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Research Article

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Qualitative phytochemical analysis and microbial inhibitory activities of pacific rain tree (*Samanea saman* (jacq.) Merr.) Pods

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Abstract In the past decades, crop diseases and human health is always at stake and the emerging problem on the use of synthetic antipathogens and medicine is one of the most difficult to combat. This current research was carried out to determine the phytochemical constituents and the inhibitory activities of *Samanea saman* (Jacq.) Merr. pods. Eight preliminary phytochemical tests was done which includes, test for alkaloids saponins, flavonoids, tannins, glycosides, steroids, terpenoids and resins. Powdered pods were subjected to ethanol and aqueous extraction. Alkaloids, saponins, tannins, glycosides, steroids, terpenoids and resins was found present on both extracts, however absence of flavonoids on both extraction was observed.The inhibiting capability of the *S. saman* pods revealed that there is a significant effects on the inhibitory effects against in-vitro bioassay of *F. oxysporum*, known to cause wilts on crops and the two bacterial pathogens *E.coli* and *S.aureus* via disc diffusion method.

Keywords *bioassay, crop wilts, disc diffusion, extracts, phytochemicals*

Introduction

In the past decades, the use of antipathogens to control crop disease has contributed to increased production of food worldwide, plant diseases were caused by many pathogens such as fungi, bacteria, nematodes and viruses. Among the list of many pathogens affecting plants, fungi is the major contributor causing about 90% yield loss and most of the agricultural plants have been reported to have at least one species of Fusarium associated disease, a destructive disease that has led to significant yield and quality losses for farmers and to contamination of its mycotoxins [1-2]. Several effective synthetic antipathogens are now being studied about their effects of on human health and environment and many research efforts have been carried out to find alternatives and environmentally safe methods can be used to control plant diseases [3]. With the increasing pesticide residues concern in agricultural products and environment as well as the incidence of pathogen-resistant chemical pesticides, the use of non-chemical based and eco-friendly methods that includes natural metabolites have assumed greater significance for a better crop yield [4]. To counteract with the highlighted challenge, mankind must develop a potent anti-pathogens against the already emerged resistant pathogen strains and even the emerging ones [5].

In the Philippines, *Samanea saman*, also known as Akasya (Filipino), Rain Tree or Monkey Pod (English) belonging to Family Fabaceae, is easily recognized by its umbrella-shaped canopy that usually reaches 15-25m (50-80ft) in height. It is an important tree in the Pacific as a shade tree on small farms, along roads, in parks and pastures [6]. Many plants possess antimicrobial activities and are used for the treatment of different diseases dated back to prehistoric tradition which uses natural substances or extracts that possesses broad spectrum of synthetic activity and

have been the source of many useful compounds [7-8]. With these findings, an emerging interest in the possible application of these phytochemicals in the development of new drugs for human and plant disease management is at stake. In addition, the knowledge shifted the direction for new drug search towards plant sources thereby leading to the recent increased studies on different solvent extracts of plant species originally used in traditional practice [2, 9].

Materials and Methods

Collection and Extraction of Samples

The pods used in were collected from the trees surrounding the college from April to May. The pods were sundried for 48 hours prior storage on a Ziploc for storage. Pods used were oven dried for six (6) hours at 70ºC and powderized using mortar and pestle separating the seeds. For the preparation of ethanol extracts, 50g grams of powdered *S. saman* pods were soaked in 250ml 95% ethanol for 48 hours at room temperature. The suspension was filtered and filtrate was subjected to a rotary evaporator for 200rpm. For the preparation of aqueous extracts, 50g of powdered *S. saman* pods were suspended in a 250ml sterilized distilled water for 24 hours. Suspension was filtered using wattman's filter paper no. 01 and filtrate was stored in a sterile amber bottles and kept refrigerated prior use.

Phytochemical Screening Test

Biochemical tests for the screening and identification of bioactive chemical constituents in the medicinal constituents under study were carried out in extracts using the standard procedures as described by [8, 10].

