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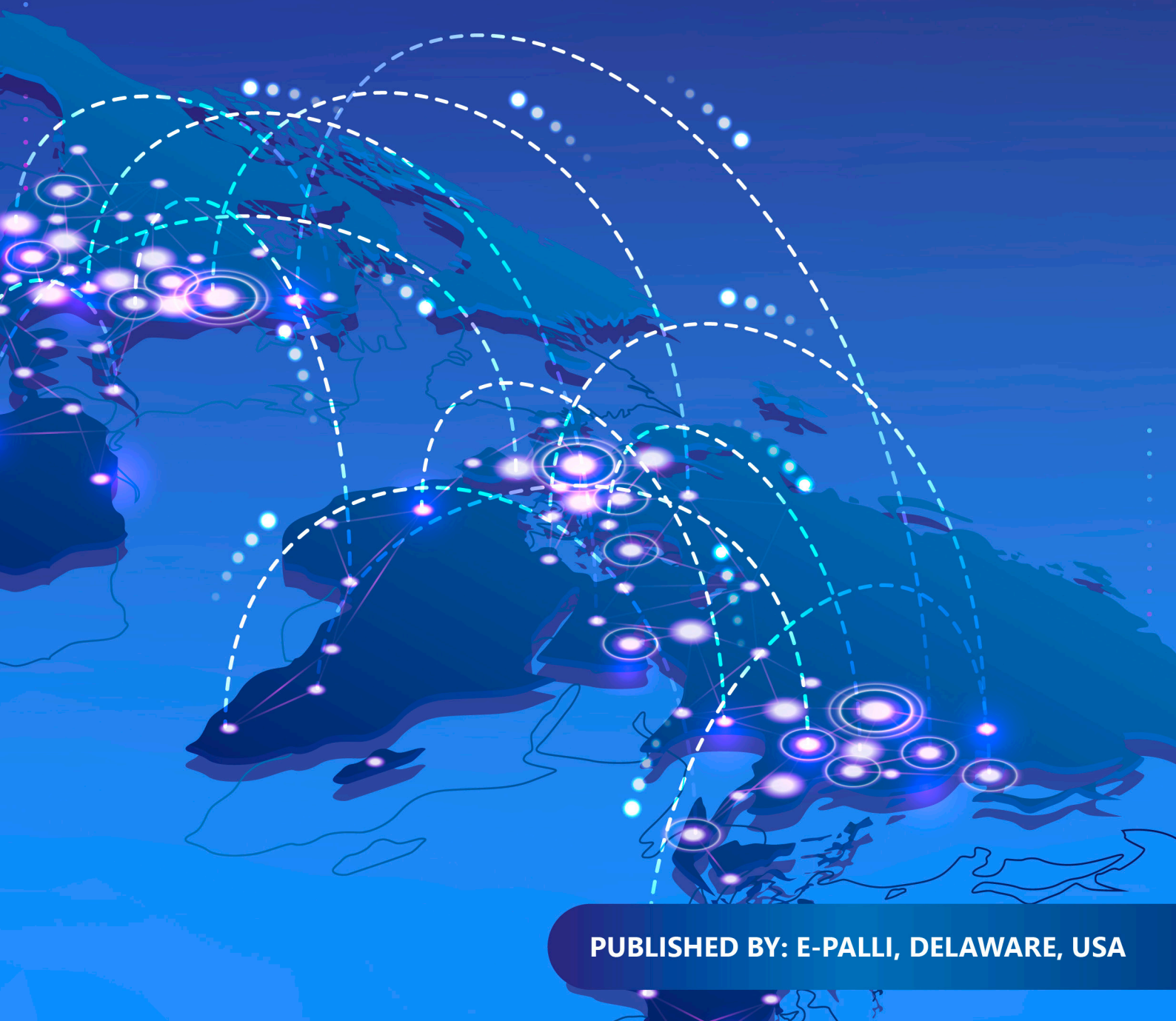

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Phytochemical Screening and Antimicrobial Assay of Selected Plant Extracts with Ethnopharmacological Potential for Wound Healing

