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## Effects of extracts of *Peristrophe bicalyculata*, *Allium sativum* and *Zingiber officinale* on mosquito larvae

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**ABSTRACT:** The problem of pollution, vector resistance to synthetic insecticides has led to the safe plant products being tested as pest control agents. The effects of extracts from *Peristrophe bicalyculata*, *Allium sativum* and *Zingiber officinale* on mosquito larvae and the percentage mortality and, LC<sub>50</sub> value were determined. The phytochemical screening of the active ingredients were carried out. The ground *P. bicalyculata* leaves, *A. sativum* bulb and *Z. officinale* rhizomes were extracted with hexane, diethylether, ethanol and water using the soxhlet extraction method. The extract were separated from the solvent by solvent recovering method. A series of concentrations of the extracts ranging from 0.1 – 1.0 mg/ml were tested against early fourth instar larvae of mosquitoes and their percentage mortalities and LC<sub>50</sub> values determined. All extracts showed various degrees of larvicidal activity to mosquito larvae at high concentration except water (aqueous) extract. The diethylether soluble extract of *Z. Officinale* showed very high larvicidal activity where 70% mortality was achieved at a concentration of 0.2mg/ml and had an LC<sub>50</sub> of 0.43mg/ml. The best lethal dosage (LC<sub>50</sub>) for *P. bicalyculata* is that for hexane extract with a value of 0.49ml/m/. The extract of *A. sativum* did not show very high activity. Photochemical analysis showed the presence of tannin, and alkaloid, in *P. bicalyculata*, flavonoid, saponin and alkaloid in *A. avitum* and Flavonoid, steroid and alkaloid in *Z. officinale*. It is concluded that extracts from *P. bicalyculata* and *Z. officinale* can be used as environmental friendly insecticide to control mosquitoes at the breeding sites.

**Key Words:** Plant extracts, larvicidal activities, phytochemical activity, LC<sub>50</sub>.

### Introduction

The mosquito constitutes a major public health problem as a principal vector of many vector borne diseases affecting humans and other animals (E Hat et al, 1999). Several mosquito species belonging to the genera *Anopheles*, *Culex* and *Aedes* are vectors for the pathogens of diseases such as Malaria, filariasis, encephalitis, dengue fever, dengue hemorrhagic fever and yellow fever. *Aedes aegypti* is the principal vector of dengue fever and it is reported to infect more than hundred million people every year in more than 110 continues in the tropics (Halstead , 2000). Interest in the control of *Anopheles gambiae* lies in the fact that it acts as the major vector of malaria which is a serious health problem in Africa and many developing countries.

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Malaria is a leading cause of premature mortality particularly in children under the age of five. According to Centre for Disease Control, about 5.3 million deaths occur annually and Hubalet and Haouzka (1999) reported that *Culex pipiens* is the vector of West Nile Virus (WNV) causing encephalitis and meningitis affecting the brain tissue and result in permanent neurological damage.

Some other species of mosquitoes carry filariasis leading to elephantiasis (disfiguring around 40 million people). There are bacterial and viral diseases transmitted by several different mosquitoes responsible for many human and animal diseases <http://www.who.int/malaria/docs/healthworkers/pdf/pg.19>. The mosquito today is considered as most deadly diseases vector known to man resulting in the death of millions of people over thousands of years and continues to kill millions per year by spreading diseases (Molly, 2005). Some of the diseases such as fever have been reasonably brought under control by vaccination. No effective vaccine is available for malaria and filariasis and the parasite is continually developing resistance to the available drugs; therefore one of the best approach to minimize the incidence of some of these diseases is achieved by application of pesticides to local habitats.

Since the discovery of DDT and other conventional pesticides, the control of mosquito species has been almost completely based on synthetic organic pesticide although they are effective, but are trailed by resistance, residual effect and outright environment pollution. They affect both target and non target organisms. (Guneady et al 1989). The resultant environmental hazards and the development of physiological resistance due to extensive use of organic insecticides has necessitated the search for biodegradable, low cost, environmentally safe an indigenous methods for the control of vectors (ICMR Bulletin 2003).

Garlic (*Allium Sativum*) has been used both as food and medicines in many cultures for many years. It is claimed to help present heart diseases such as atherosclerosis, high cholesterol, high blood pressure and cancer. It is also alleged to regulate blood sugar levels, prevent complications of Diabetes Mellitus (Silagy and Neil, 1994). It has antimicrobial activity (Groppo et al 2007). It is used in modern naturopathy for intestinal worms and other parasites, for chest problem, digestive disorder and for thrush against fungal infection (Salunkhe and Kadam 1998). It is said to consist of 84.09% water, 13.38% organic matter and 1.53% inorganic matter. It contains Allyl, Methyl sulfide (AMS) which can now be digested and results in strong smelling sulphur compound and passed to blood and is believed to act as ,mosquito repellent. (Tara 2007).

