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ANALYSIS

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PHYTOCHEMICAL ANALYSIS OF *BIXA ORELLANA* SEED EXTRACT

Nagamani. S, Gnanasoundari .A and P.Thangamathi*

PG and Research Department of Zoology, Kundavai Nachiyar Government College for Women (Autonomous), Thanjavur-612 001, Tamilnadu, India.

*Corresponding Author: thangamathi@gmail.com

Abstract

Phytochemical screening of ethanol extract of seed belongs to the family Bixaceae have been evaluated in the present study. Isolation and identification of bioactive compound from the *B. orellana* seed extract against the larvae of *A. aegypti* mosquito, the transmitters of dengue fever has been carried out. The analysis was performed using GC-MS which detects the presence of 25 different components, majorly hydrocarbons. The result shows the eight dominant compounds with a retention time relatively close and have the abundance percentage that are large enough which are identified as the most dominant compound which is abundance. The results justify the larvicidal potentials of bioactive compound from the seed aqueous extract of *B. orellana* and the need to incorporate them in vector management and control.

Keywords: *Bixa orellana*, Seed extract, GC-MS, Phytochemicals, *Aedes aegypti*

Introduction

Annatto, *Bixa orellana* L. (also known as 'achiote') is a perennial tree, native to the Neotropics (Arce, 1999) and the only species in the Bixaceae family. *Bixa orellana* L is a small evergreen tree native of the Central and Southern American rain forests (Perry, 1980). *Bixa orellana* extracts possess antiprotozoal, anthelmintic and platelet antiaggregant activity (Villar et al., 1997; Barrio et al., 2004). Root extracts have been reported to have spasmolytic activity (Mans et al., 2004). Extracts of leaves and branches have shown to be effective at neutralising the effects of snake venoms (Nunez et al., 2004). Extracts from different parts (leaves and seeds in particular) have displayed *in vitro* antimicrobial activity (Irobi et al., 1996; Castello et al., 2002; Fleischer et al., 2003). The seed extracts have been reported to exhibit chemopreventive (Agner et al., 2005) and antioxidant activity (Martinez-Tome et al., 2001). Bixin has also been found to have anticlastogenic activity (Antunes et al., 2005). The leaves of *Bixa orellana* have been reported to be used traditionally in the treatment of epilepsy (Ghani, 2003). Plants may be a source of alternative agents for control of mosquitoes, because they are rich in bioactive chemicals, are active against limited number of species including specific target-insects and are

biodegradable (Sukumar., et al., 1991) because mosquitoes develop genetic resistance to synthetic insecticides (Wattal, et al., 1981) and even tobiopesticides (Tabashnik., 1994).

Annatto extract is a natural food color, which is obtained from the outer coatings of the seeds of the Annatto tree (*Bixa orellana* L.) (Hagiwara et al., 2003). Approximately 80% of the pigments present in annatto seeds correspond to bixin (Preston and Rickard, 1980) but several other minor carotenoids have been isolated and identified, such as norbixin (Mercadante et al., 1997b; Mercadante et al., 1997a; Mercadante et al., 1999).

