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Insecticidal properties of fruit extracts isolated from *Cascabela thevetia* (L.)

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Abstract
Vector-borne diseases are widespread, and contagious. Mosquitos are deadly vectors that carry various disease-causing agents, that lead to death of many people in the past decade. On the other hand, insects like housefly and cockroach play an important role in spreading disease like cholera and amoebiasis. Even though, there are plenty of commercially available pesticides to kill these insects, they are coming up with side effects. In this regard, it is necessary to isolate a novel insecticidal agent from natural sources that is devoid of any side effects. Traditionally, plants are used to control these insects, but now it is the need of the hour to isolate a novel bioactive insecticidal compound from plants to eradicate these insects. The present study is to evaluate the chloroform and ethanolic extracts of *Cascabela thevetia* fruits tested for their insecticidal properties against the immature stages of mosquito species *Aedes aegyptii* and *Culex quinquefasciatus*, larval stage of housefly *Musca domestica* and nymphal stage of cockroach *Periplanata americana*. The chloroform extract of fruit of *Cascabela thevetia* showed good insecticidal activity against all the tested insects. The ethanolic extract not showed any significance. The study revelated that chloroform extract of *C. thevetia* could be used as insecticidal agent and there is need of isolating active principle near future.

Keywords: Vector-borne diseases, mosquito, *Musca domestica*, *Cascabela thevetia*, *Periplanata americana*, chloroform

Introduction

Cascabela thevetia is cultivated as an ornamental plant, and planted as large flowering shrub or small ornamental tree standards in gardens and parks in temperate climates. It tolerates most soils and is drought tolerant. They are distributed in various states of India like Bihar, Delhi, Gujarat, Madhya Pradesh, West Bengal, Rajasthan, Tamil Nadu and Uttar Pradesh where semi-arid climate is prevalent. Toxins from this plant have tested as biological pest control agents. Oil from the seeds of this plant have antifungal, antibacterial and anti-termite properties (Kareru *et al.*, 2010) [1]. The whole plant of *Cascabela thevetia* shows the presence for carbohydrates, saponins, alkaloids, terpenoids, tannins, phenols, steroids and cardio glycosides. These secondary metabolites contribute significantly towards the biological activities such as hypoglycaemia antidiabetic, antioxidants, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial and anti-leprosy activities (Seetharaman *et al.*, 2017) [2].

Insects act as the vector for many disease-causing pathogens. Adult flies transmit these pathogens by mechanical dislodgement from their exoskeleton, faecal deposition and regurgitation of undigested food. Cockroaches are the most important pests, which not only contaminate food by transmitting bacteria that can cause food poisoning, but they also transmit other pathogenic microorganisms such as fungi in the infested areas (Gore *et al.*, 2007). They are basically tropical insects, so far numerous pathogenic bacteria, including Salmonella sp., Shigella sp., and K. pneumoniae have been isolated from cockroaches. In addition, some parasites and fungi have been found in the external surfaces or internal parts of body of cockroaches, and some studies have shown that exposure to cockroach antigens may play an important role in asthma-related health problems (Cotton *et al.*, 2000) [3].

Houseflies are known to carry various pathogens that can cause serious and life-threatening diseases in humans and animals. Over hundred pathogens including bacteria, viruses, fungi and protozoans have been associated with houseflies (Tsagaan *et al.*, 2015). Evidence supporting the role of the housefly in transmission of diseases are mostly circumstantial, with the strongest evidence pointing to the correlation between the rise in incidence of diarrhoea and an increase in the fly population (Farag *et al.*, 2013) [4]. Houseflies usually feed and reproduce in faeces, animal manure and other decaying organic substances, and thus live in intimate association with various microorganisms including human pathogens, which may stick to body surfaces of the fly. The constant back and forth movement of houseflies between their breeding sites and human dwellings can lead to the transmission of pathogens to humans and animals (Faham *et al.*, 2018).