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ABSTRACT

Bacteria usually colonize wounds, and their low level is beneficial to the wound healing process. Thus, antibacterial agents are usually applied to wounds. In the Philippines, many herbal plants are used to speed up the wound healing process. With their number, literature revealed no scientific study yet on which among these plants could well inhibit wound bacteria. Hence, this study focused on the phytochemical screening and antibacterial activity of the ethanolic extracts of six herbal plants, namely: *Caesalpinia sappan* Linn. (Sibukao), *Jatropha curcas* Linn. (Tuba-tuba), *Lantana camara* Linn. (Kantutay), *Mimosa pudica* Linn. (Makahiya), *Moringa oleifera* Lam. (Malunggay), and *Psidium guajava* Linn. (Guava). Betadine was used as the positive control. Qualitative phytochemical analysis and antimicrobial assay on wound bacteria using the agar well diffusion method were employed from the six extracts. Results revealed that phytochemicals, namely alkaloids, saponins, flavonoids, and tannins, were present in the plant extracts except for anthraquinones. Furthermore, the plant extracts showed antibacterial activity on wound bacteria, and *P. guajava* leaf extract exhibited the most significant antibacterial effect among the treatments and is greater than the positive control (Betadine solution). On the other hand, all the plant extracts have a lower antibiotic activity index when compared with Amikacin 30mcg and Erythromycin 15 mcg. However, except for *Lantana camara* extract, the five plant extracts have a remarkably higher antibiotic activity index when compared with Penicillin 10 U. It is concluded that the plants studied have varying levels of antibacterial activities that could promote wound healing.

INTRODUCTION

A wound is defined as a loss of cellular and functional continuity of underlying living tissues in organisms. It can damage the tissues to varying degrees (Udobre et al., 2012). However, in favorable conditions, wounds undergo a normal healing process. Nayak & Pinto-Pereira (2006) posited that after an injury, the body initiates an inflammatory response that can cause the cells below the dermis (the deepest skin layer) to start and increase collagen production. The collage produced results in the regeneration of the epithelial tissues of the skin. Meanwhile, the present estimates indicate that there are nearly 6 million people across the world that suffer from chronic wounds. Unhealed wounds inflict pain by constantly producing inflammatory mediators that can increase the pain and swelling at the wound site (Kumar et al., 2007). One of the reasons for the delay in the wound healing process is infection. The infection of the wounds happens when the body's immune defenses cannot control the average bacterial growth. In most cases, infected wounds are caused by bacteria that arise from the skin and other areas of the body and the surrounding environment. According to Alam et al. (2011), the most prevalent forms of bacteria that cause wound infection are *Staphylococcus aureus* and other *staphylococci* bacteria. Similarly, unsanitary facilities and inadequate wound dressing practices may raise the risk of infection. In addition, a person's underlying health issues may prolong the healing process. For instance, the wound healing process is more likely to be impeded among diabetic individuals (Sharma et al., 2013).

The basic principles governing an effective wound healing process are highly dependent on the rapid and complete restoration of injured tissues and the suppression of exponential bacterial growth at the wound site. While these principles are highly critical, they are claimed to be challenging to enforce entirely in wound care clinical practice. Sabath (2006) posited that the techniques used to treat wounds are vital in preventing bacterial invasion. Bacterial growth at the wound site must be appropriately managed to avoid infection and promote a rapid wound healing process. In recent years, herbal medicines have gained considerable interest as good sources of organic compounds for treating a variety of diseases in many parts of the world. Similarly, these herbal medicines have been time-tested for their relative safety for human use, affordability, environmental friendliness, and accessibility. For instance, in the Philippines, many herbal plants are used to speed up wound healing. Among these herbal medicines are *Caesalpinia sappan* Linn., *Jatropha curcas* Linn., *Lantana camara* Linn., *Mimosa pudica* Linn., *Moringa oleifera* Lam., and *Psidium guajava* Linn.

Caesalpinia sappan Linn. is a little thorny tree that grows to a height of 6-9 meters and is found in the Philippines, India, Peru, and Malaya. In English, it is referred to as sappan wood or Brazil wood. Historically, the wood was used in calico printing of cotton, wool, and silk but was eventually phased out in favor of synthetic colors.

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It has been historically used to treat a broad range of diseases and is said to possess a wide variety of therapeutic characteristics. It has been found to possess anticonvulsant, anti-inflammatory, anti-proliferative, antibacterial, anticoagulant, antiviral, immunostimulant, and antioxidant properties (Sathya, 2010).

Jatropha curcas Linn. is native to the American tropics, most likely Mexico and Central America. It is a deadly, semi-evergreen shrub that grows up to 6 meters in height. It is a versatile tree with economic value due to its many industrial and medical applications. In Africa, Asia, and Latin America, *Jatropha species* are used to treat various diseases (Arekemase et al., 2011). They are utilized as antibacterial agents, and various studies have been conducted to ascertain their scientific foundation (Kalimuthu et al., 2010).

Lantana camara Linn. is a flowering shrub native to tropical America used as an ornamental plant (Agrawal et al., 2012). Various portions of the plant are utilized in folklore remedies and traditional medical systems to cure various human diseases. The leaves are used to cure itches, wounds, ulcers, swellings, bilious fever, eczema, and rheumatoid arthritis. Numerous pharmacological studies have shown that extracts of the leaves possess antibacterial activity (Agrawal et al., 2012). *Mimosa pudica* Linn. is a creeping perennial plant often cultivated for its value. It is a Brazilian native plant that has become a pantropical weed. Young plants have an upright stem, but the stem becomes creeping or trailing as they mature. The plant reaches a maximum height of 1.5 meters. Its leaves are compound bi-pinnately, with one or two pinnae pairs and ten to twenty-six leaflets per pinna. Its petioles are likewise thorny, and a study reveals that the top half of the floret petals are red, while the filaments are pink to lavender. The fruit comprises clusters of two to eight pods measuring between one and two centimeters in length and spiky on the borders (Gandhiraja et al., 2009).

