

The Antibacterial Screening of Sineuelas Aso (*Casearia grewiaefolia*) Leaves Ethyl Acetate Extract

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Abstract

Leaves of sineuelas aso (Casearia grewiaefolia) were extracted using ethyl acetate, ethanol, water and hexane solvent. The extract was screened against E. coli UPCC 1195 and S. aureus UPCC 1143 using the agar cup method. The assay was conducted in three replicates. The solvent used was also screened against E. coli. The control used was tetracycline.

Results showed that ethyl acetate extract manifested an average antimicrobial (AI) of 1.62 compared to the control i.e. tetracycline having an AI of 2.0. However, ethyl acetate solvent showed an AI of 0.9. All other extracts and solvents manifested zero clearing zone and zero antimicrobial indices.

Introduction

Background of the Study

Sineuelas aso (*Casearia grewiaefolia*) belongs to the family of the Euphorbias or Spurge family more specifically *Euphorbiaceae*. It is endemic to the plains and homesteads of Metro Vigan in the Province of Ilocos Sur. It grows from a shrub to a tree 7 meters to 12 meters high. It bears white, small and fragrant flowers and light to yellow fruits in the form of berries. The berries contain red seeds when matured and are usually eaten by insects and mostly birds. The leaves are dark green and big, ovobate in shape. According to folks, the fruits are not edible and are likened to the fruits of the *Lansium* family and the leaves are used to drive away or kill insects.

Parts of plants like leaves, barks, seeds and roots are the major locations where most of the secondary metabolites are formed. These secondary metabolites are formed resulting from primary metabolism in

Paper presented at the 33rd PSM National Convention at Heritage Hotel, Pasay City on April 28-30, 2004.

plants. Primary metabolism includes the process of photosynthesis that is usually undertaken in leaves in the presence of chlorophyll but once manufactured they are stored further in the different parts of the plant, mostly in the seeds, barks, and roots of plants. Some are simply stored in the leaves and fruits.

Secondary metabolites often referred to as natural products are important not only for the plant but also for human consumption. Humans use them as food, medicine, colorants, insecticides and many others. In order for humans to fully use their potentials, bioassays are conducted so as to determine what particular use or importance they provide. In this study, antimicrobial assay was conducted. An antimicrobial assay was performed using *Escherichia coli* and *Staphylococcus aureus*.

Review of Related Literature

Sineguelas aso or larnlamoyot as it is locally known is endemic to Ilocos. *Caesaria grewiaefolia* Vent belongs to the family Flacourtiaceae. It is a medium-sized tree growing in tropical areas of the globe. It grows well in the Philippines, Thailand, and Vietnam. It has oblong leaves which is shiny and dull underneath. The blades are moderately serrated. Its flowers are minute and white. The juicy fruits are yellow to golden yellow when matured. The seeds are red from young to maturity. They are eaten usually by birds.

Flacourtiaceae includes many plants like trees, shrubs and herbs. They are useful as multipurpose trees (MPT). Oftentimes, they are used as fuelwood, medicine, flavorings, and colorants. Because of their vulnerability to drought and rains, they are used as landscape trees and environment control and sustainability agents. This family of plants have similarities with Euphorbiaceae species only that they exude much meager sap yet stickier substances. Such characteristics are innate in Flacourtiaceae species.

The plant is a host to endophytic fungi. In Vietnam, *Caesaria grewiaefolia* is known as "tulibas". It is commonly used in landscaping.

Kato (1996) studied the pollination interactions in the lowland dipterocarp forest in Sarawak. He found that wasps of *Vespidae* were attracted to *Caesaria grewiaefolia*.

Caesaria grewiaefolia was identified by Rabena (2001) as an endemic species in Ilocos with multipurpose uses. It is accounted as fuelwood, feedant for birds, urban shed and environmental control and sustainability agent. This medium-sized tree survive in critical land and climatic conditions. It can withstand

both drought and heavy rains. Parts of the tree are used *in* many purposes. The barks of the tree are used to kill pests in households. The barks are dried under the sun and placed in places where pests are most observed and seen. Its fruits are observed to be eaten by birds particularly "pios", "panal" and "pagaw". Birds open the fruit and eat the red seeds. They are not killed instead they continually visit the fruits especially during cool temperatures of the day. Other organisms are also being studied particularly those that thrive during wet and dry months of the year and on specific locations of the tree.

Methodology

Samples A- Water Extract
 B- Ethanol Extract
 C- Ethyl Acetate Extract
 D- Hexane Extract

Test Organisms: *Escherichia coli* UPCC 1195
 Staphylococcus aureus UPCC 1143

Microbial suspensions containing approximately 600 million cells per ml were prepared from one 24-hour culture of the organisms. The suspending medium used was 0.1% peptone water.

One tenth (0.1) mL aliquots of the cell suspensions were transferred into pre-poured nutrient agar (NA). About 5 mL of the medium, melted and cooled to about 45 °C, was poured into the plate. The plate was swirled to distribute the microbial cells evenly on the plate and agar overlay was allowed to solidify. Four 3-cm wells were cut from equidistant points of the seeded plates using sterile cork borer. Two hundred (200) µL portions of the sample were transferred into each well.

The plates were incubated at room temperature for 24 hours. The clearing zone was measured in millimeters and the average diameter of the clearing zone was calculated. The antibacterial index (AI) was computed using the following formula:

$$AI = \frac{\text{Diameter of clearing zone} - \text{Diameter of Well}}{\text{Diameter of the Well}}$$

Results and Discussion

Leaves of sineguelas aso (*Casearia grewiaefolia*) were collected from Rugsuanan, Vigan City. The leaves were air dried and chopped into small pieces.

The leaves were further soaked in four different solvents; namely, water, ethyl acetate, ethanol, and hexane.

Table 1. The antimicrobial indices of sineguelas aso extracts using different solvents against *Escherichia coli* (UPCC 1195).

Samples	Antimicrobial Index (AI)
1. Water extract	0
2. Ethanol	0
3. Ethyl acetate extract	1.62
4. Hexane extract	0
5. Tetracycline	2.0
6. Ethyl acetate (alone)	0.9

Table 2. The growth inhibitions (in cm) of the sineguelas aso ethyl acetate extract against *Escherichia coli*.

Replicate	AI	Diameters (cm)
R-1	1.6	7.8
R-2	1.7	7.9
R-3	1.56	7.7
Average	1.62	7.8

Conclusion

It is concluded that the leaves of sineguelas aso (*Casearia grewiaefolia*) extracted using ethyl acetate solvent inhibits the growth of *Escherichia coli*. It manifested an antibacterial index of 1.62 at the average which is as efficient as the tetracycline, a popular medicine bought over the counter i.e. 2.0

Recommendations

It is recommended that further studies must be conducted on the screening of the fruits of sineguelas aso. A pharmacological study must be conducted on both the leaves and fruits in order to utilize the potentials of this tree. Lastly, a thin layer

chromatography must also be performed in order to determine the active principle found in the leaves which inhibited the growth of *E. coli*.

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