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Research Article

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Antibacterial and mosquito larvicidal activity of *Ficus casiguranensis* and *Ficus camarinensis* leaves

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ABSTRACT

Two Philippine endemic Ficus species, Ficus casiguranensis and Ficus camarinensis (Moraceae) were tested for their pharmacological activities. Acute oral toxicity test of the crude extracts using the OECD guidelines 425 showed their non-toxic characteristics up to 2000 mg/kg of body weight. Antimicrobial test using disc-diffusion assay showed that the crude and semi-crude extracts of both plants were active against the Gram-positive bacteria. The chloroform extract of F. casiguranensis was found to be active in all the organisms tested. A modified WHO protocol for larvicidal bioassay was used to determine the larvicidal activity of the crude and semi-crude extracts of both plants. The n-butanol extract of F. camarinensis had shown the lowest Lethal Concentration (LC_{50}) and LC_{90} at 268.5 ppm and 281.4 ppm for 24 hours and 265.2 ppm and 278.6 ppm for 48 hours, respectively.

Keywords: Moraceae, *Ficus casiguranensis*, *Ficus camarinensis*, mosquito larvicidal, antibacterial, Philippines

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INTRODUCTION

Dengue is an arboviral infection commonly spread by *Aedes* mosquitoes that had infected around 2.5 billion people and is currently becoming a serious health problem[1]. Dengue infects an estimate of 50 million people annually, and around 500,000 cases are life-threatening. Dengue is prominent in tropical and sub-tropical countries such as the Philippines, Cambodia, Malaysia, and Vietnam. These four countries in the Western Pacific region have more than a million cases and almost 5,000 deaths between the years 2001-2008. In 2008, the number of cases and deaths are higher in Cambodia and Philippines compared to the other countries in the region. Dengue imposes significant liability in health, economy, and society of the people where the infection is prevalent. The estimated count of disability-adjusted life years (DALYs) that was lost to Dengue infection in 2001 was 528 worldwide[2].

The increase in the morbidity rate of Dengue in the Philippines has lead government agencies and other organizations to devise various means of controlling the vector. One of the effective ways to control dengue is by destroying the larvae by the use of insecticides, but the insecticides that are used today are synthetic and non-selective which can pose danger to different organisms as well as the environment. The toxicity of synthetic insecticides and the development of resistance in insects had made it necessary to discover novel insecticides and further research on plant-based insecticides[3].

As per World Health Organization (WHO) reports, infectious diseases are responsible for over 50% deaths worldwide, occurring mainly in tropical and developing countries[4]. Unhealthy lifestyle, stressful living conditions, unhygienic and polluted environment contributes in making humans susceptible to infections. Antibacterial drugs used today are either synthetic or semi-synthetic which can cause toxicity and undesirable effects leading to more complications. Poor access to modern antibiotics due to logistics and socioeconomic conditions and development of resistance of bacteria to commonly used antibiotics are major problems that we face today hence the need for continuous discovery of compounds that are safe, effective, and economical.

With these rationales, there is a demand in the discovery of ways on how to combat the increasing number of dengue cases and infectious diseases using plant-based extracts or compounds. Based on our literature search, the genus Ficus (Moraceae) species showed potential as larvicidal and antimicrobial agents. Ficus species are also known to have antibacterial properties include *F.chlamydocarpa, F. cordata,* and *F. odata*[5-6]. and *F. benghalensis* and *F. sarmentosa var. henryi* have shown positive larvicidal activity against mosquito larvae[3-7]. Two of these species worth investigating are *Ficus casiguranensis* Merr. and *Ficus camarinensis* Merr. Both are endemic to the Philippines and no studies have been conducted yet. In this paper, we herein report the antimicrobial and mosquito larvicidal activities of these two *Ficus* species.

EXPERIMENTAL SECTION

2.1 Collection and authentication of plant materials

Fresh leaves of *F. casiguranensis* from Casiguran, Aurora, Philippines and *F. camarinensis* from Camarines Sur, Philippines were collected and authenticated by Dr. Wilfredo F. Vendivil, Curator II at the Philippine National Museum. A voucher specimen with control number 007035 was deposited at the Philippine National Museum.

