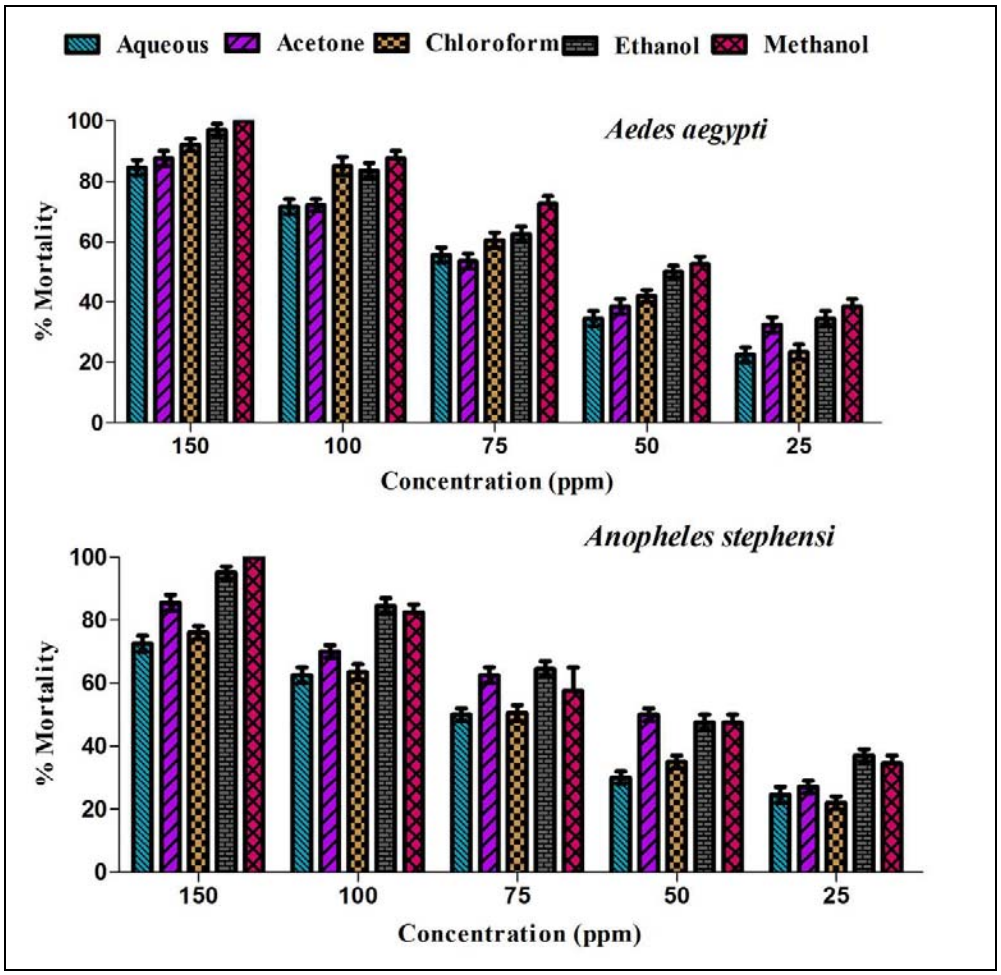
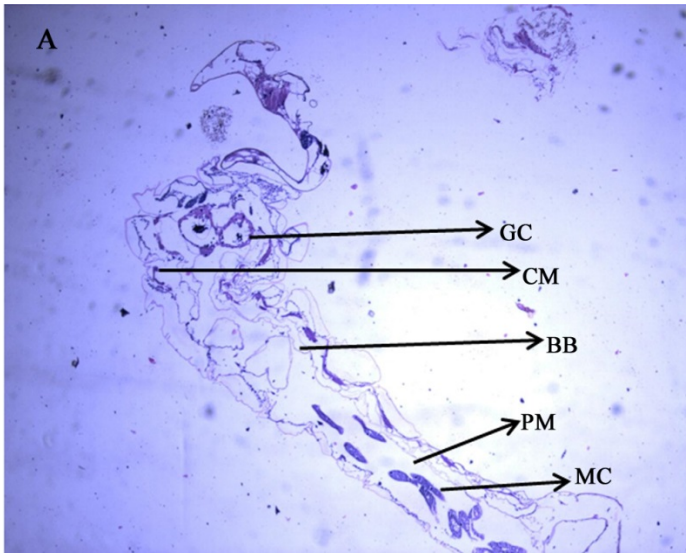