Ginger (*Zingiber officinale*) is used as spices in many dishes. It is also used for medical purposes (McGee and Harold 2004). It has been reported to be used for stomach ailments flatulence, fever and stimulates general weakness, cataracts, heart diseases, migranes, stroke, amenorrhea, chronic fatigue, cold, flu, cough and bronchitis (Blumethat 1998), depression, erectile difficulties, kidney stones and viral infections and when pounded and paste applied on abdomen, combats menstrual difficulties (Miller 1998, Lumb, 2004).

## Materials and Methods

The plant materials from *Peristrophe bicalyculata* leaves, *Allium sativum* bulb and *Zingiber officinale* rhizome were air dried under shade at room temperature 29<sup>+</sup> 1<sup>0</sup>C. They were each pounded and sieved into fine powder.

Each sample was weighed into 40g unit and wrapped in a whatman No. 1 filter paper and using 500ml of Hexane, diethylether, ethanol and water, soxhlet extraction method was used. The process carried out for 6hrs and was repeated until complete extraction was achieved. Using the solvent recovery method, the solvent was removed and crude extract of the sample were obtained.

Phytochemical screening was carried out on the plant extracts for the following. Alkanoid, flavonoid, saponin, steroid and tannin using Trease and Evans (1989), procedure. 0.25g of the concentrated extract of dried plant were dissolved in 25ml of the solvents and kept as stock (10mg/ml solution. They were then prepared into the desired series of solutions 0,1, 0.2, 0.3, 0.5 and 1.0mg/ml.

Water containers were exposed and female mosquito laid eggs which were monitored to the 4<sup>th</sup> larval instars, then used for treatment with the various extracts, and concentrations. The larval mortality was observed after 24hrs and percentage mortality calculated. Spearman Karber method was used to estimate the LC<sub>50</sub> values and associated 95% confidence units.

## Results

The result in Table 1 shows the yield of extracts of each plant from the various solvents. The highest yield was from *A. sativum* with water. Table 3 shows the active ingredients present in the plant while Table 2 shows the effect of the various extracts on the mosquito larvae.

In the three plants, water extract had the highest yield followed by ethanol, while hexane had the least yield. Hexane extract of *P. bicaliculata* had 50% mortality at concentrate of 0.3mg/ml. *A. sativum* had LC<sub>50</sub> corresponding to 95% confidence intervals with – diethyl ether was 50% at 0.3. *Z. officinale* had LC<sub>50</sub> of 70% at 0.2 concentration with Diethylether.

**Table 1: The percentage yield of extracts from 40% of the plant material**

| Plant                           | Solvent       | Initial weight of sample (g) | Wt of Extract (Yield) | % Yield |
|---------------------------------|---------------|------------------------------|-----------------------|---------|
| <i>Peristrophe bicaliculata</i> | Hexane        | 40                           | 2.12                  | 5.30    |
|                                 | Diethyl ether | 40                           | 2.44                  | 6.10    |
|                                 | Ethanol       | 40                           | 6.75                  | 16.88   |
|                                 | Water         | 40                           | 8.15                  | 20.38   |
| <i>Allium sativum</i>           | Hexane        | 40                           | 1.12                  | 2.80    |
|                                 | Diethyl ether | 40                           | 1.85                  | 4.63    |
|                                 | Ethanol       | 40                           | 4.05                  | 10.13   |
|                                 | Water         | 40                           | 12.45                 | 31.13   |
| <i>Zingiber officinale</i>      | Hexane        | 40                           | 1.64                  | 4.10    |
|                                 | Diethyl ether | 40                           | 2.33                  | 5.88    |
|                                 | Ethanol       | 40                           | 4.13                  | 10.38   |
|                                 | Water         | 40                           | 11.35                 | 18.28   |

## Discussion

The assays of the twelve extracts from *P. bicaliculata*, *A. sativum* and *Z. officinale* against the early fourth inster larvae of mosquitoes showed that the most effective extract was that of diethylether form *Z. officinale*. It demonstrated a high larvae mortality, at a concentration of 0.2mg/ml, the extract produced 70% mortality and LC<sub>50</sub> of 0.43mg/ml.