Mosquitoes are carriers(vectors) for many diseases such as dengue, malaria, chikungunya, encephalitis, zika and yellow fever. Mosquito-borne diseases are caused by the bite of the infected mosquito. Factors such as urbanization, global travel and human population growth have increased the potential for mosquitoes to proliferate and hence cause disease on wider scale (James gathany, 2016). Integrated vector management is a key strategy to control mosquito-borne diseases. The traditional vector control is becoming less effective due to certain challenges. Challenges include increasing resistance of mosquito to the insecticides and they are also adapting to control methods, therefore it is more difficult to control the mosquito vectors. Therefore, it is necessary to develop some alternative approaches such as

larvicide. This involves the use of insecticides that kill mosquitoes at their stage- before they develop into adult. (World Health organization, 2017) [11].

Materials and methods

Collection & Processing of sample

The fruits of *Cascabela thevetia* were identified and collected from Tiruvannamalai district, Tamil Nadu during the month of November 2019. The fruits were sliced and shade dried. The shade dried fruits were coarsely powdered using electrical blender and stored in an airtight container for further analysis.

Preparation of fruit extract

The powdered fruit (500g), (w.v 1:3) was soaked in Chloroform (1.5 litre) 48 hrs. The filtrate was filtered using Whatman's filter paper and concentrated by vacuum rotary evaporator. Finaly the extracts were obtained as paste form and stored at 4 C°. The remaining residue was further extracted with ethanol in a similar manner.

Mosquito Larvicidal activity

Mosquito larva was collected from stagnant water sources using dipping method (using net). Field collected larva were reared separately under laboratory conditions in Entomology Research Institute, Loyola college. They were placed in the water and protected within nets. They were provided with starch as a nutrient for growth. They were reared in a closed room under normal room temperature. Mosquito larvicidal bioassay was done using World Health Organization (WHO) standard protocols with some modifications. 20ml of distilled water was taken in six different beakers. Ten 3rd instar larvae of *Aedes aegypti* were introduced into each beaker. Three different concentrations (50µl, 100µl and 200µl) of each sample were introduced into those beakers and mortality % was recorded after 24hrs. (Figure 1)

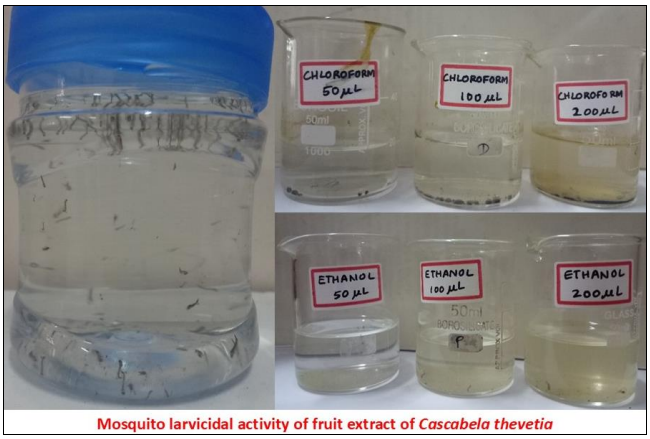

Fig 1: Mosquito larvicidal activity of fruit extracts of *Cascabela thevetia*

Housefly Larvicidal activity

Houseflies often lay their eggs in rotting, decayed or fermenting organic material with moisture content between 50-85%. Fresh poultry manure with 75-80% moisture content were used as the medium for rearing the larva of *Musca domestica Linn.* Standard method of larvicidal bioassay was carried out with some minor modifications. This bioassay was based on exposing housefly larva to food contaminated with plant extracts. The bait was prepared by mixing 2g of milk powder with 2ml of water containing chloroform and ethanol extracts (5mg) separately in two different containers. The 3rd larval instars were allowed to feed on these baits. The controls were kept with baits free of plant extract. All containers were incubated at room temperature for 24hrs and then the percentage mortalities were estimated. (Figure 2)