Moringa oleifera Lam. is a highly coveted plant that grows in various tropical and subtropical regions. It has a broad variety of high nutritional value and therapeutic applications. Its different components include a variety of essential minerals, vitamins, proteins, and other phenolic compounds (Farooq et al., 2007). The plant leaves, seeds, roots, bark, flowers, fruits, and immature pods all work as cardiac and circulatory stimulants and are said to have anti-inflammatory, antitumor, antiepileptic, antipyretic, and antiulcer properties (Farooq et al., 2007). *Psidium guajava* Linn. is a tropical American native evergreen shrub that grows in tropical and subtropical regions. It is a phytotherapeutic plant that has been used in folk medicine for centuries. It is thought to have active ingredients that aid in the treatment and management of various ailments. Numerous plant components have traditionally been used to cure several ailments, including vomiting, malaria, gastroenteritis, wounds, ulcers, coughs, toothache, and sore throats (Biswas et al., 2013).

With these number of herbal plants, literature revealed no scientific study yet on which among these plants could well inhibit wound bacteria. This prompted the researchers to investigate the phytochemical screening and antimicrobial activity of selected herbal plants used in folk medicine on wound healing. If proven effective, these plants could be processed and could be cheap alternative sources of drugs to cure wound infections and probably hasten the epithelial repair of injured tissues. This study aimed to determine the phytochemicals and antimicrobial activities of selected plant extracts with ethnopharmacological potential for wound healing. Specifically, this sought to (1) determine the presence of secondary metabolites in the selected plant extracts; (2) determine if the selected plant extracts are effective in inhibiting the growth of wound bacteria; (3) determine which of the selected plant extracts are significantly effective in inhibiting the growth of wound bacteria; and (4) determine the antibiotic activity indexes of the plant selected plant extracts.

LITERATURE REVIEW

Wound Bacteria

Infected wounds are wounds in which bacteria or other microorganisms have colonized, causing either a delay in wound healing or wound deterioration. Bacteria typically contaminate most wounds. However, infected wounds result when the body's immune defenses are overwhelmed or cannot cope with average bacterial growth. Infection of wounds caused by surgery is a severe health risk, as studies have shown that 70 percent of the deaths of patients who have undergone surgery are caused by surgical site infections (Udobre et al., 2012). Most infected wounds are caused by bacteria originating either from the skin, other body parts, or the outside environment. The skin contains bacteria (normal flora) which are generally harmless if the skin is intact. However, the protective barrier formed by the skin is disrupted when there is a wound, and this normal flora can colonize the injured area. This results in further tissue damage and may prolong wound healing by promoting more inflammation, which prolongs the process of wound healing. The most common bacteria causing wound infection is *Staphylococcus aureus* and other groups of staphylococci (Alam et al., 2011). Contamination from other parts of the body may also cause wound infection. Poor wound dressing techniques and unhygienic conditions may increase the risk of wound infection (Sharma et al., 2013).

Wound Healing

A wound can be defined as a break in the continuity of the soft tissues like skin, mucous membranes, and the tissue surface. An external wound is a wound with a varying degree of damage to the tissue, including the skin. An internal wound damages the underlying tissue to a varying degree, leaving the skin intact (Udobre et al., 2012). According to Nayak and Pinto-Pereira (2006), wound healing is the process of repair that follows injury to the skin and other soft tissues. After an injury, an inflammatory response occurs, and the cells below the dermis (the deepest skin layer) increase collagen (connective tissue) production. Later, the epithelial tissue

(the outer skin layer) is regenerated. There are three stages to wound healing: inflammation, proliferation, and remodeling. The proliferative phase is characterized by angiogenesis, collagen deposition, epithelialization, and wound contraction.

Delay in the Wound Healing Process

Wound healing is impaired in diabetic patients with infection or hyperglycemia (Sharma et al., 2013). The guiding principles for wound healing include the rapid and complete repair of the created defect and preventing the bacterial invasion during the period the natural barriers are defective. Although these principles appear to be individual goals, they are impossible to attain separately in the clinical care of wounds. In practice, the maneuvers employed to promote rapid wound healing are intimately related to preventing bacterial invasion (Sabath, 2006).

Caesalpinia sappan Linn.