Mosquito spread various vector-borne diseases such as malaria, filariasis, Japanese encephalitis and dengue fever, which are transmitted by the three genera of mosquitoes namely *Anopheles, Culex and Aedes*. 40 million people in India suffer from mosquito borne diseases annually. There are over 3000 mosquito species belonging to 34 genera in the world. Of these, only about 300 transmit human and animal diseases. These diseases devastate Indian economy every year1. Dengue, dengue haemorrhagic fever and chikun-gunya are transmitted by *Aedes aegypti*. The symptoms of the disease are severe pain in the joints and muscles, skin eruptions. However, dengue fever is rarely fatal. The species breed, profusely in rainwater storage containers like cisterns, barrels, pots, etc. Dengue outbreaks are often associated with urban areas due to irregular potable water supply. Dengue fever continues in recurrent epidemic afflicting millions and causing thousands of deaths annually which is transmitted by *Aedes aegypti*. Mosquito are a serious threat to public health through which several dangerous diseases are transmitted in both animals and human beings2 Plants may be an alternative source of mosquito repellent agents since they constitute a rich source of bioactive chemicals (Ahn, Chang and Kim, 2002). A number of research has been focused on plant extract or phytochemicals as potential sources of biological control agents and commercial mosquito repellent agents. One of the approaches for control of these mosquito Borne diseases is the interruption of disease transmission by either killing, preventing mosquitoes to bite human beings (by using repellents) or by causing larval mortality in a large scale at the breeding centres of the vectors. Conventional pesticides such malathion, DDT and pyre-throides that are generally used for mosquito control are known to cause the problem of environmental pollution, residual effects and resistance by their indiscriminate use. Development of resistance to malathion (Guneady et al., 1989).

Mosquito control is very necessary because mosquitoes are not only nuisance as biting insects, but are also involved periodically in transmitting disease to humans and animals. Dengue

is the most important human viral disease transmitted by arthropod vectors. Annually, there are an estimated 50-100 million cases of dengue fever (DF), and 250,000 to 500,000 cases of dengue haemorrhagic fever (DHF) in the world (Rigau-Pérez et al., 1998). Over half of the world's population lives in areas at risk of infection, and these are popular tourist destinations. DF and DHF are caused by the four dengue viruses DEN 1, 2, 3 and 4, which are closely related antigenically. Infection with one serotype provides life-long immunity to that virus but not to the others. Dengue viruses are maintained in an urban transmission cycle in tropical and subtropical areas by the mosquito *Ae. aegypti*, a species closely associated with human habitation. In some regions other Aedes species, such as *Ae. albopictus* and *Ae. polynesiensis* are also involved (Gubler, 1995 and 1997).

Renewed interest has been shown in the development of alternative strategies, including the use of suitable type of natural insecticides derived from traditional botanical pest control agents. Seed of , *B. orellena* L. is regarded both as a notorious weed and a popular ornamental garden plant. It has found various uses in folk medicine in many parts of the world. B. orellena also produces a number of metabolites which some have been shown to posses useful biological activities (Ghisalberti, 2000). Mintweed or Chan, *H. suaveolens* has recently been shown to posses insecticidal properties (Singh and Upadhyay, 1993). Therefore, it is a challenging attempt to study these two plants for their phytotoxic acitivities as biological control agents. In the present study, the biological control against *Ae. aegypti* by the two extracts from *H. suaveolens* and *L. camara* was investigated and evaluated for their potential of insecticide in the control of *Ae. aegypti*.

Materials and Methods

Seed collection

The seeds of *Bixa orallena* were collected from plants growing in Thanjavur district of Tamil Nadu, India. The seeds of *B. orallena* were collected in sterile polythene bags and store in a laboratory.

Seed Powder preparation

The seeds were air dried under shade for 10-12 days at room temperature of 25 ±2°C. The dried seeds (100 g) were powdered mechanically using commercial electrical stainless steel

blender (Preethi, India/model- 2008) and the powder was kept separately in air tight containers and stored in a deep freezer.

Gas Chromatography – Mass Spectroscopy

(GC-MS) analysis the powdered sample (20 g) were soaked and dissolved in 75 ml of ethanol for 24 h. Then the filtrates were collected by evaporated under liquid nitrogen. The GC-MS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µ m df capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised upto 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

Results

Phytochemicals may serve as eco-friendly insecticide because they are relatively safe, in expensive and readily available in many parts of the world. Several plants are used in traditional for the mosquito larvicidal activity in many parts of the world. Our results showed that the ethanol seed extract of *B. orellana* (Figure 1) was subjected to GC-MS and the isolated the active compounds such as 2,4-Imidazolidinedione, 1-[[(5-nitro-2-furanyl1)methylene]amino]-Upiol (22.88%), Pthalic acid,allyl ethyl ester (10.27%), Pentadecanoic acid (7.78%), 9,12 – Octadentadienoic acid (5.54%), Haptadecanoic acid (5.42%), Benzene (3.51%), 9,12-Octadecadien-1-ol-Octadecanic acid (0.97%), Acetic acid (0.64%) were reported to possess Phytochemical properties (Table 1).