Fig 4: Larvicidal activity of peel extracts of *A. hypogaea* against *A. aegypti* and *A. stephensi*

A. Control



B. Treated

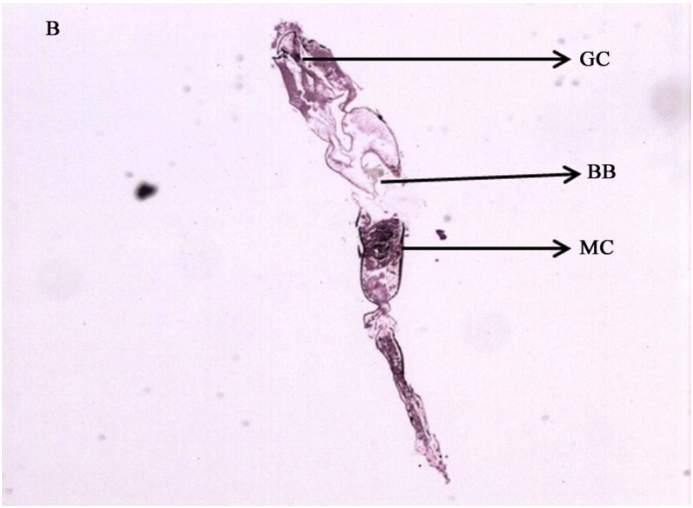


Fig 5: Longitudinal section of the digestive tract of 4th instar larvae of *A. aegypti* treated with methanol extract of *A. hypogaea* compared with control.

4. Discussion

An insecticide does not have to cause high mortality on target organisms in order to be acceptable [23] but it should prevent breeding. Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive and are readily available throughout the world [24]. The biological activity observed in the present study might be due to the various compounds, including phenols, terpenoids and alkaloids existing in plants [25]. These compounds may jointly or independently contribute to produce larvicidal activity against the mosquito species.

Phenols are generally known to be important sources of potent insecticides, fungicides, bactericides and herbicides for pest control. Triterpenoids are also credited with mosquito larvicidal activities [26]. These hydrocarbons inhibit the development stages of mosquitoes [27].

Phenolic compounds are synthesized in plants partly as a response to ecological and physiological pressures such as pathogen and insect attack [28]. The basic structural feature of phenolic compounds is an aromatic ring bearing methyl ester, hexadecanoic acid, eicosanoic acid, ethyl ester with more hydroxyl groups [29].

Plant phenolic compounds are classified as simple phenols or polyphenols based on the number of phenol units in the molecule [30].

In the present study the different solvent extracts of groundnut peel showed very high, moderate and low larvicidal effects. However, highest mortality was observed in methanol and ethanol peel extracts when compared to the other extracts. These results are in accordance with the observations of Elumalai *et al.,* [18] who reported the larvicidal potential of the whole plant extracts of *Tridax procumbens* against the larvae of *A. aegypti*, *A. stephensi* and *C. quinquefasciatus.* The larvicidal property of the peel extracts may be due to the presence of alkaloids, flavonoids and tannins and phenolic compounds [31].

The degenerating effect of the extract on the mosquito influenced the overall failure in adult emergence, maybe due to interference in chitinous cuticle formation as interpreted by Saxena and Yadev [32], noted that pupation followed prolonged larval stage in *A. stephensi* when treated with acetone extract of *Ocimum sanctum* along with abnormal larvae, intermediate pupae and several morphogenetic aberrations. These observations are further substantiated by histopathological changes observed in the fourth instar of *A. aegypti* when exposed to methanol peel extract of *A. hypogaea*. Neem compounds showed remarkable effects on caecum and gut of *A. aegypti* [22], which are in accordance with the present study.