Caesalpinia sappan Linn. is a small thorny tree, 6-9 m high, in the Philippines, India, and Peru. In the study of Sathya (2010), a preliminary phytochemical analysis of the aqueous extract revealed the presence of flavonoids, saponins, sterols, glycosides, triterpenoids, and tannins. Ethanol showed a positive test for flavonoids, saponins, glycosides, triterpenoids, and tannins. It is being used traditionally for many ailments and is reported to have a wide variety of medicinal properties. Its anticonvulsant, anti-inflammatory, anti-proliferative, antimicrobial, anticoagulant, antiviral, immunostimulant, and antioxidant activities have been reported. According to Ayurveda, the heartwood is valuable in vitiated conditions of Pitta, burning sensation, wounds, ulcers, leprosy, skin diseases, diarrhea, dysentery, and diabetes. A decoction of the heartwood is commonly used in Kerala, India, for its anti-thirst, blood purifying, antidiabetic properties, and the plant is one of the ingredients in many traditional Ayurvedic formulations (Sathya, 2010).

Jatropha curcas Linn.

Jatropha curcas Linn. (Euphorbiaceae) is native to the American tropics, most likely Mexico and Central America. It is a multipurpose tree of commercial significance because of its several industrial and medicinal uses. *Jatropha species* are used in traditional folklore medicine to cure ailments in Africa, Asia, and Latin America (Arekemase et al., 2011). They are used as antimicrobial agents, and scientists have carried out several works to find out their scientific basis (Kalimuthu et al., 2010). Preparations of all parts of this plant in the form of the decoction are used in traditional medicine and for veterinary purposes. The decoction of leaves is used against cough and as an antiseptic after birth. Latex has antimicrobial properties against many species. The oil of this plant is used traditionally for the treatment of sciatica dropsy, paralysis, rheumatism, dysentery, diarrhea, and certain skin diseases (Kalimuthu et al., 2010).

Lantana camara Linn.

Lantana camara Linn. is commonly known as wild sage. It is a flowering shrub native to tropical America and is cultivated throughout the world as an ornamental plant. Different parts of the plant are used in folklore remedies and traditional medicine systems to treat various human ailments. The leaves are used to treat itches, cuts, ulcers, swellings, bilious fever, eczema, and rheumatism. Many pharmacological investigations indicated that extracts of the leaves exhibit antibacterial properties (Agrawal, 2012). In the study of Agrawal (2012), the extract of L. camara Linn. leaves in methanol exhibited good antibacterial and inhibited the growth of all the isolates used. The aqueous extract was effective against all the bacterial cultures except *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Mimosa pudica Linn.

Mimosa pudica L. is a creeping annual or perennial herb often grown for its curiosity value, as the compound leaves fold inward and droop when touched and reopen within minutes. It belongs to the Fabaceae family. Mimosa pudica is native to Brazil but is now a pantropical weed. The other names given to this plant are Humble plant, Shame plant, Touch me not, Sleeping grass, Prayer plant. The species epithet "pudica" is a Latin equivalent for "Bashful" or "Shrinking" because of its curious nature and easy procreation. The stem is erect in young plants but becomes creeping or trailing with age. The plant grows to a height of 1.5m (5 ft). The leaves are bipinnately compound, with one or two pinnae pairs and 10-26 leaflets per pinna. The petioles are also prickly, and on close examination, it is seen that the floret petals are red in their upper part, and the filaments are pink to lavender. The fruit consists of clusters of 2-8 pods of 1-2cm long each, prickly on the margins (Gandhiraja et al., 2009).

Moringa oleifera Lam.

Moringa oleifera Lam. is a highly valued plant distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain essential minerals and are a good source of protein, vitamins, β – carotene, amino acids, and various phenolics (Farooq et al., 2007). The Moringa plant provides a rich and rare combination of zeatin, quercetin, kaempferol, and many other phytochemicals. It is essential for its medicinal value. Various parts of the plant, such as the leaves, roots, seed, bark, fruit, flowers, and immature pods, act as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer (Farooq et al., 2007). Other critical medicinal properties of the plant include antispasmodic, diuretic, antihypertensive, cholesterol-lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial, and antifungal activities (Nickon et al., 2003).

The phytochemical screening indicated the presence of phenolics, flavonoids, tannins, and glycosides in the extracts. The leaves of M. *oleifera* contain alkaloids, which showed potential antimicrobial properties by intercalating with bacterial DNA. It is believed that the higher reducing power of the aqueous extract could be due to the better solubility of the antioxidant components in water, whereas the principal antibacterial activity in

organic solvent extracts as compared to aqueous extracts indicates that the active components responsible for the bactericidal activity are more soluble in organic solvents. These studies provide evidence to support the traditional medicinal uses of the plant (Vinoth et al., 2012).

Psidium guajava Linn.