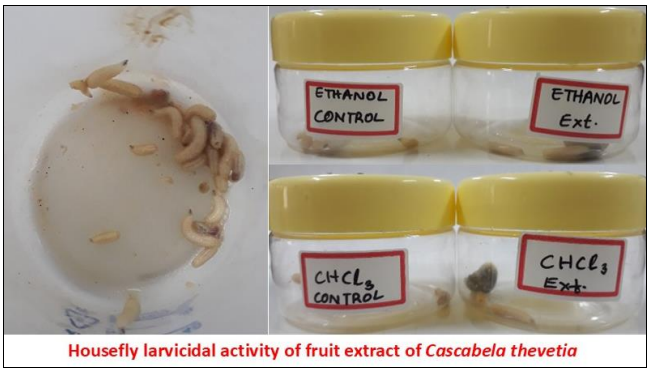

Fig 2: Housefly larvicidal activity of fruit extracts of *Cascabela thevetia*

Cockroach Nymphicidal activity

Cockroach nymphs were collected from drainage manhole by using net. The Nymphicidal bioassay was carried out with standard method proposed by World Health Organisation (WHO) with some modifications. 2g of sugar was mixed with 2ml of distilled water containing chloroform and ethanol extracts (5mg) separately. Cotton balls were soaked in these solutions and were placed in two different containers. The controls were kept with cotton balls soaked with sugar solution free of plant extract. Now five cockroach nymphs were introduced in each container and the containers were incubated at room temperature for 24hrs. The mortality rate was checked after 24hrs. (Figure 3)

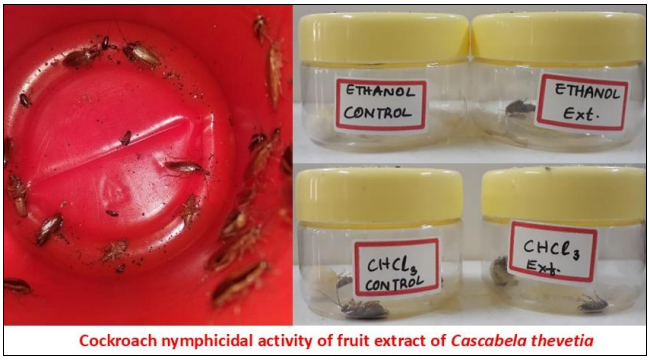

Fig 3: Cockroach nymphicidal activity of fruit extracts of *Cascabela thevetia*

Results

Extraction of fruit material

The phytoconstituents in the fruit of *Cascabela thevetia* was extracted using chloroform and ethanol as the solvents. The yield was around 18gms for chloroform and 22gms for ethanol.

Insecticidal activity

The larvicidal bioassay of chloroform and ethanol extracts of *Cascabela thevetia* against the 3rd instar larva of mosquitoes and houseflies were carried out and the

chloroform extract showed more larvicidal activity than the ethanol extract which are shown below. The chloroform extract showed mortality percentage of 100% at 5mg/ml concentration, whereas ethanol extract showed 60% mortality at 5mg/ml concentration against mosquito larva. Similarly, the nymphicidal bioassay against the cockroach nymphs was carried out and the chloroform extract showed more activity which is shown below (Table-1,2) (Figure 4,5)

Table 1: Mosquito larvicidal activity of chloroform fruit extract of *Cascabela thevetia*

Concentration(mg/ml)	Total dead	Mortality (%)
1.25	3	30
2.5	7	70
5	10	100
Control	2	20

Table 2: Mosquito larvicidal activity of ethanolic fruit extract of *Cascabela thevetia*

Concentration(mg/ml)	Total dead	Mortality (%)
1.25	1	10
2.5	3	30
5	6	60
Control	1	10