The present study reveals that even relatively short term exposure of larvae to the peel extracts was found to markedly increase the mortality of the larvae overtime and thus reduce the total number of viable adults leading to a possible reduction in the total population dynamics of vectors [33]. It is evident from the present study that the peel extract of *A. hypogaea* might have a promising larvicidal efficacy.

5. Conclusion

The bioactive compounds from the peel extracts of *A. hypogaea* showed higher efficiency in reducing mosquito menace due to their larvicidal toxicity. The crude extract of *A. hypogaea* showed potent larvicidal activity against *A. aegypti* and *A. stephensi.* Hence the large biomass of the agricultural waste could be exploited for integrated mosquito control programs.

6. References

1. Suwannee P, Amara N, Maleeya K, Ushavadee T. Evalutions of larvicidal activity of medicinal plant extracts to *Aedes aegypti (Diptera: Culicidae)* and other effects on a non-target fish. Insect Science 2006; 13:179-84.
2. Service MW. Mosquitoes (Culicidae). In: Lane RP, Crosskey RW. Medical insects and arachnids. Chapman & Hall, London, 1999, 723-5.
3. Halstead SB. Lancet, 2012; 380, 1535.
4. World Health Organization. World Malaria Report (WHO, Geneva), 2011.
5. Peter RJ, Van den, Bossche P, Penzhorn BL, Sharp B, Trick. Fly and mosquito control-lessons from the past, solutions for the future. Vetnary parasitol 2005; 132:205-15.
6. Rahuman AA, Venkatesan P. Larvicidal efficacy of five *cucrbit aceous* plant leaf extracts against mosquito species. Parasitol Research 2008; 103:133-9.
7. Govindarajan M, Jebanesan A, Pushpanathan T, Samidurai K. Studies on effect of *Acalyphaindica* L. (Euphorbiacea) leaf extracts on the malarial vector,

