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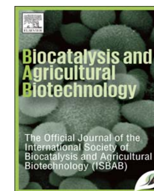

Intrinsic studies of *Euphorbia antiquorum L.* latex extracts against human bacterial pathogens and mosquito vector *Aedes aegypti*, *Culex quinquefasciatus* (Diptera: Culicidae)

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A B S T R A C T

At present scenario, due to the rapid outbreak of endemic diseases and developing resistance against infectious diseases drive the researchers to search for new natural insecticides from plant origins. Plant latex is a naturally abundant resource secreted by laticifer cells containing various groups of secondary metabolites having diverse biological activities. The aim of this study is to evaluate antimicrobial and mosquitocidal activity of the latex extracts of *Euphorbia antiquorum*. The chloroform, methanol and aqueous latex extract were screened for their toxicity against developmental stages of mosquito larvae and pathogenic bacteria. The latex extracts responded good inhibitory activity against the tested human pathogens. In the mosquitocidal study, the latex extracts, particular chloroform extract show dose-dependent activity and causing 100% mortality in 24hr against *Aedes aegypti* and *Culex quinquefasciatus*. In addition, the chloroform extract was subjected to phytochemical profiling studies. Fourier Transform Infrared Spectroscopy (FTIR) analysis infers the presence of aliphatic, aromatic, ketones, phenols, alcohols, esters, ethers, proteins and terpenoids. Gas Chromatography - Mass Spectrometry (GC-MS) analysis of the chloroform extract gave a tentative identification of 82 compounds. Squalene, a terpenoid was identified as the major compound in the GC-MS spectrum that could be attributed to enhanced bactericidal and larvicidal activity. These results suggest that latex is an effective stratagem to combat the diseases caused by vectors and microbes and need further investigation for the development of an effective insecticide.

1. Introduction

Diseases such as flu, influenza, dengue, malaria, tuberculosis, gonorrhea, tuberculosis, pneumonia, septicemia, urinary tract infections, Japanese encephalitis, dengue fever, chikungunya, filariasis and yellow fever caused by virus, bacteria, fungus and parasites are very hard to treat with antibiotics (Roopan et al., 2013; Benelli, 2015). This is due to the pathogens causing infections are resilient and have developed several ways to develop resistance against drugs (Stephen et al., 2011). Around the world, such diseases pose a great risk as most of the deaths happening worldwide can be attributed to these diseases particularly in developing countries (Tallury et al., 2010). In particularly, mosquito-borne diseases cause a severe concern in the society due to their disease transmitting capability and more than 700 million of people are affected per year.

In the Indian scenario, mosquito-borne diseases are well prevailing in all over the states due to favorable ecological conditions (Patil, 2012). Dengue fever is caused by an RNA virus belonging to family Flaviviridae and the principal vector responsible for transmission of Dengue worldwide is *Aedes Aegypti* (Erum et al., 2010). *Culex quinquefasciatus* is a vector for *Wuchereria bancrofti* which cause lymphatic filariasis. According to WHO, both the diseases are spread all over the India resulted in a high number of cases and death were reported between the year 2010 -– 2016 (Bhavna, 2013; Cecilia, 2014; Ramkumar et al., 2016). Though there is an extensive knowledge of the molecular pathogenesis of diseases and use of advanced therapeutics, the morbidity and mortality associated with bacterial and parasitic diseases still remain high.

Conventional synthetic insecticides organochlorines, carbamates, organophosphates, and pyrethroids temephos, fenthion, malathion, and dichlorodiphenyltrichloroethane (DDT) are very toxic, expensive cost, adapting resistance and lethal to nontarget organisms (Anyaele

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and Amusan, 2003). Keeping in view of the negative effects of synthetic products it is very imperative to develop plant-based drugs as a new generation of drugs in pharmaceutical perspective.

The biological control program (Biopesticides/ Bioinsecticides) have shown promise of being eco-friendly, potentially high toxicity against vectors and an alternative approach to synthetic insecticides (Chitra et al., 2014; Ravindran et al., 2015). Botanical insecticides contain chemical constituents' alkaloids, terpenoids, saponins, tannins, phenolics, essential oils and enzymes which act as repellents, feeding deterrents, toxins, and growth regulators (Pavela and Govindarajan, 2016). There are numerous studies conducted by researchers on different plant extracts against different instar larval stages of mosquitoes. (Govindrajan and Rajamohan, 2014; Sharma et al., 2014).

