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Anti-microbial Properties of Selected Plant Leaf Extracts Against *Aspergillus niger* (van Tieghem), *Pseudomonas aeruginosa* (Schroeter) and *Staphylococcus aureus* (Rosenbach)

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ABSTRACT

With growing reports of multidrug-resistant pathogens causing untreatable human infections, the need for new antimicrobial therapies is becoming increasingly important. This study was conducted to investigate the antimicrobial properties of the leaf extracts of *Premna odorata* Blanco, *Petersianthus quadrialatus* Merr., *Shorea astylosa* Foxw., and *Tridax procumbens* Linn. The medicinal importance of these plants remains understudied despite their abundant distribution and endemism in the Philippines. A disk diffusion assay was utilized to test the antimicrobial properties of *T. procumbens* leaf extract against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. On the one hand, the fungal colony and spore germination assay was used to test *P. odorata, P. quadrialatus*, and *S. astylosa* leaf extracts against Aspergillus niger. Different concentrations of leaf extracts were prepared and compared with positive and negative controls. *T. procumbens* minimally inhibits the growth of *P. aeruginosa* (10 mm) and *S. aureus* (10 mm), while *S. astylosa* leaf extracts revealed the most significant inhibition on colony growth (5.38 mm) and spore germination (15). P. odorata and *P. quadrialatus* showed the least (28.35 mm; 82.17) and moderate (10.97 mm; 49.5) inhibitory potentials, respectively. The discovery of new antimicrobial compounds from these plant extracts is seen as a potential resolve to the pressing problem of antimicrobial resistance.

Keywords: Anti-microbial, Tridax procumbens, Staphylococcus aureus, Pseudomonas aeruginosa, Premna odorata, Petersianthus quadrialatus, Shorea astylosa, Aspergillus niger

INTRODUCTION

Improper use of antibiotics and antifungal drugs remains the foremost factor for antimicrobial resistance (AMR) and multi-drug resistance (MDR), compromising medical efficacy and human health. There is a rapid emergence of AMR and MDR among pathogens, which necessitates discovering novel antimicrobial therapies and alternative clinical approaches (Aslam et al., 2018; Kumar et al., 2020). This global crisis has refocused scientific attention on drug discovery from traditional medicine species (Prakoso et al., 2018).

Selected pathogens of concern, such as the *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Aspergillus niger*, have exhibited drug resistance over the years. *S. aureus*, the leading cause of skin and soft tissue infections, is resistant to methicillin (Stapleton & Taylor, 2002). Another opportunistic pathogen causing healthcare associated infections (HAI), infections, *P. aeruginosa*, is reported to be resistant to antibiotics (Pang et al., 2019; Wu & Li, 2015). Likewise, the black mold disease-causing *A. niger* demonstrated resistance against specific antifungal agents (Van Der Linden et al., 2011; Baker, 2006). The discovery of new antimicrobial compounds from plant extracts is seen as a potential resolve to this pressing problem. Novel antimicrobials such as ceftolozane exhibits specific activity against *Pseudomonas aeruginosa* in patients with hospital-acquired and ventilator-associated pneumonia and ceftazidime with the novel β-lactamase inhibitor avibactam produce an additive effect in patients infected with Gram-negative bacteria (Cheesman et al., 2017).

P. odorata is an endemic and a well-known medicinal plant in the Philippines. Various applications of the plant include its use as analgesic, antipyretic, and anti-inflammatory. *P. quadrialatus* is an endemic Philippine tree of the family Lecythidaceae, locally known as Toog, Magtalisai, and Kapullan. (Dayan, et. al., 2005). Yakal (*Shorea astylosa* Foxw.) is a medium to large tree about 25 to 30 meters tall. It is one of the species of family Dipterocarpaceae, native in the Philippines, commonly

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found in Luzon, particularly Quezon and Camarines; Samar; Negros; and Mindanao, particularly Zamboanga, Agusan, and Davao. *Tridax procumbens* belong to the family-Asteraceae, tribe-Heliantheae, genus-Tridax, is a perennial plant. The extracts of *Tridax procumbens* have been reported to have various pharmacological effects, antimicrobial activity against both gram-positive and gram-negative bacteria, and stimulate wound healing.

Several endemic plants such as *Premna odorata* (Common Name: Alagao), *Petersianthus quadrialatus* (Common Name: Toog), and *Shorea astylosa* (Common Name: Yakal) are widely used as traditional medicine in the Philippines (Fernando et al., 2008). These are traditionally used to treat microbial diseases, although pharmacological evidence is scarce. Their ethnomedical applications as an analgesic, antipyretic, anti-inflammatory, antinociceptive, or anti-viral indicate their homeopathic properties (Dianita & Jantan, 2017; Nunez et al., 2021; Ecosystem Research and Development Bureau (ERDB), 2012; Dayan et al., 2005). Reports indicated that *Tridax procumbens*, an invasive alien plant species (Shabana et al., 2020), have medicinal properties and may be used to treat wounds, viral infections, inflammations, diabetes, and arthritis (Suseela et al., 2002; Kumar et al., Diwan et al., 1982; Diwan et al., 1983; Taddel & Rosas, 2000; Udupa et al., 1991).