Test for alkaloids

Extracts were dissolved individually in diluted Hydrochloric acid and filtered. Filtrates were treated with Wagner's reagent (Solution of Iodine in Potassium Iodide). Formation of a reddish brown colored precipitate indicates the presence of alkaloids.

Test for Saponins

To test the saponins, 2g of powdered sample is boiled together with 20ml of distilled water in a water bath and filtered. 10ml of the filtered sample was mixed with 5ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing is then mixed with 3 drops of olive oil and for the formation of emulsion which indicates the presence of saponins.

Test for Flavonoids

To determine the presence of flavonoids, 2 to 3 drops of 1% NH3 solution was added to the aqueous extract of each sample in a test tube. A yellow coloration is observed if flavonoid compound is present.

Test for Tannins

Tannins were determined by boiling 0.5g of powdered sample in 20ml distilled water in a test tube and filtered. 0.1% $FeCl_3$ was added to the filtered samples and observed for brownish to green or a blue to black coloration which shows the presence of tannins. Green coloration indicates the presence of gallotannins while brown coloration indicates the presence of pseudotannins.

Glycosides

Test for glycosides was determined by preparing 1ml of concentrated H_2SO_4 in a test tube where 5 ml of aqueous extract from the sample was mixed with 2ml of glacial acetic acid containing 1 drop of $FeCl_3$. The above mixture was carefully added to 1ml of concentrated H_2SO_4 so that it is underneath the mixture. If cardiac glycoside is present in the sample, a brown ring will appear indicating the presence of the cardiac glycoside constituent.

Evaluation of the Microbial Inhibitiory Activities of Rain Tree Pods

Dilution of Extracts

Each extracts were diluted on the following concentrations: for the antibacterial assay, 10ppm, 100ppm and 1000ppm was used and for the antifungal assay, 1mg/ml, 5mg/ml and 10mg/ml was used.

Source of Microbial Cultures

Pure cultures of *Fusarium oxysporum* was obtained from the Laboratory of fungal collection of RM-CARES, Research and Extension, CLSU. Bacterial strains, *E. coli* and *S. aureus* was obtained from the bacterial culture collection of Department of Biological Sciences, College of Arts and Sciences, CLSU.

Antifungal Assay

Antifungal assay was carried out using 2ml of prepared ethanol extracts at different concentrations were aseptically poured on a sterile standard plate and approximately 15-20 ml of sterilized Potato Dextrose Agar (PDA) was added, aseptically swirled on a clockwise and counter clockwise motion to homogenize mixtures. Mixture was then allowed to cool and solidified. Each plates containing mixtures were aseptically inoculated with a 10-mm fungal disk. Inoculated standard plates were incubated on a room temperature (28-32°C) and growth was measured using calibrated Vernier caliper for every 24-hours for 5 days.

Antibacterial Assay

The antibacterial assay was done following the Kirby-Bauer Method against *E. coli* and *S. aureus*. 6mm of sterile paper discs were soaked on the ethanol and aqueous extracts for about 30-40 mins to allow absorption of the extracts. The discs were aseptically inoculated onto the plates with the test organisms with proper labels each disc. Zones of inhibition was observed at 8^{th}, 16^{th} and 24^{th} hour of incubation.

Statistical Analysis

Analysis was laid out Completely Randomized Design (CRD) with three (3) replications per treatment combination. The results presented are the means ± standard deviation of three replicates. The recorded data were treated statistically using the one way analysis of variance (ANOVA). The means were compared by Least Significant Difference test at $p < 0.05$ using SPSS v.20.

Results and Discussion

Phytochemicals are naturally occurring constituents of plants. Researchers have been widened to prove the so-called bioactivities of these constituents. The presence of these phytochemical constituents were carried out in this present study**.** *Samanea saman* pods exhibited low to high presence of the different biochemical constituents present. Table 1 showed that alkaloids present on both the ethanol and aqueous extract are moderate in amount.