Psidium guajava Linn. is an evergreen shrub native to tropical America that has neutralized South East Asia. It is an important food crop and medicinal plant available in tropical and subtropical countries. It is a phytotherapeutic plant used in folk medicine that is believed to have active components that help to treat and manage various diseases. It contains essential phytoconstituents such as tannins, triterpenes, flavonoid: quercetin, pentacyclic triterpenoid: guajanoic acid, saponins, carotenoids, lectins, leucocyanidin, ellagic acid, amritoside, beta-sitosterol, uvaol, oleanolic acid, and ursolic acid. Many pharmacological studies have demonstrated the ability of this plant to exhibit antioxidant, hepatoprotective, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, anti-inflammatory, and antinociceptive activities, supporting its traditional uses. It suggests a wide range of clinical applications. The many parts of the plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and several other conditions (Biswas et al., 2013).

P. guajava contains several chemical constituents, which possess antibacterial, antidiarrheal, antimycobacterial, antihyperglycemic, antimalarial, cytotoxic, and antioxidant activities (Roy et al., 2006). A recent study showed that the *P. guajava* aqueous extract possessed antibacterial activity against *Salmonella* typhi and *Klebsiella pneumoniae*. However, no effect on the growth of *Escherichia coli, Staphylococcus aureus*, and *Streptococcus fecalis* organisms (Geidam et al., 2010).

Phytochemicals in Antibacterial Activity

The medicinal and pharmacological actions of medicinal herbs are often dependent on the presence of bioactive compounds called secondary herbal metabolites (Kumar et al., 2007). Saponin and tannins are reported to possess antibacterial activities. Phytochemical analysis of the methanol and distilled water extracts of *Lantana camara* showed the presence of saponin, tannin, and flavonoid, which could be the active principle. The secondary metabolites like tannin, flavonoids, and steroids showed antibacterial activities (Udobre et al., 2012).

The mode of action of antibacterial effects of saponins seems to involve membranolytic properties rather than simply altering the surface tension of the extracellular medium, thus being influenced by microbial population density (Usman & Osuji, 2007). Flavonoids are phenolic structures containing one carbonyl group; since plants synthesize them in response to microbial infection, it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms (Roy et al., 2006). Alkaloids isolated from the plant are commonly found to have antibacterial properties (Biswas et al., 2013).

MATERIALS AND METHODS

Collection of Plant Sample

Healthy, intact, and young leaves of *Jatropha curcas, Lantana camara, Mimosa pudica, Moringa oleifera, and Psidium guajava* were collected in the vicinities of the researchers' residences. The Caesalpinia sappan bark was purchased in the public market of Koronadal City, Philippines. The leaves were thoroughly washed with running water to remove dirt on the plants and dried with a clean cloth.

Ethanolic Extraction

The leaves of the plants were cut into small pieces using scissors. The bark of *C. sappan* was chopped into small pieces. The plant parts were separately placed in a beaker and were soaked in ninety-five percent (95%) ethanol for 24 hours. Filtration was done on each sample, and the filtrates were concentrated in a rotary evaporator at a temperature of 45oC. The concentration of the extract was computed and expressed as grams of plant parts per mL of extract. The concentrated ethanolic crude extracts were placed in vials with labels and were stored in the refrigerator to prevent the growth of microorganisms.

Phytochemical Screening

The phytochemical screening of the plant extracts was carried out based on the procedures of Guevarra (2005).

Test for Alkaloids

An equivalent of twenty (20) grams of plant material from the stock plant extract was placed in an evaporating dish. This was evaporated to a syrupy consistency over a steam bath. Five (5) mL of 2M hydrochloric acid (HCl) was added and stirred for about 5 minutes and was allowed to cool. One (1) mL of the filtrate was tested with 2 to 3 drops of Dragendorff's reagent. Another one (1) mL of the filtrate was tested with 2 to 3 drops of Meyer's Reagent. The relative amount of precipitation was observed as follows:

(+) slight turbidity, (++) definite turbidity, (+++) heavy precipitation

Confirmatory test: Three (3) mL of the crude extract was obtained, and 28% ammonia solution was added until the solution turned alkaline to litmus paper. The alkaline solution was extracted three times with small portions of less than ten (10) mL of chloroform. The lower chloroform extract was collected, and the upper aqueous layer was reserved for the quarternary and amine oxide test. The chloroform extract was evaporated to dryness over a water bath. Five (5) mL of 2M HCl was added, stirred over a water bath for about 2 minutes, and allowed to cool. The solution was then filtered, and the filtrate was separated into two portions. One (1) portion was tested with Mayer's reagent and the other with Dragendorff's reagent. The result was recorded as (+), (++), and (+++). Positive results indicated the presence of primary, secondary, or tertiary alkaloids.