This study investigates the antimicrobial potentials of the ethanolic leaf extracts of *P. odorata*, *P. quadralatus*, and *S. astylosa* against *A. niger* and *T. procumbens* against *P. aeruginosa* and *S. aureus*. The results aimed to provide baseline data and preliminary evidence of their pharmacological applications.

METHODOLOGY

Plant samples

P. odorata is an endemic and a well-known medicinal plant in the Philippines. Various applications of the plant include its use as analgesic, antipyretic, and anti-inflammatory. Compounds associated with anti-inflammatory and anti-nociceptive properties were previously isolated from its leaves. Preliminary phytochemical screening of *P. odorata* crude leaf extracts showed the presence of steroids, terpenoids, flavonoids and hydrolysable tannins (Montoya, 2012). It is one of the species of family Lamiaceae, native in the Philippines, Nepal, India to Myanmar, China, Taiwan, Indo-China, Thailand, Japan, Malaysia and Australia. This plant is also referred to several names such as Fragrant Premna, Alagaw, Alagao, and Adiyo (Philippine Medicinal Plants, ND) (Fig. 1).

P. quadrialatus is an endemic Philippine tree of the family Lecythidaceae, locally known as Toog, Magtalisai, and Kapullan. (Dayan, et. al., 2005). It is a deciduous, medium-sized to fairly large tree that grows up to 40 m tall and 100 cm in diameter. It has been found to strong as akle, ipil and molave. Because of its appearance and high quality, Toog is now recognized in the local and world market under the trade name Philippine Rosewood. However, it is considered a vanishing timber (Philippine Flora, 2009). It is

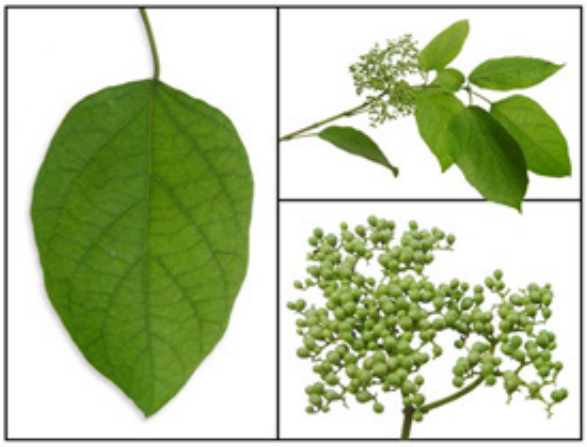

Figure 1. Alagao (*Premna odorata Blanco*) leaves and fruits (Stuart, 2015).

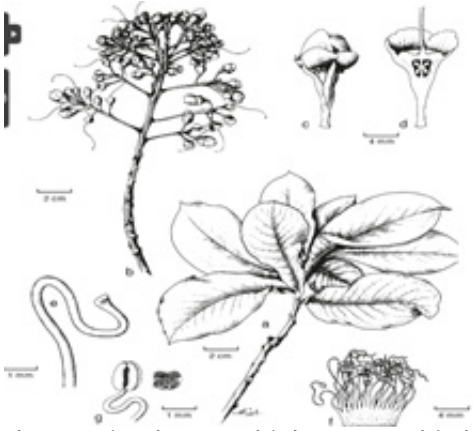

Figure 2. Philippine Rosewood (*Petersianthus quadrialatus*) a. Habit; b. inflorescence; c. flower bud with winged calyx tube; d. longitudinal section of ovary; e. style and stigma; f. stamen; g. anther (Flora Malesiana, n.d.)

Figure 3. Yakal (*Shorea astylosa*) tree and leaves from Tree Facts *Shorea astylosa* Foxw. (Energy Development Corporation, 2012).

Figure 4. *Tridax procumbens*
Source: https://lithophytes.files.wordpress.com/2012/12/dscn4440edited.jpg

fairly common and grows scattered in primary rainforests, near riverbanks or on hillside, in swampy and cool places and grows in an elevation that ranges from sea level up to about 400 m. It thrives in an area where rainfall is evenly distributed throughout the year. It requires well-drained, clayish, sandy and loamy soils (Philippine Flora, 2009). Isolation of stigmasterol, unsaturated triglycerides, β-amyrin fatty acid ester and α-amyrin fatty acid ester from the leaves of *P. quadrialatus*. (Ragasa, et. al., 2014).

Yakal (*Shorea astylosa* Foxw.) is a medium to large tree about 25 to 30 meters tall. It is one of the species of family Dipterocarpaceae, native in the Philippines, commonly found in Luzon, particularly Quezon and Camarines; Samar; Negros; and Mindanao, particularly Zamboanga, Agusan, and Davao. It can also be found in primary forests at low altitudes (Philstar, 2012). This plant is also referred to as Yamban, Gisok, and Dungon-dungon (Energy Development Corporation, 2012).