2.2 Extraction and fractionation procedure

The fresh leaves of *F. casiguranensis* and *F. camarinensis* leaves were air-dried away from direct sunlight and were pulverized using a Wiley Mill with fine mesh. The ground leaves (651 g *F. casiguranensis* and 1122 g *F. camarinensis*) were extracted exhaustively with technical grade MeOH in a percolator. Each MeOH extraction was done for 72 hours. For *F. casiguranensis*, a total of 8.3 L MeOH was used while 8.5 L was used for *F. camarinensis*. The combined filtrates were concentrated under reduced pressure at temperatures below 50°C to obtain the crude methanolic extract. The percentage yield of each extract was computed. The extracts were stored in a tightly sealed container and refrigerated.

The respective plant crude methanolic extract was suspended in distilled water and partitioned using hexane (thrice). The hexane upper layers were collected, dried with anhydrous Na_2SO_4, and concentrated under reduced pressure to obtain the hexane extract. The aqueous layer was then partitioned with $CHCl_3$ (thrice). The combined organic layer was dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain the $CHCl_3$ extract. The remaining aqueous layer was partitioned with *n*-BuOH (thrice) and the collected organic layer was concentrated under reduced pressure.

The crude and semi-crude fractions were subjected to phytochemical screening by thin-layer chromatography using chemical spray reagents.

2.3 Acute Oral Toxicity Test (OECD guidelines 425, 2005)

Following the OECD guidelines 425 for acute oral toxicity test using main test for extracts or chemical compounds that have no established level of safe dose, twelve (12) female Swiss mice were bought at the Philippine Food and Drug Administration and housed at UST-RCNAS Animal House. According to OECD, female Swiss mice are generally slightly more sensitive than their male counterparts. This experiment was approved by the Bureau of Animal Industries (BAI) and given an animal research permit with the reference number 08-2013-43. The mice were acclimatized for 7 days and were given food, water, and the cages were cleaned daily. Three doses were used in the experiment, 175 mg/Kg, 550 mg/Kg, 2000 mg/Kg, respectively. The methanolic extracts of *F. casiguranensis* and *F. camarinensis* were dissolved in Tween (Polysorbate) 80. The mice were fasted 3 hours before the administration of the extracts and 1 hour after administration. The amount administered was 0.4 mL which is the average capacity of the stomach of the mice. The extracts were administered using an oral gavage. After the administration the animals were observed for 48 hours before increasing the dose.

2.4 Gross Necropsy

The female Swiss mice were brought to a licensed veterinarian for gross necropsy. The mice were euthanized using cervical dislocation, dissected, and the organs were observed. After the observation, the organs and the body of the mice were placed in a yellow plastic bag and disposed in Research Institute for Tropical Medicine (RITM).

2.5 Antimicrobial Assay

The test organisms used in this experiment are the following: *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 25922), *Pseudomonas aureginosa* (ATCC 27853), and *Kliebsiella pneumoniae* (ATCC 13883).

The Disc-Diffusion assay was used to determine the antimicrobial activity of the crude and semi-crude extract of *F. casiguranensis* and *F. camarinensis*. Standardization was done using 0.5 Mcfarland to adjust the turbidity of the

inoculum. After having the same turbidity as the standard. the inoculum was streaked onto the Mueller-Hinton Agar. Three sterile filter papers of 6 mm diameter (Schleicher & Schuell CAT number 2017-006 Grade AA discs) were saturated with 20 µL of the extract (250 mg of the crude extract dissolved in 1 mL of methanol). A concentration of 5 mg of the extract per disc was obtained. Then, One sterile filter paper with the same diameter was saturated with methanol as the negative control. After completely drying the discs, the plate was inverted and incubated for 24 hours at 37°C. The zone of inhibition was measured with a metric ruler (Orion) and reported as an average of three measurements.

2.6 Larvicidal bioassay

Third instar *Aedes aegypti* larvae were bought in University of the Philippines (UP) department of parasitology for the experiment. Using modified WHO guidelines for larvicidal bioassay, each cup was filled with 50 mL of water and extracts. Ten (10) larvae were placed per cup. The concentration per cup was 1000 ppm, 750 ppm, 500 ppm, and 250 ppm of the extract. For the positive control, BTI or *Bacilus thuringensis israelis* was used and for the negative control distilled water was used. They were observed and recorded after 24 and 48 hours.

2.7 Data Analysis

The data collected was calculated using probit regression of LC_{50} and LC_{90} using SAS version 9.0.