Plant latex has been of interest to mankind for millennia, with much of the research on its bioactive properties focused towards pharmaceutical applications (Rajkuberan et al., 2015, 2016a). Moreover, latex in the plant itself has the ability to cause toxicity against insects and butterflies. (Konno, 2011). *Euphorbia antiquorum* is a succulent plant belonging to the largest and the most diverse family in the plant kingdom, Euphorbiaceae. It is characterized by having 3 winged branchlets and stipular spines on sinuate repend wings. Stipules are transformed into spines. Latex present in branches contains β-amyrin, cycloartenol, euphol, euphadienol and euphorbol. Juice contains diterpene esters, euphorbin. Stem-bark and latex contain triterpenoids, taraxerol and taraxerone, friedelanol and *epi*-friedelanol, euphol (Rajkuberan et al., 2016b; Ghani et al., 2005). Therefore, the study emphasizes the antibacterial and the larvicidal activity of *E. antiquorum* latex extracts.

2. Materials and methods

2.1. Latex collection and processing

The plant *E. antiquorum* is located in and around Bharathidasan University campus (10.6747°N, 78.7442°E) and taxonomically identified in the Botanical Survey of India, South circle, TNAU campus, Coimbatore, India (Fig. 1a & b). The latex was collected in a clean, sterile bottle at early morning by cutting the leaf phyllode of *E.antiquorum* using a razor blade (Fig. 1c). After collection of the latex, it is poured on a Petri plate and kept in a hot air oven at 50 °C for 48 h for drying of latex. Subsequently, after drying, the dried latex materials were homogenized in a mortar and pestle for fine powder formation. 1gm of *E.antiquorum* latex powder was taken and mixed with 100 ml of chloroform and kept in an orbital shaker for 6 h. After then, the latex mixtures were filtered through Whatman filter paper no: 1 for removing any particles. The remaining dried mixtures were successively fractioned with double distilled water and methanol. The collected solvent mixtures were concentrated in a rotary vacuum evaporator for removal of solvents. The obtained crude extracts were further stored at −4 °C until further use.

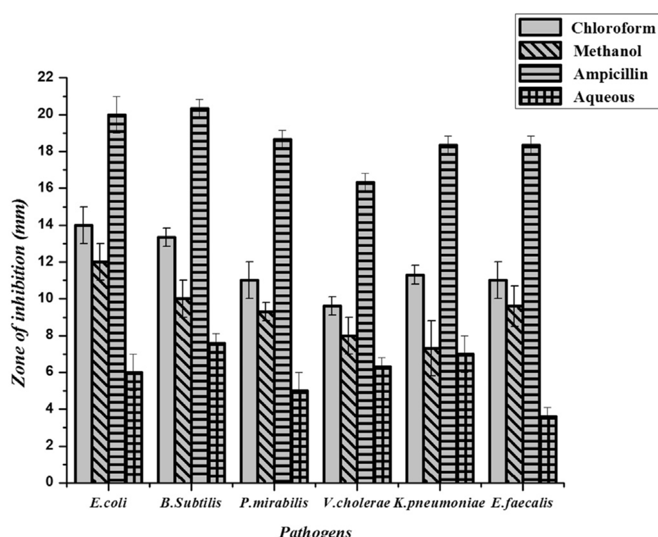

Fig. 2. Antibacterial activity of *E.antiquorum* latex extracts against tested pathogens. Zone values are mean ± SD of three replicates.

Fig. 3. Larvicidal activity of *E.antiquorum* latex extracts (Chloroform).

2.2. Antimicrobial activity of latex extracts

The antibacterial activity of crude latex extracts was tested for their antibacterial efficacy against selected human pathogens by using the agar well diffusion method. The pathogens *Escherichia coli* (MTCC-2622), *Bacillus subtilis* (MTCC- 2387), *Proteus mirabilis* (MTCC-1429), *Vibrio cholerae* (MTCC-3946), *Klebsiella pneumoniae* (MTCC-9544), *Enterococcus faecalis* (MTCC-3159) were procured from the Microbial Type Culture Collection (MTCC), Chandigarh, Government of India.

100 mg of crude latex extracts was suspended separately in 1 ml of chloroform, methanol and distilled water. The fresh bacterial culture of 100 µl having 10^8 CFU/ml was inoculated into the agar medium and

a *E.antiquorum* **b Leaf phyllode of *E.antiquorum*** **c Latex collection**

Fig. 1. a *E.antiquorum,* b Leaf phyllode of *E.antiquorum,* c Latex collection.

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