Tridax procumbens belong to the family-Asteraceae, tribe-Heliantheae, genus-Tridax, is a perennial plant. It is available in all seasons. It has been known by several names like coat buttons in English, ghamra in Hindi, Jayanti veda in Sanskrit, herbecaille in French, vettukaayapoondu in Tamil. It is a weak straggling herb about 12-24cm long with few leaves 68cm long and grows on road sides, hedges and in wastes globally. The leaves of this plant including other aerial parts except flowering tops have been claimed to be useful in the treatment of inflammatory conditions and has a tendency to heal wounds, anti-diabetic activity, anti-arthritic activity, preventing hair loss, diarrhea and serve as insect repellent. (Kumar et. al. 2012) Tridax procumbens Linn (Compositae) is common grass found in tropical southern part of Nigeria, growing primarily during raining season.

The extracts of *Tridax procumbens* have been reported to have various pharmacological effects, antimicrobial activity against both gram-positive and gram-negative bacteria, and stimulate wound healing. Traditionally, the local Yoruba population of Western States of Nigeria uses the leaf of the plant as treatment to reduce blood pressure. (Taddel and Rosas, 2000, Udopa et al;1991, Diwan et al; 1982, and Diwan et al; 1983). *Tridax procumbens* is known for several potential therapeutic activities like antiviral, antibiotic efficacies, wound healing activity, insecticidal and anti-inflammatory activity (Suseela et al 2002).The Ethno pharmacological and traditional use of plants often results in the discovery of new biologically active molecules (Alisi et al., 2008). Plants have a long history of use in the treatment of cancer (Mohammad et al., 2006).

Fresh and healthy leaves of the plant, *P. odorata*, *P. quadrialatus*, and *S. astylosa* and *T. procumbens* were

collected between August and October, 2017 at Cadayona Farm, Tugbok District, Mintal, Davao City. The taxonomy of the studied plant species: P. odorata, P. quadrialatus, S. astylosa, and T. procumbens were certified and verified by a botanist. The researchers also ensured that the plant specimens were healthy and free from any discoloration in any part.

Microbial cultures

Pure cultures of *P. aeruginosa* (ATCC27853) and *S. aureus* (ATCC25923) were obtained from the Department of Science and Technology Regional Office XI (DOST XI) located in Friendship cor. Dumanlas Rds., Bajada, Davao City. Also, A. niger samples were identified by a plant pathologist. Experiments using the plant and microbial cultures were conducted at the Davao Doctors College (DDC), Davao City, Philippines.

Leaf extract preparation

Fresh leaves of *P. odorata*, *P. quadrialatus*, *S. astylosa* and *T. procumbens* were washed with tap water and distilled water separately. After washing, the leaves were air-dried at room temperature for seven days prior to cutting into smaller pieces. A commercial mechanical grinder was used to powderize the leaves. Next, 250 g of powdered leaves of *P. odorata*, *P. quadrialatus*, *S. astylosa* and *T. procumbens* were soaked in 800 ml of 80% ethanol for 48-72 hours. Ethanol was used in this study because this solvent was able to extract hydrophilic bioactive compounds due to its polarity (Do et al., 2014; Ehiowemwenguan et al., 2014). The extracts were filtered using Whatman filter paper (No.1) while hot, concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in a desiccator. The extracts were then kept in sterile bottles, under refrigerated conditions (2^0 to 4^0 C) until further use for antimicrobial assays. The dry weight of the plant extracts was obtained by the solvent evaporation and used to determine concentration in mg/ml (Bhalodia et al.,2011).

Preparation of Different Concentrations of Plant Extracts

Different concentrations (100%, 75%, 50% and 25%) of *P. odorata, P. quadrialatus, S. astylosa*, 40 ml of the P. odorata, P. quadrialatus, and S. astylosa extracts (40 ml) were prepared in sterilized vials at room temperature (Guevara, 2005). The prepared 40 ml leaf extracts of *P. odorata, P. quadrialatus, and S. astylosa* were diluted by adding 80 ml distilled water (1:1 ratio) to obtain the 50% concentration.

T. procumbens leaf extracts were prepared in different concentrations such as 25 µg/ml, 50 µg/ml, 75µg/ml, and 100 µg/ml with dimethyl sulfoxide (DMSO). One hundred grams (100 g) of leaves were washed, air-dried, and macerated. The leaves were soaked in 100ml ethanol for 24-72 hours (Guevara, 2005). The macerated leaves were separated from the extract mixed with ethanol. Afterwhich, pure extract from the ethanolic extract was derived via rotaevaporation (RE100-Pro Digital Rotary Evaporator, 3500 rpm for 30 minutes). The beaker in rotaevaporation was rotated at a variable speed of 0 – 220 rpm. Finally, the beaker was placed in a water bath without exceeding the boiling point. After the evaporation process, pure *T. procumbens* leaf extract was obtained.