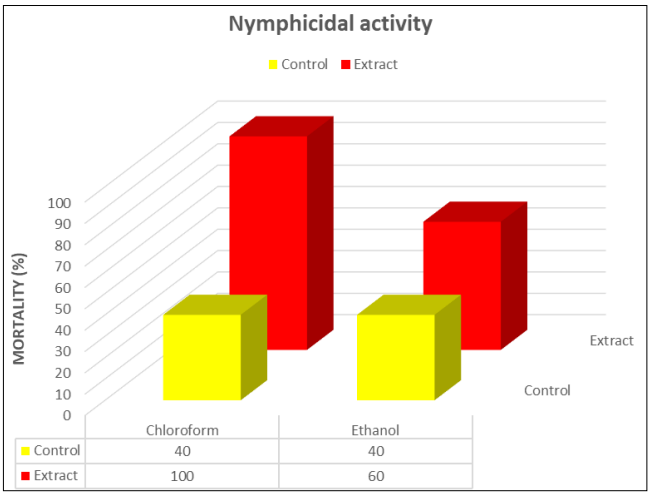

Fig 4: Cockroach nymphicidal activity of fruit extracts of *Cascabela thevetia*

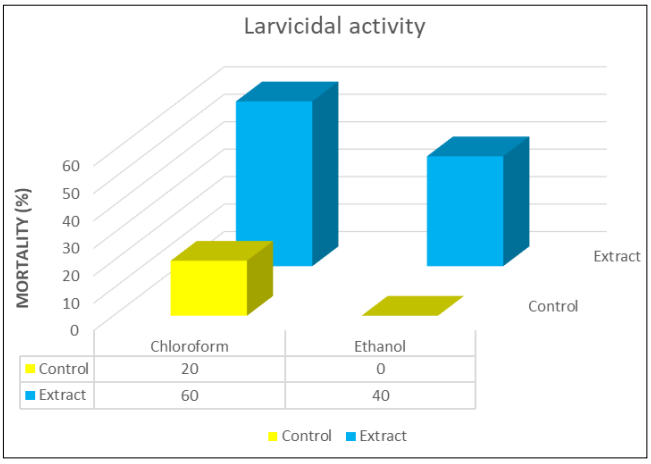

Fig 5: Housefly larvicidal activity of fruit extracts of *Cascabela thevetia*

Discussion

The fruit extracts of *Cascabela thevetia* were prepared by using Chloroform and ethanol as the solvents. The preliminary phytochemical analysis showed that the chloroform extract contained phenols, saponins, flavonoids, terpenoids and steroids, whereas the Ethanol extract contained only phenol. Various medicinal properties have been attributed to natural herbs. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products (Ivanova *et al.*, 2005) [5]. Secondary metabolites of plants serve as defence mechanisms against predation by many microorganisms, insects and herbivores (Cowan *et al.*, 1999) [6]. The phenolic compounds are one of the largest and most ubiquitous groups of plants of plant metabolites that possess an aromatic ring bearing one or more hydroxyl constituents (Singh *et al*., 2007). Phenolic compounds are widely found in the secondary products of medicinal plants, as well as in many edible plants (Hagerman *et al*., 1998) [7]. A number of studies have focused on the biological activities of phenolic compounds, which are potential antioxidants and free radical scavengers (Chanda and Dave, 2009) [8]. They possess various biological properties such as: anti-apoptosis, anti-aging, anticarcinogen, antiinflammation, cardiovascular protection and improvement of endothelial function. Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoids, phenolic acids, tocopherols, etc. (Ali *et al*., 2008) [9].

Plant products have been successfully exploited as insecticides, insect repellents and insect antifeedants. Higher plants are a rich source of novel natural substances that can be used to develop environmentally safe methods for insect control (Morand *et al*., 2014) [10]. The recently developed new isolation techniques have led to an interest in plants as the source of new larvicidal compounds. Mosquitoes act as a vector for many diseases like dengue, chikungunya, malaria, filariasis, etc. Therefore, it is necessary to control the proliferation of mosquitoes. One of the most convenient approaches to control the mosquitoes is the usage of phytoconstituents as the insecticidal agents. Similarly, cockroaches and houseflies also play a vital role in transmitting various disease-causing pathogens and are responsible for many diseases such as diarrhoea, typhoid and other communicable diseases.

Larvicidal and nymphicidal activities of fruit extracts of *Cascabela thevetia* were carried out by using standard methods recommended by the World Health Organisation (WHO). The chloroform extract showed more insecticidal activity when compared with ethanol extract. The result obtained indicate that the phytoconstituents found in *Cascabela thevetia* were biocidal, therefore, it can be used as an alternative to synthetic insecticides.

Conclusion

Plants have been used for medicinal purposes since time immemorial. This is due to the presence of various phytoconstituents which possess different pharmacological activities. Therefore, plants which possess these valuable phytoconstituents has been used to treat various diseases. The current study was carried out with the fruit extracts of *Cascabela thevetia* which were extracted using chloroform and ethanol as the solvents. The insecticidal activities of these extracts were also carried out against the 3rd instar larva of mosquito (*Aedes aegypti*) and housefly (*Musca domestica*). The chloroform extract showed more larvicidal

activity in comparison to the ethanol extract. The nymphicidal activity of these extracts was also carried out against the nymphs of cockroach (*Periplanata americana*) and the chloroform extract showed more activity than the ethanol extract. Hence from this study, it was observed that the fruits of *Cascabela thevetia* proved to be a good insecticidal agent.

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