Anopheles stephensi Liston (Diptera: Culicidae). Parasitol Research 2008; 103:691-695.
8. Govindarajan M, Jebanesan A, Pushpanathan T. Larvicidal and ovicidal activity of *Cassia fistula* Linn. Leaf extract against filarial and malarial vector mosquitoes. Parasitol Research 2008; 102:289-292.
9. Pandey V, Agrawal V, Raghavendra K, Dash AP. Strong larvicidal activity of three species of Spilanthes (Akarkara) against malaria (*Anopheles stephensi* Liston, *Anopheles culicifacies*, species C) and filaria vector (*Culex quinquefasciatus* (Say). Parasitol Research 2007; 102(1):171–174.
10. Pushpalatha E, Muthukrishnan J. Larvicidal activity of a few plant extracts against *Culex quinquefasciatus* and *Anopheles stephensi.* International Journal of Malarial research 1995; 32(1):14-23.
11. Tonk S, Bartarya R, Maharajkumari K, Bhatnagar VP, Srivastava SS. Effective method for extraction of larvicidal component from leaves of *Azadirachta indica* and *Artemisia annua* Linn. Journal of Environtal Biology 2006; 27(1):103–105.
12. Chaubal R, Pawar PV, Hebbalkar GD, Tungikar VB, Puranik VG, Deshpande VH, Deshpande NR. Larvicidal activity of *Acacia nilotica* extracts and isolation of D-pinitol—a bioactive carbohydrate. Chemistry & Biodiversity 2005; 2(5): 684–688.
13. Sharma P, Mohan L, Srivastava CN. Larvicidal potential of *Nerium indicum* and *Thujaorier telis* extracts against malaria and *Japanese encephalitis* vector. Journal of Environtal Biology 2005; 26(4):657–660.
14. Jyoti M, Galgut, Jaya Peter, Sharique A, Ali. Estimation of resveratrol in *Arachis hypogaea* fruit skin extracts by High-Performance Thin-Layer Chromatography. Biosci Biotech Res, 2011, 33-36.
15. Harborne JB. Phytochemical Methods. Chapman and Hall, London, 1998, 6.
16. Serkar J, Pal S, Bhattacharya S, Biswas M. Thin layer chromatographic profiling and evaluation of analgesic activity of *Psidium guajava* leaf extracts in mice. Journal of Advance Pharmacutical Education and Research 2011; 2:177- 183.
17. Dean M. Lympatic filariasis, the quest to eliminate, bulletin a 4000- year old disease Hollis NH, Publishing Company 2001.
18. Elumalai D, Kaleena PK, Mujeera fathima, Narashkumar C). Phytochemical sereening and larvicidal activity of *Tridax procumbens* (L) against *Anopheles stephensi* (Liston), *Aedes aegypti* (L) and *Culex quinquefasciatus* (Say). International Journal of Bioscience Research 2013, 2.
19. Arivoli S, Samuel T. Bioefficacy of *Citrullus Colocynthis* (L.) Schrad (cucurbitaceae) whole plant extracts against *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* (Dipters: Culicidae). International Journal Current Research 2011; 3(4):296-304.
20. WHO 2005. Communicable disease tool kit, Sudan. World Health Organization, WHO/CDS, 2005.
21. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 1925; 18:265-267.
22. Kaewnang OE, Ngampongsaim A, Subhadhirasakul S, Srichana T. Toxicity of fixed oil and crude extract from sa-dao-thiam Azadirachta excelsa (Jack) seed kernel to *Aedes aegypti* (L.) Songklanakarin, Journal of Science and Technology 2011; 33(1):43-49.
23. Kabaru JM, Gichia L. Insecticidal activity of extracts derived from different parts of the mangrove tree *Rhizophora mucronata* (Rhizophoraceae) Lam. against three arthropods. African journal of Science and Technology 2001; 2(2):44-49.
24. Bowers WS, Sener B, Evans PH, Bingol F, Erdogani. Activity of Turkish medicinal plants against mosquitoes *Aedes aegypti* and *Anopheles gambiae*. International Journal of Tropical Insect science 1995; 16(3/4):339-342.
25. Park IK, Lee HS, Lee SG, Park JD, Young-Joon Ahn. Antifeeding Activity of Isoquinoline alkaloids Identified in *Coptis japonica* roots against *Hyphantri acunea* (Lepidoptera: Arctiidae) and *Agelastica coerulea* (Coleoptera: Galerucinae). Journal of Economic Entomology 2000; 93(2):331-335.
26. Gbolade A. Plant derived insecticides in the control of malaria vector. In: "Phytomedicine in malaria and sexually transmitted diseases: Challenges for new millennium" ,edited by C.O. Adewunmi and S.K. Adesina, Drug research and production unit, Faculty of Pharmacy, Obafemi, Awolowo University, lle-fe, Nigeria 2000, 48-50.
27. Miller LL, Schultz GC. Weeds branch, Agnote Rajuence No.477. Current Sciences 2002; 81:15-29.
28. Diaz Napal GN, Defago M, Valladares G, Palacios S. Response of *Epilachna paenulata* to two flavonoids, Pinocembrin and quercetin, in a comparative study J Chem Ecol 2010; 36:898–904.
29. Chirinos R, Betalleluz-Pallardel I, Huamán A, Arbizu C, Pedreschi R, Campos D. HPLC-DAD characterisation of phenolic compounds from Andean oca (*Oxalis tuberosa* Mol.) tubers and their contribution to the antioxidant capacity. Food Chem 2009; 113:1243-1251.
30. Wang C, Zuo Y. Ultrasound-assisted hydrolysis and gas chromatography–mass spectrometric determination of phenolic compounds in cranberry products. Food Chem 2011; 128:562–568.
31. Anees AM, Larvicidal activity of *Ocimum sanctum* Linn. (Labiatae) against *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say). Parasitology Research 2008; 103(6):1451-1453.
32. Saxena SC, Kaushik RK. Total development arrest of fourth instar larvae of *Culex* quinquefasciatus treted with penfluron. Current Science 1988; 57: 1196-1199.
33. Ansari MA, Vasudevan P, Tandon M, Razdan RK. Larvicidal and mosquito repellent action of peppermint (*Mentha piperita*) oil. Bioresources Technology 2000; 71:267–71.