Evaluation of Leaf and Root Extracts of *Melia dubia* L. against Larvae of *Culex quinquefasciatus* and Five Important Human Pathogens

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Mosquito larvicidal activity and antimicrobial screening of ethyl acetate extract of leaves and root of *Melia dubia* belongs to the family of *Meliaceae* have been evaluated in the present study. Exposure of the larvae to these extracts for 12 hours led to 98 and 96% mortality, respectively. The results obtained show that this plant material exhibited significant activity and could be considered as potent natural larvicidal agent. The results of *in-vitro* antimicrobial screening of the crude ethyl acetate extract exhibited a wide range of activity on *E.coli*, *Salmonella typhi*, *S. paratyphi*, *Klebsilla penemonia*, *Staphylococcus aureus*. The extracts of the leaves and root inhibited the growth of *K. pneumonia*, *E. coli* and *Staphylococcus aureus* while only the leaf extract was active against *S. typhi* and *S. paratyphi*. The results obtained in this present study have lent scientific justification to some of the uses of the plant in ethno-medicine.

Key words: Melia dubia, larvicidal activity, Culex quinquefasciatus, Antibacterial activity,

Melia dubia commonly known as Malaivembu in Tamil is a member of the family Meliaceae. Every part of the plant is being used as traditional herbal medicines, such as anthelmintics, treatment of leprosy, eczema, asthma, malaria, fevers and venereal diseases (Govindachari, 1992) as well as cholelithiasis and acariasis (Kokwaro, 1976). It is well known as a rich and valuable source of bioactive limonoids (Awang et al., 2007). Although hundreds of limonoids have been isolated from various plants but, their occurrence in the plant kingdom is more abundantly in Meliaceae. Ongoing studies show that limonoids are highly oxygenated, modified terpenoids and have recently attracted attention because

compounds belonging to this group have exhibited a range of biological activities like insecticidal, insect antifeedant especially on some of the forest insect pests and growth regulating activity on insects as well as antibacterial, antifungal, antimalarial, anticancer, antiviral and a number of other pharmacological activities on humans (Koul et al., 2004, Endo et al., 2002, and Nakagawa et al., 2001). In the present study, ethyl acetate extracts of Melia dubia leaf and root were assayed for their larvicidal and antimicrobial, activity. The antimicrobial activity was determined by the agar well diffusion method. The larvicidal activity was against the larvae of the mosquito Culex quinquefasciatus.

MATERIALS AND METHODS

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The larvae used to test for the larvicidal activity were obtained from colonies of *Culex*

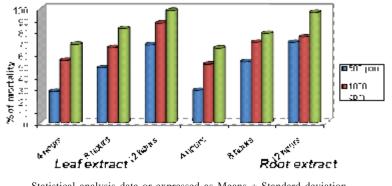
quinquefasciatus mosquitoes cultured and maintained in the laboratory at a temperature of $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and 80 - 90% relative humidity. The larvae were fed with mice feed and yeast powder in the ratio of 3:1. They were transferred to another clean bowl for three days at 24hrs interval and the water was aerated with the aid of an air pump. Larvicidal activity of the mosquito C. quinquefasciatus was assessed by following the standard WHO method (1996). The ethyl acetate extracts of leaf and root of Melia dubia for assayed against larvicidal activity was carried out at different concentrations ranging from 500, 1000 and 1500 ppm in distilled water. Twenty five third instar larvae of C. quinquefasciatus were collected separately and transferred gently to the test medium and simultaneously a control was maintained with ethanol-fresh tap water mixture. The larval mortality in both treated and control were recorded every 4 h, 8 h and 12 h. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. The experiments were replicated three times and conducted under laboratory conditions at 25 -30°C and 80 - 90% relative humidity.

A total of five bacterial cultures (E.coli, Salmonella typhi, Salmonella paratyphi, Klebsilla penemonia, Staphylococcus aureus) were used in this study. The bacterial strains were grown in nutrient broth at 37°C and they were stored on nutrient agar slants for future use. Anti-bacterial activity of plant extracts was tested by a modified well-in agar method (Sinclair and Dhingra, 1995). From the nutrient broth, the inoculum suspension was swabbed uniformly over the Muller Hilton Agar by using of sterile cotton swab. Subsequently,

using a sterile borer, well of 0.5 cm diameter was made in the pathogen inoculated media. Different concentrations, i.e., 20, 40 and 60 µl of each extract were aseptically filled into the well. Later the plates were placed at room temperature for an hour to allow diffusion of extract into the agar. Then the plates were incubated for 24 h at 37°C. The results were recorded by measuring the diameter of inhibition zone at the end of 24-48 h.

RESULTS AND DISCUSSION

The larvicidal activity of ethyl acetate extracts of leaf and root of M. dubia against C.quinquefasciatus mosquito larvae were given in Fig 1. The larvicidal activity of ethyl acetate extracts of leaf and root of M. dubia showed 98.27% and 96.65 of death with the use of 1500 ppm concentrations, respectively, after 12 hrs. In 12 hrs, 1000 ppm concentration killed more than 78% of the larvae in both extracts. In 12 hrs, 500 ppm concentration killed more than 65% of the larvae in both extracts. Among the two extracts, the leaf extract of M. dubia was found more lethal than root extracts. This work demonstrates the potency of M. dubia in the control of mosquito larvae. The high mortality recorded for leaf extract might be attributed to deficiency of dissolved oxygen in the water. The plant allelochemicals may be quite useful in increasing the efficacy of biological control agents because plants produce a large variety of compounds that increase their resistance to insect attack (Senthil Nathan et al., 2005). This result compared favourably with that from other species, for example, Yogananth et al., (2012) and Chanthuru et al., (2014) has showed



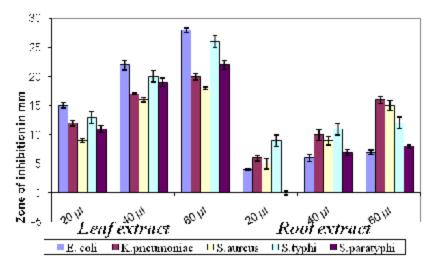
Statistical analysis data or expressed as Means ± Standard deviation

Fig. 1. Larvicidal activity of Melia dubia against Culex quinquefasciatus

larvicidal activity against the mosquito C. quinquefasciatus.

The result of the anti-bacterial activity tests of *M. dubia* leaf and root extracts are presented in Fig 2. The leaf extract at the concentration of 60 µl showed appreciable zone of activity against all the bacterial pathogens tested i.e., *E.coli, S. typhi, S.paratyphi, K. penemonia, S. aureus* (28, 20, 18, 26 and 22 respectively). The ethyl acetate extracts of root exhibited minimum activity in some of the organisms, namely *E.coli* and *S. paratyphi* (7 and 8 mm respectively). Among

these two extracts, the leaf extracts showed the maximum antibacterial activity than root extracts. Agreement with the earlier findings ethyl acetate extracts of *Andrographis paniculata* (Chanthuru *et al.*, 2014). The results of the study also supports the traditional application of the plant and suggests that the plant extracts possess compounds with antibacterial properties that may be used as antibacterial and antifungal agents in novel drugs for the treatment of gastroenteritis, uretritis, dysentry, typhoid fever, allergy, anaemia and skin disorder



Statistical analysis data or expressed as Means \pm Standard deviation **Fig. 2.** Antibacterial activity of *Melia dubia* against bacterial pathogens

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