Phytochemical Screening

The extracts were subjected for a phytochemical screening in order to detect bioactive compounds. Air dried and powdered plant materials were brought and screened to a private laboratory institution of the University of the Immaculate Concepcion-Science Resource Center for the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarin, saponins, gum, mucilage, carbohydrates, reducing sugars, starch, protein, and amino acids (Kumar et al.,2009; Nariya et al., 2011).

Antimicrobial property

Media preparation

A mixture of 5.7 g nutrient agar and 9.5 g Muller-Hinton Agar were placed on a 500 ml Erlenmeyer flask. It was added with 250 ml distilled water then heated using a hot plate until both agar mixtures were clear. Next, the Erlenmeyer flasks containing the cooked agar were covered with cotton and aluminum foil before its placement in the autoclave for 45 minutes to 1 hour at 121°C. After autoclaving the agar, it was cooled and poured unto the sterile Petri dishes to solidify.

Meanwhile, 39 g of powdered Potato Dextrose Agar (PDA) was suspended in 1 L distilled water. The solution was thoroughly mixed and boiled until it was homogenized. The mixture was sterilized in the autoclave at 121°C for 15 minutes. Then, it was cooled down to room temperature and was poured into each Petri plate (Aryal, 2017).

Agar Disk diffusion method

The agar disk diffusion method was used to determine the antibacterial activities of T. procumbens against Pseudomonas aeruginosa and Staphylococcus aureus. The test was performed by applying a bacterial inoculum of approximately 1-2x108 CFU/mL to the surface of a large (150 mm diameter) Mueller-Hinton Agar plate and Nutrient Agar by spreading. Replicates (N=3) and control set-ups with fixed concentrations and paper antibiotic disks, respectively were prepared and incubated at 37°C for 24 hours. For the control set-up, the antibiotic Vancomycin served as the positive control while distilled water served as the negative control. The test disks were soaked in the Petri dishes containing different concentrations of leaf extracts for 15 minutes before being placed on the agar plates. The zones of inhibition around each of the antibiotic disks were measured in millimeters (mm) using a caliper.

Colony diameter method

A 2 ml volume of leaf extracts with 50% concentration was aseptically poured onto Petri plates (per plant sample), followed by the addition of 18 ml of melted Potato Dextrose Agar (PDA) (Raji & Raveendran,

2013). Before the agar medium congealed, the plates were swirled gently to achieve thorough mixing of the contents. The plant extracts were substituted with Fungicide X as positive control and distilled water as the negative control. In a solid medium, the inoculum discs of *A. niger* were inoculated at the center of each Petri dish. The inoculated Petri dishes were stored at room temperature. Then, the diameters of the fungal colonies were measured on the 1st, 3rd, 5th, and 7th day of incubation with the aid of a Vernier caliper (Rao & Srivastava, 1994). For evaluation, the mean values of the three readings and fungi's radial growth per day were divided by the total number of observation days (Brancato et al., 1953).

Spore germination assay

A 0.1 ml of leaf extracts with 50% concentration was placed in the cavity slide with the spores from the prepared subculture. Each slide was stored in a moist Petri dish at room temperature to maintain enough humidity (Das et al., 2009). Six replicates were maintained for each treatment, including the controls. The slides were examined at an interval of three hours for a total six-hour duration. Percent spore germination of each treatment was calculated using this formula (Kiraly et al., 1974):

$$\text{Percent spore germination} = \frac{\text{no. of spores germinated}}{\text{total no. of spores examined}} \times 100$$

The leaf extracts of *P. odorata*, *P. quadrialatus*, and *S. astylosa* were tested against *A. niger*. The fungal growth of A. niger was compared among the five treatments: 50% ethanolic extract of P. odorata, P. quadrialatus, S. astylosa, fungicide X (positive control), and distilled water (negative control). The treatments were replicated six times and incubated for seven days. The fungal colonies' diameter was measured on the 1st, 3rd, 5th, and 7th day of observation.

Data analysis

Descriptive statistics were included in the data analyses. The parameters, treatments, replicates were clearly described. The descriptive statistics included mean values, standard deviation or variance, minimum and maximum values of the range. One-way Analysis of Variance (ANOVA) was used to compare the variance among different treatments observed. It is used when one categorical independent variable has three or more levels and one continuous dependent variable (Boduszek, 2017). The data obtained from the growth of *A. niger* through PDA and spore germination was statistically analyzed using R and R Studio at $p \leq 0.05$ level of significance.

Biological safety

The microorganisms were treated as potential pathogens. All the experiments were done in a biosafety cabinet. Proper handling procedures, storage, and disposal of microorganisms and plant materials were established to prevent contamination and infection. Decontamination and disposal of plant materials were done by use of autoclave (121°C, 15 psi for 15 minutes). All the standard microbiology laboratory safety protocols were followed.

RESULTS AND DISCUSSION

Figure 5 shows the fungal colony growth on PDA with the different treatments.

