The Antimicrobial Assay of Coumarins from Kakawate *(Gliricidia sepium)* Jacq. Kunth ex Walph. Leaves against *Staphylococcus aureus, Escherichia coli,* and *Trichophyton mentagrophytes*

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Abstract

To determine the active principle found in the leaves of kakawate (Gliricidia sepium) Jacq. Kunth. ex Walph., the kakawate leaves were soaked separately in distilled water and petroleum ether. Pure compounds coumarins were isolated, characterized, and identified by mass spectroscopy (MS), ultra violet (UV) spectrophotometry, infrared (IR) spectrophotometry, proton (JH) and carbon 13 (13C) nuclear magnetic resonance (NMR) spectroscopy.

Bioassay of the coumarins against microorganisms were tested against Escherichia coli, Staphylococcus aureus, and Trichophyton mentagrophytes. The coumarins manifested antimicrobial index of 14.5 against the fungus Trichophyton mentagrophytes.

Introduction

Most of our medicines are prepared and fonnulated synthetically but a lot of side effects have been observed among users. Natural Products Chemistry gives the answers to the problem. Scientists found alternatives to the existing technology and geared their studies to the utilization of renewable sources like plants, animals, and microorganisms believed to contain potent bioactive substances that eradicate diseases. Due to the use of these bioactive substances, residues easily disintegrate and do not become part of the food chain, hence, are not harmful to the environment.

Objectives of the Study

The main objective of the study was to determine the bioactivity of the active principle found in the leaves of kakawate *(Gliricidia sepium)* Jacq. Kunth. ex Walph and

its effects on microorganisms particularly *Escherichia coli, Staphylococcus aureus,* and *Trichophyton mentagrophytes.*

Review of Related Literature

Kakawate *(Gliricidia sepium)* Jacq. Kunth. ex Walph. is a small tree widely grown throughout the Philippines. It was introduced to the Philippines by the Spaniards in the mid 1500 during the Manila-Acapulco Trade Relations. It is commonly called madre de cacao. It is named Bunga Jepun in Malaysian Peninsula for its flowers are like the cherry blossoms of Japan. English-speaking countries call it Mexican lilac. In Mexico, kakawate is known as madero Negro and matarraton in Colombia, South America (Calle and Nathan, 1987).

James and Ubina (1990) found out that kakawate water extract was effective against budworms giving 17% and 43.5% larval mortality 24 and 48 hours after treatment, respectively. For ethanol extracts, tobacco roots and kakawate bark both gave 34% mortality against cutworm. The biology and control of the bee mite, *Varroa jacobsoni* (Oudemans) were studied. The life cycle occurs primarily in the bee cell. The female mites attach themselves to the emerging adult bees while the males stay inside the cell. Tetradiform strips, naphthalene and leaves of *Gliricidia sepium* (Jacaq.) Steud. (madre de cacao) were tested for the control of bee mites. High mortalities were observed in *G. sepium* (Cervancia and Aspiras, 1987).

Several plant species including kakawate that were suspected to have fungicidal properties were collected and crude water extracts thereof were screened for their fungicidal properties in vitro against *Aspergillus flavus* and *Aspergillus parasiticus.* The crude extracts of kakawate *(Gliricidia sepium)* was found to be effective against *A. flavus* and *A. parasiticus.* Aflatoxin formation was not detected on potato dextrose broth when the crude plant extracts were inoculated with *A. flavus* or *A. parasiticus* (Garcia and Garcia, 1988).

The steam distillate of fresh mature leaves was found to possess an odorous viscous oil exhibiting antifungal properties against *Trichophyton mentagrophytes* in vitro.

Methodology.

1. Microbial Bioassay

Culture
Microbial suspension
Nutrient agar

Potato dextrose agar (PDA)
Seeding
Incubation
Detennination of Clearing Zone
Antimicrobial Index (Al)

Materials and Methods

Microbial suspension containing approximately 600 million cells per mL were prepared from 24-hour old culture of *E. coli*, and *S. aureus*, and from a 5-day old culture of *T. mentagrophytes*.

microbial suspension
suspending – 0.1% peptone water

0.1 mL suspension + 5 mL medium (melted, 45C)

nutrient agar (N.A.) plate

or

potato dextrose agar (P.D.A.) plate

cut 1.0 cm well

put 200 uL (coumarin)

30 ug (standard reagent)

nutrient agar incubated
37C, 24 hours

potato dextrose agar incubated
30'C, 5 days

measure the clearing zone

One-tenth mL of *E. coli* and *S. aureus* suspension were transferred into nutrient agar plates, while suspensions of *T. mentagrophytes* were transferred into potato dextrose agar plates. Two hundred uL of samples and 30 ug of standard antimicrobial agent were used.

$$\text{Antimicrobial Index} = \frac{\text{Diameter of Clearing} - \text{Diameter of well}}{\text{Diameter of Clearing}}$$

Results and Discussion

Pure compounds isolated from kakawate *(Gliricidia sepium)* leaves were tested on three microorganisms. *Escherichia coli* and *Staphylococuss aureus* were prepared from 24-hour old culture and *Trichophyton mentagrophytes* was prepared from 5-day culture. *E. coli* and S. *aureus* suspension were transferred to nutrient agar plates while *T. mentagrophytes* was transferred to potato dextrose agar plates. Two hundred uL of kakawate coumarins were assayed against the microorganisms; 30 ug of standard antimicrobial agent were used. To test the effect of the sample on *E. coli,* chloramphenicol was used as standard. Tretracycline was used against S. *aureus* and clotrimazole against *T. mentagrophytes.*

Table 1. Results of observation **on the** effects of *Gliricidia sepium* on *Escherichia coli, Staphylococcus aureus,* and *Trichophyton mentagrophytes.*

SAMPLE	*E. COLI*			*S. AUREUS*			*T. MENTAGRO-PHYTES*		
Gliricidia sepium	1	2	**AI**	1	2	AI	1	2	AI
Coumarin	-	-	0	-	-	0	14	15	14.5
Chi			2.0						
Ctr									2.0
Tet						2.5			

Legend: Chi – Chloramphemcol
Ctr – Clotrimazole
Tet - Tetracycline

Table 1 shows that the bioassay of the chloramphenicol manifested an antimicrobial index (AI) of 2.0 against *E. coli*. Tetracycline showed a value of 2.0 against *T. mentagrophytes*. Kakawate coumarin tested in two replicates showed negative results in *E. coli* and *S. aureus*. However, it showed antimicrobial indices of 14 and 15 against *T. mentagrophytes*. On the average, the Al is 14.S which indicates a close value to the reference, that is, clotrimazole, which is 2.0.

Conclusion

It is concluded that the pure compound coumarins isolated from kakawate *(Gliricidia sepium)* leaves show bioactivity. It can inhibit the growth of fungus *Trichophyton mentagrophytes*. It is further concluded that coumarins isolated from kakawate is a fungicide.

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