On the other hand results exuded by ethanol extracts on the presence of saponins is greatly higher than aqueous extracts. However, flavonoids is basically absent on both ethanol and aqueous extract. Moreover, tannins, glycosides, steroids, terpenoids and resins on ethanol extracts are present in moderate appreciable amounts while on aqueous extracts only glycosides and resins showed moderate presence but significantly lower on the presence of steroids and terpenoids. Biochemical constituents plays a significant role in human health. For instance, the presence alkaloids in perceptible amounts has been testified to act as a pain reliever and a contemporary anaesthetic in ophthalmology with stimulating result and antipyretic effects as other functions [11].

As reported by Cheeke (1983) [12], the presence of saponins includes major biological effects such as erythrocyte hemolysis, enzyme inhibition, cholesterol and bile acid metabolism, antifungal activity, anti-carcinogenic and effect on the reproduction which is evidently present on *S. saman* pods.

Table 1: Results of the phytochemical analyses on *Samanea saman* pods using the test solvent extracts.

Mycochemical Test	Ethanol Extracts	Aqueous Extracts
Alkaloids	++	++
Saponins	+++	++
Flavonoids	−	−
Tannins	++	+
Glycosides	++	++
Steroids	++	+
Terpenoids	++	+
Resins	++	++

Key: − (Absent), + (Low in abundance), ++ (Moderate in abundance), +++ (High in abundance).

Biochemical constituents present on the ethanol and aqueous extracts of *S. saman* pods has showed inhibitory effects on both antifungal and antibacterial bioassays. Tannins are known antimicrobial agents that could inhibit the growth of microorganisms by precipitating out the microbial protein and thus depriving them of nutritional proteins needed

for their growth and development [13]. Table 2 projected inhibitory effects against *Fusarium oxysporum*, a wilt-causing fungi present on agricultural crops.

After 5 days of incubation, it was observed evidently that ethanol extracts at 10mg/ml has exhibited a mean diameter of 39.51mm which is significantly lower that the results showed by the ethanol extracts having 1mg/ml (53.85mm) and 5mg/ml (48.05mm) against the control with 56.93mm. The observation on the aqueous extracts at different levels of concentration also indicates that at 10mg/ml, the growth of *F. oxysporum* can be stunted at 41.96mm compared to the results of 1mg/ml (48.96mm) which is comparable to the results of the control (48.98mm) and 5mg/ml (46.69mm).

Table 2: Mean diameter (mm) of mycelial growth of *Fusarium oxysporum* against different treatments.

Treatments	**Days of Incubation (mm)**				
Ethanol Extracts	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
1mg/ml	12.19±0.65^{b}	16.99±0.79^{b}	27.62±2.04^{c}	42.66±4.65^{b}	53.85±2.48^{c}
5mg/ml	12.18±1.33^{b}	15.21±0.62^{b}	21.90±2.05^{b}	42.35±1.57^{b}	48.05±4.87^{b}
10mg/ml	11.13±1.37^{a}	14.58±1.06^{a}	19.41±1.04^{a}	33.68±2.95^{a}	39.51±3.12^{a}
Control	12.99±0.61^{b}	20.40±1.04^{c}	39.25±2.51^{d}	51.37±4.12^{c}	56.93±1.70^{d}
Aqueous Extracts	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
1mg/ml	12.16±0.41^{b}	19.75±0.58^{b}	30.11±2.34^{c}	38.92±1.45^{c}	48.96±1.84^{c}
5mg/ml	13.18±0.54^{c}	18.20±0.77^{b}	25.84±1.32^{b}	36.98±1.59^{b}	46.69±1.77^{b}
10mg/ml	11.48±0.55^{a}	16.25±0.61^{a}	22.12±1.02^{a}	32.77±1.60^{a}	41.96±1.99^{a}
Control	13.21±0.80^{c}	21.23±0.83^{c}	33.70±0.75^{d}	38.63±5.06^{d}	48.98±5.97^{c}

Note: Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance.