In Table 1, the Tukey HSD pairwise comparison among the treatments on fungal growth revealed that the S. astylosa leaf extract is not significantly different from the positive control (Fungicide X). On the 7th day, P. odorata (T1) had a mean value of 2.84 cm, P. quadrialatus (T2) had a mean value of 1.1 cm, S. astylosa (T3) had a mean value of 0.54 cm, distilled water (T4) had a mean value of 3.21 cm,

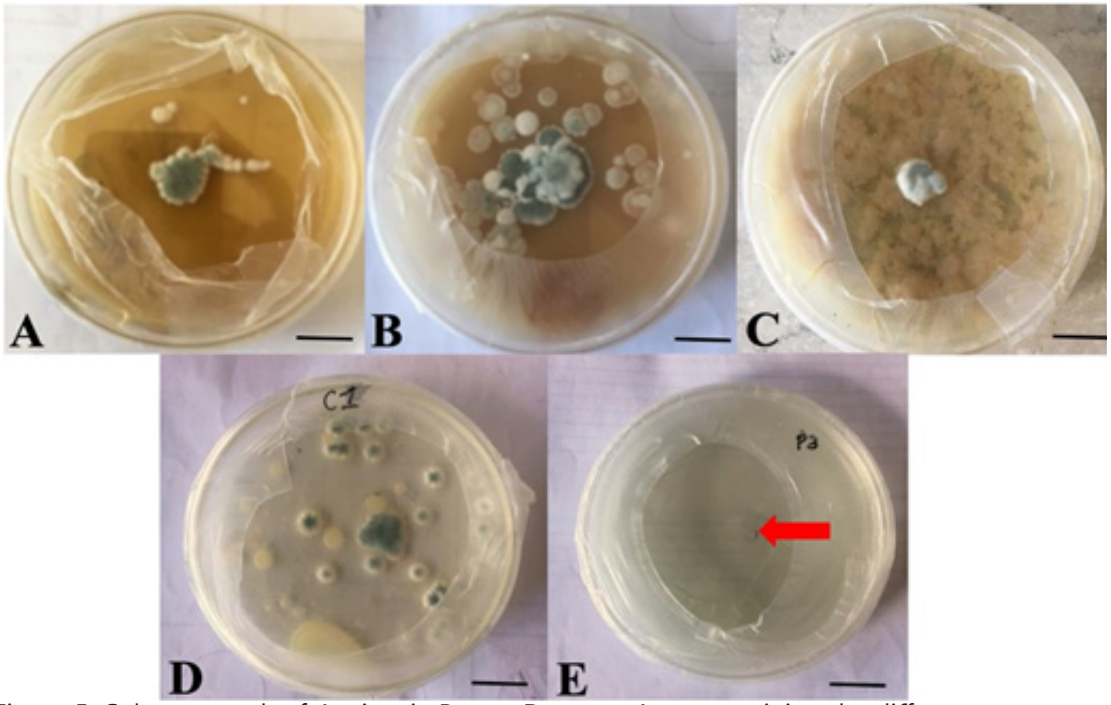

Figure 5. Colony growth of *A. niger* in Potato Dextrose Agar containing the different treatments: A – *P. odorata* leaf extract (50%); B – *P. quadrialatus* leaf extract (50%); C – *S.astylosa* leaf extract (50%); D - Distilled water; and, E - Fungicide X. (Red arrow: inoculum disk). Bar = 10 mm.

Table 1

Antifungal activity P. odorata, P. quadrialatus, and S. astylosa leaf extracts against Aspergillus niger after 7 days.

Treatment	n	Colony Diameter Growth (mm) of A. niger			Spore Germination Count		
		Mean ± SD	Min	Max	Mean ± SD	Min	Max
T^1- P. odorata	6	28.35^{e} ± 5.58	21	36	82.17^{c} ± 11.48	66.00	99.00
T^2 - P. quadrialatus	6	10.97 ± 1.9	7.9	12.9	$49.5^{b,d}$ ± 6.12	42.00	57.00
T^3 - S. astylosa	6	5.38 ± 0.59	4.5	6.1	$15.0^{c,f}$ ± 5.22	7.00	21.00
T^4 - Distilled Water	6	32.13^{b} ± 1.25	30.1	33.9	349.0 ± 48.62	276.00	401.00
T^5 - Fungicide X	6	0.00 ± 0.00	0.00	0.00	0.00^{d} ± 0.00	0.00	0.00
p-value[a]		0.000004*			0.000189*		

a. One-Way ANOVA: Significant *p*-value (*) at 0.05 level
Tukey HSD Post-Hoc Test: Not significantly different to b. T^1, c. T^2, d. T^3, e. T^4, and f. T^5 at 0.05 level

and Fungicide X (T5) had no growth recorded. This data indicates that *S. astylosa* leaf extract effectively inhibited the colony growth of *A. niger* at a 50% concentration.

The total number of spores, number of spores germinated, and percentage of spore germination per treatment were recorded. Results revealed that the lowest spore count and spore germination percentage was observed in S. astylosa leaf extract. The spore germination indicated that the activity of S. astylosa leaf extract and fungicide X were significantly different and significantly lower compared to other treatments, as shown in Table 1. The phytochemical screening showed that S. astylosa contains flavonoids, saponins, and tannins, providing insight into its antimicrobial activity.