RESULTS AND DISCUSSION

3.1 Extraction and phytochemical screening of the plant materials

Extraction of the air-dried leaves of *F. casiguranensis* and *F. camarinensis* afforded the crude methanolic extracts in a dark-green paste consistency. *F. casiguranensis* obtained a 15.36% yield from based from the ground leaves while 14.62% was obtained from *F. camarinensis*.

The crude and semi-crude extracts were then subjected to phytochemical screening using TLC and chemical spray reagents. The results have indicated the presence of terpenoids, higher alcohols and essential oils (vanillin-sulfuric acid spray), steroids (acetic anhydride-sulfuric acid spray) and phenols, tannins and flavonoids (potassium ferricyanide-ferric chloride spray).

3.2 Acute Oral Toxicity Test (OECD guideline 425, 2005)

All of the test animals survived the given doses (175 mg/Kg, 550 mg/Kg, 2000 mg/Kg) administered to them. This concluded that both extracts are safe at 2000 mg/Kg body weight. A licensed veterinarian certified that the animals also underwent gross necropsy showing that the organs are morphologically normal. however, the weight of the organs were not documented by the veterinarian as they were seen to be normal.

3.3 Antimicrobial Test

The antimicrobial disc-diffusion assay utilized six microorganisms which are composed of three Gram-positive bacteria namely *Staphylococcus aureus*, *Bacillus cereus, Staphylococcus epidermidis*, and three Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*.

Results showed that the crude and semi-crude extracts of both plants were active against the Gram-positive bacteria. The chloroform extract of *F. casiguranensis* was found to be active in all the organisms tested. Using the Guideline to Phytochemical Screening antimicrobial chapter as a reference, The chloroform extract of *F. casiguranensis* was very active against *S. aureus* (27 mm), *B. cereus* (30 mm), *E. coli* (24 mm), and *K. pneumoniae* (24 mm). It was active against *S. epidermidis* (14 mm), and partially active against *P. aeruginosa* (10 mm). To the best of our knowledge, this is the first study on the antimicrobial activity of the two endemic *Ficus* species. However, the antimicrobial activity of some *Ficus* species had been documented in different literature. The compound (9,11), (18,19)-disecoolean-12-en-28-oic acid isolated from *Ficus benjamina var. comosa* was discovered to be showing significant antimicrobial activity against *S. typhimurium* (MTCC-98), *C. albicans* (IAO-109), *S. aureus* (IAO-SA-22), *E. coli* (K-12) and low activity against *A. niger* (lab isolate ICAR), *A. brassicola*.[(8)] The recent experiment gives a significant basis for the use of extracts from *Ficus chlamydorcapa and Ficus cordata* for the treatment of infections associated with the studied microorganisms[8]. *Ficus auriculata* showed potential antibacterial activity even though it was comparatively weaker than standard antibiotics[9].

3.4 Larvicidal Bioassay

A modified WHO protocol was used to test for the larvicidal activity of the crude and semi-crude extracts of both plants. The set-up used ten (10) 3rd to 4th instar mosquito larvae submerged in 50 mL of distilled water with different concentration of the plant extract per cup namely 1000 ppm, 750 ppm, 500 ppm, and 250 ppm of the extract. This experiment determined the LC_{50} and LC_{90} of the extracts using a probit linear regression. The butanol

extract of *Ficus camarinensis* had shown the lowest LC_{50} and LC_{90} at 268.5 ppm and 281.4 ppm for 24 hours and 265.2 ppm and 278.6 ppm for 48 hours, respectively. While the positive control, *Bacillus thuringiensis israelis* have a LC_{50} of 0.0195ppm and LC_{90} of 0.0251ppm[10]. LC_{50} is the concentration at which half of the sample will be killed. It is also known as the median lethal concentration. Likewise, LC_{90} is the concentration at which 90 percent of the sample will be killed.

CONCLUSION

The extracts of *F. casiguranensis* and *F. camarinensis* showed to be a promising source of antibacterial agent/s and mosquito larvicide. The result of the acute oral toxicity of the plant extract revealed that it was safe even at 2000 mg/Kg per body weight and did not cause any morphological change in the organs of the mice. The antibacterial activity of the Ficus species mostly showed effect on gram positive bacteria; however, the chloroform fraction of *Ficus casiguranensis* showed activity on both gram positive and gram negative bacteria. Furthermore, it also has the highest activity among the different extracts. The mosquito larvicidal activity of the plant extracts showed activity against *Aedes aegypti* larvae. These shows that the two Philippine endemic *Ficus* species are new biologically-active materials worthy of further scientific investigations.

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