The four extracts from *A. sativum* did not show very high activity, they still caused mortalities at some concentrations. All the extracts showed some larvicidal activities to mosquito larvae except the aqueous extract.

The plant extracts have shown that they can kill up to 90% of the larval population tested. This can help reduce the mosquito population drastically. Considering that a large proportion of the human population living in mosquito borne diseases prone areas suffer from varying degrees of poverty, the discovery of cheap and available plant extracts that could control the mosquito population could be of great value. These plants grow widely and offer an opportunity for developing alternatives to rather expensive and environmentally hazardous inorganic insecticides.

A large number of plant extracts have been reported to have mosquitocidal or repellent activities against mosquito vectors but very few plants products have shown practical utility for mosquito control (Sukumer et al,

1991). The effects of the various aqueous extracts of *balanites aegypti* (desert dates) on the mortality of *Culex pipiens* mosquito larva with kernel extract treatment, there was less than 50% mortality on the first day even at the highest concentration. At the end of the second day, 2% concentration killed up to 90% of the larvae. But not up to 100% (Bishau and Zeen 2005).

**Table 2: Effect of Extracts on Mosquito Larva**

| Plant                           | Solvent       | Concentration (mg/ml) |     |     |     |     | LC <sub>50</sub>   |
|---------------------------------|---------------|-----------------------|-----|-----|-----|-----|--------------------|
|                                 |               | 0.1                   | 0.2 | 0.3 | 0.4 | 1.0 |                    |
| <i>Peristrophe bicalyculata</i> | Hexane        | 10                    | 40  | 50  | 70  | 90  | 0.49 (0.37 – 0.60) |
|                                 | Diethyl ether | 0                     | 0   | 20  | 30  | 80  | 0.70 (0.36 – 0.83) |
|                                 | Ethanol       | 0                     | 0   | 10  | 10  | 30  | 1.0 (0.85 – 1.14)  |
|                                 | Water         | 0                     | 0   | 10  | 10  | 10  | 1.10 (0.99 – 1.20) |
| <i>Allium sativum</i>           | Hexane        | 0                     | 0   | 10  | 70  | 80  | 0.63 (0.49 – 0.76) |
|                                 | Diethyl ether | 0                     | 0   | 50  | 50  | 70  | 0.68 (0.52 – 0.83) |
|                                 | Ethanol       | 0                     | 0   | 10  | 30  | 40  | 0.97 (0.74 – 1.07) |
|                                 | Water         | 0                     | 0   | 0   | 0   | 0   | 0.0 (0.0 – 0.0)    |
| <i>Zingiber officinale</i>      | Hexane        | 30                    | 30  | 40  | 90  | 90  | 0.63 (0.49 – 0.76) |
|                                 | Diethyl ether | 0                     | 70  | 70  | 80  | 90  | 0.43 (0.32 – 0.54) |
|                                 | Ethanol       | 0                     | 0   | 0   | 10  | 30  | 1.01 (0.59 – 1.45) |
|                                 | Water         | 0                     | 0   | 0   | 0   | 20  | 1.08 (0.68 – 1.47) |

**Control (+) Hexane (0.1mg/ml) ( - ) Tap Water**

Lethal concentrations with the corresponding 93% confidence intervals are shown in parenthesis.

**Table 3: The active ingredients present in the plants**

| Plant                           | Flavonoid | Saponin | Steroid | Tannin | Alkaloid |
|---------------------------------|-----------|---------|---------|--------|----------|
| <i>Peristrophe bicalyculata</i> | —         | —       | —       | +      | +        |
| <i>Allium sativum</i>           | +         | +       | —       | —      | +        |
| <i>Zingiber officinale</i>      | +         | —       | +       | —      | +        |

**Key:** + = Present  
— = Absence

From this work, the plant extracts from *Z. officinale* and *P. bicalyculata* with diethylether and hexane at as low concentrations as 0.4mg/ml can give a 90% mortality in 24 hours. This shows a higher larvicidal effect suggesting that the *Z. officinale* extracts and *Z. officinale* extracts could be effectively used for the control of mosquito larvae in public health operations.

Previous studies have shown that many plants extracts possess insecticidal properties (Sukuma *et al* 1991). Some possess flavonoid, Saponin, Steroid, Tannin and Alkaloid. The active ingredients in *Z. officinale* are flavonoid, steroid and alkaloid while in *P. bicalycula*, are Tannin and alkaloid.

Developing alternatives to rather expensive and environmentally hazardous insecticides is necessary. This result is useful in the search for new biodegradable and environmentally friendly larvicidal natural compounds. This may yield promising results in mosquito borne disease and mosquito management programs.

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