Aspergillus niger is a naturally ubiquitous, filamentous fungus of the Phylum Ascomycota, which can cause the black mold disease on certain fruits such as grapes and peanuts, and vegetables such as onions (Baker, 2006). Rotting caused by black mold is the primary cause of rotting in stored yellow onions in Texas, USA, between 1974 and 1976 (Miller and Dillon, 1979). In Japan, onions stored over the summer had losses of 33% (Tanaka and Nonaka, 1981). In the Philippines, onion bulbs exhibit clusters of black fungal spores from along veins and on or between the outer papery scales of the bulbs (Philippine Council for Agriculture, Forestry and Natural Resources Research and Development, 2018). *A. niger* can grow in a wide range of temperatures (6-47°C), making it ubiquitous in warm and humid places (Palacios-Cabrera et al., 2005). Also, Bui-Klimke and Wu (2015) reported that A. niger could produce ochratoxin, a mycotoxin linked with the human diseases Balkan endemic nephropathy (BEN) and chronic interstitial nephropathy (CIN), and other renal diseases.

In this study, three Philippine endemic plants were investigated. *P. odorata* belongs to the Family Lamiaceae, locally known as Alagao (Philippine Medicinal Plants, 2021). Various plant applications include its use as an analgesic, antipyretic, and anti-inflammatory. Compounds associated with anti-inflammatory and antinociceptive properties were previously isolated from its leaves. Initial phytochemical screening of *P. odorata* crude leaf extract showed steroids, terpenoids, flavonoids, and hydrolyzable tannins (Montoya, 2012). In the study of Lirio et al. (2014), P. odorata crude methanolic extract and sub-extracts showed that 1-heneicosyl formate present in the leaf extracts has significant inhibitory activity against *M. tuberculosis* H37RV (MIC= 8 mg/ml). It also has antimicrobial, anti-inflammatory, and chemopreventive activities (Pinzon et al., 2011).

Secondly, *P. quadrialatus* belongs to the Family Lecythidaceae, locally known as "Toog" (Dayan et al., 2005). It is a deciduous tree that grows up to 40 m tall and 100 cm in diameter. Because of its appearance and high lumber quality, it is now recognized in the local and world market under the trade name Philippine Rosewood (Philippine Flora, 2009). However, it is considered vanishing timber, calling for comprehensive conservation actions and sustainable use. The study of Ragasa et al. (2014) revealed that *P. quadrialatus* leaf extracts yield unsaturated triglycerides and a mixture of beta and alpha-amyrin fatty acid ester.

The third endemic tree, *S. astylosa*, is locally called "Yakal," "Yamban," "Gisok," and "Dungon-dungon" (Energy Development Corporation, 2012). It is a medium to large-sized species about 25 to 30 meters tall. It belongs to the family Dipterocarpaceae and is widely distributed in Quezon and Camarines, Samar, Negros, Zamboanga, Agusan, and Davao. Among the endemic plants investigated, *S. astylosa* leaf extract (50%) inhibited the growth and spore germination of A. niger.

In this study, the phytochemical screening (see Supplemetal Data) of the leaf extracts revealed flavonoids, saponins, and tannins. Flavonoids are secondary metabolites in class polyphenol and present in several plants and diets (Wang et al., 2018). Aboody and Mickymaray (2020) reported that flavonoids show various pharmacological functions, including antioxidant, anti-diabetic, anti-obesity, anti-hyperlipidemic, anti-inflammatory, antiosteoporotic effects, antiallergic and antithrombotic, hepatoprotective, neuroprotective, renoprotective, chemopreventive and anticancer, antibacterial, antifungal, and anti-viral

activities. They are recognized as antioxidants and possess free radical quenching properties. Also, they perform as chelators of divalent cations. It has free radical scavenger properties that inhibit lipid peroxidation, capillary permeability, platelet aggregation, and fragility. Flavonoids regulate biological systems by inhibiting many enzymes, such as hydrolase, lipase, α-glucosidase, aldose reductase, cycloxygenase, xanthine oxidase, hyaluronidase, alkaline phosphatase, arylsulphatase, lipoxygenase, Ca+2-ATPase, cAMP phosphodiesterase, and several kinases.

Flavonoids from *Erythrina burtii* showed antifungal activity (Yenesew et al., 2005). Aquilegia vulgaris leaf extracts contain 4-methoxy-5,7-dihydroxyflavone-6-C-glucoside (isocytisoside) and antifungal activity against A. niger (Bylka et al., 2004). Thus, this study's findings provide preliminary evidence of S. astylosa's pharmacological benefits upon discovering flavonoids and antimicrobial activities.

Meanwhile, saponins are secondary metabolites in plant species. They act as inactive precursors and are readily converted into active biological antibiotics against pathogens. They are glycosylated compounds and can be divided into three major groups: triterpenoids, steroid or steroidal glycoalkaloids, and triterpene saponin. These compounds were found in *Capsicum frutescens* and showed antifungal activity against sixteen different fungal strains (Renault et al., 2003). Deterioration of cooked foods caused by yeast and fungi is restrained by plant extracts containing saponins (Tamura et al., 2012).