Discussion

Mosquito borne diseases are one of the most public health problems in the developing countries. It can be controlled by preventing mosquito bite using repellent, causing larval

mortality and killing mosquitoes. The leaves of *Bixa orellana* have been reported to be used traditionally in the treatment of epilepsy (Ghani, 2003). In this study, a methanol extract of *Bixa orellana* leaves was prepared to investigate whether it had any effect on the central nervous system and any role in controlling seizures in mice. As *Bixa orellana* leaves extracts have been reported to be useful in headaches, the extract was also investigated for analgesic activity using the acetic acid-induced writhing model. When administrated intraperitoneally to mice, acetic acid causes algesia by liberating noxious endogenous substances, including serotonin, histamine, prostaglandin, bradykinin and substance P that sensitise pain nerve endings (Collier et al., 1968).

The reported traditional use of *Bixa orellana* leaves in treating dysentery (Perry, 1980; Joshi, 2000) prompted us to screen the extract for antidiarrhoeal and antidysenteric activity. Evaluation of antidiarrhoeal activitywas performed using castor oil-induced diarrhoea model and gastrointestinal motility test in mice. Castor oil causes diarrhoea through its active metabolite ricinoleic acid (Ammon et al., 1974), The biological control of *Ae. aegypti* by *Argemone mexicana* L. seed was investigated (Sakthivadivel and Thilagavathy, 2003). The acetone fraction of the petroleum ether extract of *A. mexicana* seeds exhibited larvicidal and growth inhibiting activity against the 2nd instar larvae of *Ae. aegypti*. This activity occurred at higher concentrations (200, 100, 50 and 25 ppm). Mosquito larvicidal activity of *Piper longum* fruit-derived material against fourth-instar larvae of *Ae. aegypti* was examined by Lee et al. (2002). A crude methanol extract of *P. longum* fruits was found to be active against the larvae, and the hexane fraction of the methanol extract showed a strong larvicidal activity of 100% mortality. The biologically active component of *P. longum* fruits was characterized as pipernonaline by spectroscopic analyses.

Conclusion

Previously, synthetic chemical spray, such as DDT, was most applied to kill or reduce the number of mosquitoes, which damaged the environment as well. Natural products from plants are explored to replace the chemically synthetic insecticides. *B. orallena* seed is proposed for this purpose. *B. orallena* have lot compounds and control to the *Ae. Agepti*. This study aimed to use the extracts of *B. orallena* seed extract is biologically control dengue fever mosquito (*Aedes aegypti* Linn.) which is the vector of the yellow fever and the deadly hemorrhagic fever diseases.

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Table 1: Phytochemical compounds identified in *B. orallena* seed extract

S.No	RT	Name of the Compound	Molecular formula	MW	Peak Area %
1	19.444	2,4-Imidazolidinedione, 1-[[(5-nitro-2-furanyl1)methylene]amino]-Upiol	$C_8H_6N_4O_5$	238	22.88
2	19.492	Pthalic acid,allyl ethyl ester	$C_{13}H_{14}O_4$	234	10.27
3	24.045	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	7.78
4	24.927	9,12 – Octadentadienoic acid	$C_{18}H_{34}O_2$	282	5.44
5	24.395	Haptadecanoic acid	$C_{17}H_{34}O_2$	270	5.42
6	6.480	Benzene	C_8H_{10}	106	3.51
7	26.267	9,12-Octadecadien-1-ol-Octadecanic acid	$C_{18}H_{34}O$	266	0.97

Figure 1: GCMS analysis of Bixa orellana seed extract.