Test for *quarternary bases and amine oxide*: The alkaline

aqueous layer obtained in the confirmatory test was acidified with 2M HCl. The solution was then filtered, and the filtrate was divided into two portions. One portion was tested with Mayer's reagent and the other portion with Dragendorff's reagent. The results were observed and recorded as above. A score of (++) or (+++) in both Mayer's and Dragendorff's tests was taken to indicate the presence of both quarternary and amine oxide bases. A (+) score was recorded as the result of incomplete chloroform extraction and thus was considered negative for quarternary bases.

Test for Saponins (Froth Test)

A volume of the plant extract equivalent to two (2) grams was transferred into a test tube. In a separate test tube, one (1) mL of "gogo" extract was placed, and this served as the standard. Ten (10) mL distilled water was added to each test tube, stoppered, and shaken vigorously for 30 seconds. The solutions in the test tubes were allowed to stand for ten (10) minutes, and the formation of a "honeycomb" froth was evaluated. The result for the plant extract was compared with that of the standard.

Positive result: If the "honeycomb" froth is greater than two (2) cm in height from the surface of the liquid and persists after ten (10) minutes, the sample was considered positive for saponins. *For plant extracts with poor frothing effects,* a small amount of 5% sodium carbonate solution was added to basify the extract. The formation of a stable and dense froth indicated the presence of free fatty acids.

Test for Flavonoids

An equivalent of ten (10) g of plant material from the stock plant extract was allowed to evaporate in incipient dryness over a steam bath and allowed to cool at room temperature. The residue was defatted with nine (9) mL petroleum ether. Petroleum ether was then discarded. The defatted aqueous layer was diluted with ten (10) mL of 80% ethyl alcohol, filtered, and the filtrate was divided into three (3) test tubes. One (1) portion was taken as control.

Test for leucoanthocyanins by Bate-Smith and Metcalf method: One (1) portion of the alcoholic filtrate was treated with 0.5 mL concentrated HCl (12M). A change in color was observed. The solution was warmed for 15 minutes in a water bath. Any further change in color observed within an hour was recorded and compared with the control.

Positive result: A strong red or violet color indicates the presence of leucoanthocyanins.

Test for γ-benzopyrone nucleus: Wilstatter "cyanidin" test: The other portion of the alcoholic filtrate was treated with 0.5 mL concentrated HCl (12M). Three (3) to four (4) pieces of magnesium turnings were added. Any color change observed within ten (10) minutes was recorded and compared with the control tube. If definite coloration was observed, an equal volume of water and one (1) mL of octyl alcohol were added. The mixture was shaken and was allowed to stand. The color of each layer was noted.

Positive result: Colors ranging from orange to red, to crimson and magenta, and occasionally to green or blue.

Test for Tannins

An equivalent of ten (10) g of plant material was taken from the stock plant extract and was evaporated to incipient dryness over a steam bath. Twenty (20) mL of hot distilled water and five (5) drops of 10% sodium chloride solution were added to salt out undesirable constituents. The solution was filtered, and the filtrate was divided into three (3) test tubes. One (1) test tube served as blank, and an aqueous solution of tannic acid served as the reference standard.

Gelatin Test: Three (3) drops of gelatin salt reagent were added to another test tube. The same was done for the aqueous solution of tannic acid. The formation of any precipitate indicated the presence of tannins.

Ferric Chloride Test: The third test tube was treated with three (3) drops of ferric chloride solution. The same was done to the reference standard. A blue-black color indicated the presence of hydrolyzable tannins, while a brownish green color indicated the presence of condensed tannins.

Test for Anthraquinones (Borntrager's Test)

A portion of crude extract equivalent to one (1) g of plant material was evaporated to incipient dryness over a water bath. The residue was taken with ten (10) mL of distilled water and filtered. The filtrate was treated twice with five (5) mL portions of benzene. The benzene extract was divided into two (2) portions. One (1) portion served as the control. The other portion was treated with five (5) mL of ammonia solution, shaken, and compared with the control. A red coloration in the lower alkaline layer indicated the presence of anthraquinones.

In Vitro Antimicrobial Screening of the Plant Extracts on Wound Bacteria

Isolation of Wound Bacteria

Three days after wound induction on *Mesocricetus auratus (hamster)*, bacteria in the wound were aseptically inoculated in the sterile nutrient broth using a cotton swab. The nutrient broth was prepared by mixing 1 gram peptone, 0.6 grams beef extract, and 200 mL distilled water. The culture was incubated for 24 hours at 37oC.