On the other hand, *T. procumbens* leaf extract at 100% concentration partially inhibited the growth of P. aeruginosa and S. aureus. T. procumbens is a perennial plant that belongs to the family Asteraceae. The leaves of this plant have been reported to have anti-viral, anti-inflammatory, anti-diabetic, and anti-arthritic activities. It has been reported to aid in wound healing, prevent hair loss and serve as an insect repellent. It has antimicrobial activity against gram-positive and gram-negative bacteria and stimulates wound healing (Suseela et al., 2002; Kumar et al., 2012).

Tannin, flavonoids, luteolin, terpenoid, and alkaloids were isolated from *T. procumbens* leaves (Kethamakka and Deogade, 2014; Cushnie and Lamb, 2005; Cowan, 1999). Tannin can inhibit the extracellular microbial enzyme, deprive substrates required for microbial growth, and directly inhibit oxidative phosphorylation (Fenner and Freeman, 2020). Luteolin can inhibit DNA topoisomerase I and II activity, which further inhibits nucleic acid and

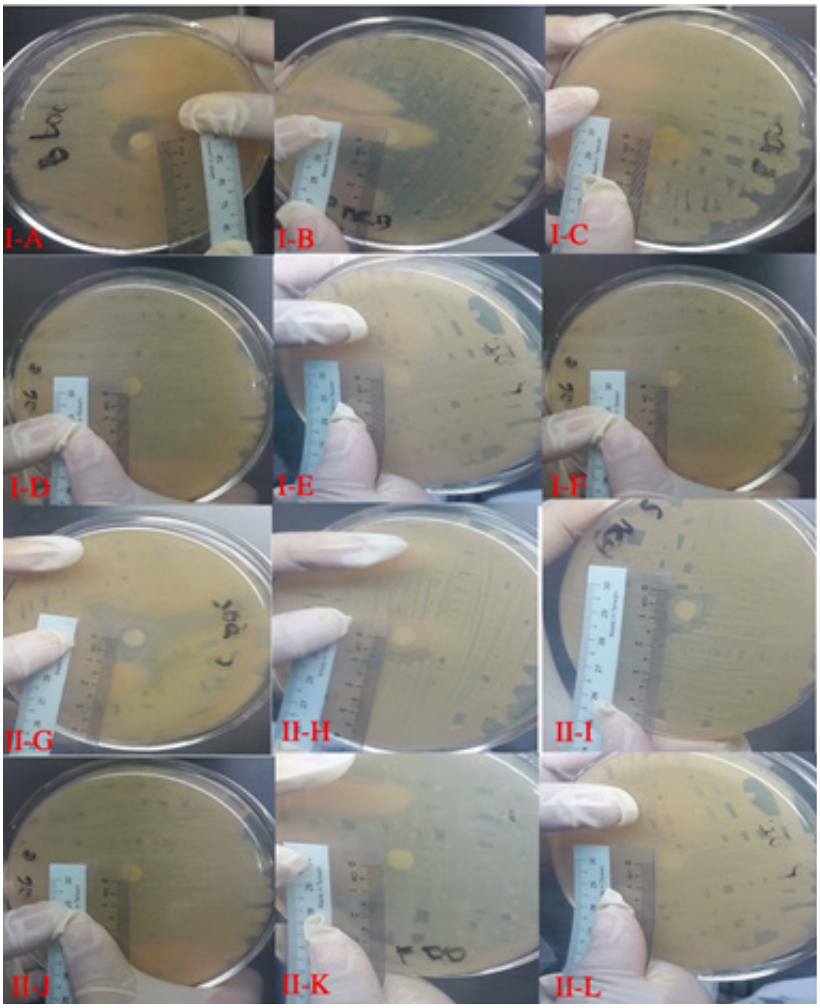

Figure 2. *T. procumbens* tested against I. *P. aeruginosa*: A. Positive Control Vancomycin; B. Negative Control (dH2O); C. 100% *Tridax procumbens* leaf extract; D. 75% *Tridax procumbens* leaf extract; E. 50% *Tridax procumbens* leaf extract; and, F. 25% *Tridax procumbens* leaf extract; and II. *S aureus*: G. Positive Control Vancomycin; H. Negative Control (dH2O); I. 100% *Tridax procumbens* leaf extract; J. 75% *Tridax procumbens* leaf extract; K. 50% *Tridax procumbens* leaf extract; and, L. 25% *Tridax procumbens* leaf extract.

protein synthesis, especially against *S. aureus* (Wang and Xie, 2010). In addition, terpenoid is effective against gram-positive and gram-negative pathogens by interfering in bacterial membrane permeability (Sathya Bama et al., 2012). Flavonoids inhibit cytoplasmic membrane function and energy metabolism (Cushnie and Lamb, 2005). Lastly, the alkaloids can affect the virulence gene regulatory systems such as quorum sensing and virulence factors such as sortases, adhesins, and secretion systems. It also inhibits the transcriptional regulator in bacteria (Cushnie et al., 2014).