The extracts of *S. saman* in Table 3 were found to have inhibitory effects on the gram negative bacteria *E. coli* and the gram positive bacteria *S. aureus*. After 24 hours of observation it was found out that at 1000ppm ethanol extracts of *S. saman* against *E.coli* and *S. aureus* inhibited 13.35mm and 11.10mm respectively, significantly higher inhibition compared to the results exuded by 10ppm (11.11mm, 9.55mm) and 100ppm (12.11mm, 10.43mm).

Table 3: Zones of inhibition exhibited by Ethanol and Aqueous extracts of *S. saman* against *E. coli* and *S. aureus*

Treatments	**Zones of Inhibition (mm)**					
	E.coli			***S. aureus***		
Ethanol Extracts	**8 hours**	**16 hours**	**24 hours**	**8 hours**	**16 hours**	**24 hours**
10ppm	6.37±0.30^{a}	8.33±0.62^{a}	11.11±0.11^{a}	7.07±0.33^{a}	7.95±0.65^{a}	9.55±0.21^{a}
100ppm	6.81±0.54^{b}	8.55±0.51^{b}	12.11±0.67^{b}	8.22±0.57^{b}	9.250.18^{b}	10.43±0.44^{b}
1000ppm	7.50±0.41^{c}	9.76±0.51^{c}	13.35±0.46^{c}	8.15±0.58^{c}	10.16±0.45^{c}	11.10±1.05^{c}
Control	7.56±0.23^{d}	11.21±1.25^{d}	22.36±1.81^{d}	9.17±0.76^{d}	13.73±1.80^{d}	22.23±1.61^{d}
Aqueous Extracts						
10ppm	6.45±0.53^{a}	7.27±0.66^{a}	9.03±0.68^{a}	6.20±0.34^{a}	6.70±0.38^{a}	9.36±0.38^{a}
100ppm	7.52±0.72^{b}	8.54±0.60^{b}	10.38±0.43^{b}	7.63±0.17^{b}	8.29±0.51^{b}	10.18±0.32^{b}
1000pm	7.46±0.77^{c}	9.42±0.57^{c}	11.18±1.25^{c}	7.06±0.96^{c}	8.78±0.89^{c}	10.38±0.73^{c}
Control	8.73±0.57^{d}	13.58±1.28^{d}	22.43±1.36^{d}	8.45±0.40^{d}	13.99±1.36^{d}	21.78±1.48^{d}

Note: Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance

The same trend was observed on the aqueous extracts against *E. coli* and *S.aureus*. At 1000ppm, the extracts inhibited 9.42mm and 9.36mm, significantly higher inhibition than 10ppm (9.03mm and 9.36mm) and 100ppm (10.38mm and 10.18mm). The presence of tannins in plants can cause negative effect on productivity, reduced nutrient availability, reduced digestibility, impaired digestive physiology and may be mucosal perturbations for those who will intake such plants. While the occurrence of terpenoids in plants could cause cytotoxic effects, growth hormones and tumor promoters and plants containing alkaloids have high nitrogen organic constituents which can be attributed to their ability to become poisonous and even addictive [14].

The results from this study apparently highlighted the scientific foundation for the possible use of *S. saman* pods in an ethno-medication and the probable intervening effectiveness of ethanol and aqueous extracts. Thus, the ethanol and aqueous extracts of *S. saman* pods appears to be a better source of natural but narrow spectrum antimicrobial. In conclusion, the phytochemical components and antimicrobial activity results of the present study suggests that *S. saman* pods could serve as a good source energy giving foods or raw materials, food and biodiesel industries. This also suggests that the therapeutic potency of *S. saman* may be dependent on the extraction solvent used and it is strongly suggested that other extraction method be used in which our laboratory in currently aligned, and finally the extraction and characterization of the detected phytochemicals in the *S. saman* pods might result in the elucidation of its active therapeutic compound.

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