Antimicrobial Screening of the Extracts by Agar Well Diffusion Assay (Guevarra 2005)

Muller Hinton Agar (MHA) was used for the antimicrobial activity test. MHA was prepared by weighing 38 grams of the powdered agar into 1000 mL of distilled water in a clean conical flask. It was cooked in low flame, and 15 mL of the medium was poured into test tubes and autoclaved at 121oC, 15 psi pressure for 15 minutes. Petri dishes, pipettes, culture tubes, steel cylinders (borer), and forceps were individually wrapped in paper and were sterilized in the autoclave for 15-20 minutes at 121 oC and 15 psi together with the previously prepared culture media. 0.5 mL of the incubated wound bacteria were inoculated on a sterile test tube containing nutrient broth. After which, the inoculum density of the test organisms was compared with the 0.5 McFarland Nephelometer barium sulfate turbidity standard containing approximately 1.5 x 108 CFU / mL of the test organism. Wound bacteria

was added if the turbidity was lesser than the McFarland standard.

The base plate was prepared by transferring 15 mL of the sterile MHA on a sterilized Petri dish and was allowed to solidify. The seeded top agar was prepared by transferring 1mL of the standardized test organism to 99 mL of sterilized MHA. Only 5 mL of the seeded top agar was added to each solidified plate. There were eight treatments in this study, namely: Treatment A - *Caesalpinia sappan extract,* Treatment B - *Jatropha curcas extract,* Treatment C - *Lantana camara extract,* Treatment D - *Mimosa pudica extract*, Treatment E - *Moringa oleifera extract*, Treatment F - *Psidium guajava extract*, Treatment G – Betadine solution (positive control), and Treatment H – Sterile distilled water (negative control).

After the top agar had solidified, four 6mm holes were aseptically bored into the seeded plate. The agar plugs were removed inside the cork borer by pushing them out with the help of a metal rod and were discarded aseptically. With a marking pen, the underside of the Petri dishes under each well was labeled specifically with letters to identify treatments to be delivered. Two (2) drops (approximately 0.1 mL) of each of the prepared plant extracts were delivered to the agar wells using a sterile Beral pipette. When all the treatments were delivered on the agar, the plates were inverted and wrapped individually with clean paper. These were then placed in the incubator for 24 hours at 36.5oC.

Determination of Antibiotic Activity Index of the Plant Extracts (Disc Diffusion Method)

The antibiotic activity index of the extracts was determined by the Kirby-Bauer agar diffusion method based on the National Committee for Clinical Laboratory Standards. The base plate was prepared by transferring 15 mL of the sterile MHA on a sterilized Petri dish and was allowed to solidify. The seeded top agar was prepared by transferring 1mL of the standardized test organism to 99 mL of sterilized MHA. Only 5 mL of the seeded top agar was added to each solidified plate. The sterile discs made from Whatman #1 filter paper (diameter 6mm) were soaked in plant extracts and were placed on the MHA agar surface with flamed forceps and gently pressed down to ensure contact with the agar surface. Amikacin 30 mcg, Erythromycin 15 mcg, and Penicillin 10 U were used as positive controls. The discs were spaced far enough to avoid reflection waves from the edges of the Petri dishes and overlapping rings of inhibition.

Ethical Consideration

The researchers conducted this study in complete accordance with established research protocols. The researcher ensured that potentially hazardous biological agents risk assessment form and human and vertebrae animal tissue form were approved prior to the conduct of the study.

Data Gathering

After all the plates were incubated, they were examined for the presence of zones of inhibition. The diameter of the zone of inhibition for both in the agar well and in the discs was measured using a plastic ruler in mm. The activity index of the ethanolic plant extracts was calculated as follows:

Activity index (A.I.) = Mean of zone of inhibition of the extract / Zone of inhibition obtained for standard antibiotic drug.

Used agars and microbial cultures were decontaminated in the autoclave prior to disposal.

Statistical Analysis

The antimicrobial activity was determined by measuring the diameter of the zone of inhibition and the mean of triplicates ± Standard Deviation (S.D.) of three replicates. One-way Analysis of Variance (ANOVA) was used to determine if there was a significant difference among the treatment means.

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemicals are non-nutritive plant compounds that provide the plant with disease-preventative characteristics. Qualitative analysis was performed on the phytochemical components, including alkaloids, flavonoids, saponins, tannins, and anthraquinones. Table 1 summarizes the results of the phytochemical screening. Note. CS - *Ceasalpinia sappan* Linn. (Sibukao); JC - *Jatropha curcas* Linn. (Tuba-tuba); LC - *Lantana camara* Linn. (Kantutay); MP- *Mimosa pudica* Linn. (Makahiya); MO - *Moringa oleifera* Lam. (Malunggay); PG - *Psidium guajava* Linn. (Guava);