On the other hand, *T. procumbens* was tested against P. aeruginosa and S aureus. Table 2 shows the zones of inhibition (ZOI) of the different concentrations of T. procumbens leaf extract. The 100% leaf extract had a partially active result (ZOI=10 mm). Based on the equivalent antibacterial activity level by Guevarra (2005), T. procumbens leaf extract at 100% concentration was partially active in inhibiting the growth of *Pseudomonas aeruginosa* and *S. aureus*.

In this study, *S. aureus* was found to be susceptible to the ethanolic leaf extract of *T. procumbens*. The antibacterial activity of ethanolic extract of *Tridax procumbens* can be attributed to the presence of flavonoids and tannins which are substances known to have several mechanisms of action such as inhibition of DNA gyrase, inhibition of cytoplasmic membrane function, inhibition of energy metabolism, etc. (Cushnie and Lamb, 2005). It is a common opportunistic bacteria in human skin and has been known to cause severe problems due to its methicillin resistance. Its major route of infection includes open wounds, especially excisional wounds that may harbor bacterial species causing delayed wound healing. Also, *S. aureus* is the leading cause of skin and soft tissue infection resulting in significant morbidity and mortality from septic shock, endocarditis, pneumonia, and bacteremia (Prakoso et al., 2018; Stapleton & Taylor, 2002).

Meanwhile, the leaf extracts of *T. procumbens* also demonstrated inhibition activity against *P. aeruginosa*. This data presents clinical importance as the nosocomial pathogen is known to have increasing antibiotic resistance. As it is opportunistic, it exploits immunocompromised hosts to mount an infection. It can cause disease in the urinary tract, respiratory system, skin, soft tissue, bone and joint, gastrointestine, and blood, particularly in patients with severe burns, tuberculosis, cancer, and Acquired Immunodeficiency Syndrome (AIDS) (Pang et al., 2019; Wu and Li, 2015). *P. aeruginosa* has high intrinsic antibiotic resistance, enabling it to survive in various environmental settings, including medical facilities (Balcht and Smith, 2010). Multi-drug resistant strains are emerging, such as carbenicillin-resistant, cephalosporins-resistant, ceftazidime-resistant, and ciprofloxacin-resistant (Public Health Agency of Canada, 2012). With the severity of P. aeruginosa infections and the limited antimicrobial interventions used to treat them, finding alternative prevention and treatment strategies is an urgent priority (Gellatly & Hancock, 2013).

Traditionally, indigenous people in other countries like Nigeria use the leaves of this plant as a treatment to reduce blood pressure (Taddei and Rosas, 2000, Udopa et al.,1991; Diwan et al., 1982, and Diwan et al., 1983). The Philippine Medicinal Plants website (2021) mentioned that Indians utilized it as an anticoagulant, repellent, antidiarrheal, and anti-dysenteric. The leaf extract can be used on fresh wounds to stop bleeding and as a hair tonic. In Ayurveda, it is used for liver disorders, arthritis, and heartburn. Local uses in Guatemala revealed that the leaf extract is used for colds, inflammation, vaginitis, stomach pains, and diarrhea. The whole plant is used for protozoal infections, treatment of chronic ulcers. The whole dried plant is used in Africa for fever, cough, backache, diarrhea, and epilepsy. Dried leaves are used for malaria, gastrointestinal mycosis, and dressing wounds.

CONCLUSION

S. astylosa leaf extract demonstrated the most significant inhibition of fungal growth (5.38 mm) and spore germination (15) of *A niger*. However, *P. odorata* and *P. quadrialatus* showed the least (28.35 mm; 82.17) and moderate (10.97 mm; 49.5) inhibitory potentials, respectively. *T. procumbens* minimally inhibits the growth of *P. aeruginosa* (10 mm) and *S. aureus* (10 mm). In the qualitative phytochemical screening of S. astylosa, results indicated presence of flavonoids, saponins, and tannins. Among these phytochemical components, flavonoids may act as the antifungal component. Meanwhile, 100% ethanolic leaf extract of *T. procumbens* inhibited *P. aeruginosa* and *S. aureus*. There is a significant difference (Significant p-value (*) at 0.05 level) in the mean zone of inhibition (ZOI) of *P. aeruginosa* and *S. aureus* compared with the positive and negative controls. It is recommended that further studies be made on method development such as extraction procedure and processing of samples to potentially increase the antimicrobial activities of these

Table 2

The Zone of Inhibition (ZOI) of T procumbens leaf extract against P. aeruginosa and S. aureus after 24 hours of incubation.

Test Organism	n	Zone of Inhibition (mm) Tridax procumbens leaf Extract				Positive Control (mm)	Negative Control (mm)
		100%	75%	50%	25%		
Pseudomonas aeruginosa	1	10	0	2	4	18	2
Staphylococcus aureus	1	10	0	0	8	20	16

plant samples against more indicator strains.

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