Table 1. Results of Phytochemical Screening

Phytochemicals	Tests		Results					
Alkaloids			C.S.	JC	LC	MP	MO	PG
	Mayer's Test		-	+	+	+	+	+
	Dragendorff's Test		-	+	+	+	+	+
	Confirmatory Test	Mayer's Test	-	++	+	-	+	++
		Dragendorff's Test	-	+	+	-	+	+++
	Quarternary Bases and amine oxides	Mayer's Test	-	+++	+	++	+	++
		Dragendorff's Test	-	+++	+	+	+	++
Saponins	Froth Test		-	+	+	+	+	+
Flavonoids	Leucoanthocyanins "Bate-Smith and Metcalf Method		+	+	-	+	-	+
	y-benzopyrene nucleus Wilstatter "cyanidin" Test		+	+	+	-	-	+
Tannins	Gelatin Test		-	+	-	+	-	+
	Ferric Chloride Test		+	+	+	+	+	+
Anthraquinones	Borntrager's Test		-	-	-	-	-	-

Alkaloid Mayer's and *Dragendorff's Test:* (+) slight turbidity; (++) definite turbidity; (+++) heavy precipitation; Confirmatory Test: (+) primary alkaloid; (++) secondary alkaloid; (+++) tertiary alkaloid; Test for quarternary bases and/or amine oxide: (+) absent; (++) or (+++) present; *Test for Saponins, Tannins, and Anthraquinones*: (+) present; (-) absent.

The phytochemical analysis of the plant extracts revealed the presence of the following secondary metabolites: saponins, flavonoids, alkaloids, and tannins. According to Biswas et al. (2013), the different secondary metabolites such as saponins, flavonoids, alkaloids, and tannins account for the majority of plants' antibacterial activity. Saponins' antibacterial effect is due to their capacity to inhibit the membranolytic characteristics of bacteria rather than just modifying the surface tension of the extracellular medium (Usman & Osuji, 2007). Likewise, flavonoids are phenolic compounds with a single carbonyl group generated by plants in response to microbial infection. Hence, it is unsurprising that they are efficient antibacterial agents against a diverse array of microorganisms in vitro (Roy et al., 2006).

Meanwhile, the antibacterial activities of alkaloids derived from plants are frequently reported (Biswas et al., 2013). Similarly, research using various plant extracts has shown that phytochemicals such as flavonoids and tannins are known to aid the wound healing process. This is mainly due to their astringent and antibacterial qualities, which contribute to wound contraction and epithelialization rate increase (Usman & Osuji, 2007).

Antimicrobial Activity Screening

Wound bacteria isolated from an incised Mesocricetus auratus (hamster) were treated with ethanolic extracts from leaves of Jatropha curcas, Lantana camara, Mimosa pudica, Moringa oleifera, and Psidium guajava, and the bark of Caesalpinia sappan. Figure 1 showed the zones of inhibition of the treatments on wound bacteria in the different replicates. As shown in Figure 1, P. guajava exhibit the highest zone of inhibition among the treatments. Furthermore, Guevarra, 2005 made an inference on the results of the Zones of Inhibition produced on the test discs. <10 mm may be expressed as inactive, 10-13 mm as partially active, 14-19 mm as active, and >19 mm as very active. Table 2 showed that Psidium guajava was very active among the eight treatments, followed by Mimosa pudica and betadine as active, Caesalpinia sappan, Jatropha curcas, and Lantana camara as partially active, and Moringa oleifera and distilled water as inactive.

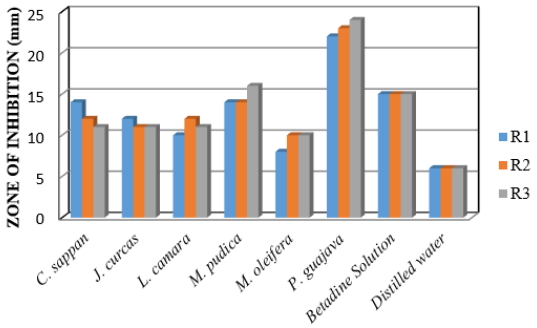

Figure 1. Zones of inhibition of the treatments on wound bacteria

Table 2. Treatment means + S.D. and their interpretations based on 6 mm test discs.

Treatments	Mean + S.D.	Interpretation (Guevarra, 2005)
Caesalpinia sappan Bark Extract	12.30 + 1.530 ^{c}	Partially Active
Jatropha curcas Leaf Extract	11.30 + 0.577 ^{c}	Partially Active
Lantana camara Leaf Extract	11.00 + 1.000 ^{c}	Partially Active
Mimosa pudica Leaf Extract	14.67+ 1.150 ^{b}	Active
Moringa oleifera Leaf Extract	9.33 + 1.15d	Inactive
Psidium guajava Leaf Extract	23.00 + 1.000 a	Very Active
Betadine Solution	15.000 + 0.000 b	Active
Distilled Water	6.00 + 0.000 e	Inactive
Values with different superscripts on the same column have a different interpretation of zones of inhibition based on Guevara (2005) in the 6 mm test disc.		

Table 3. One-way analysis of variance (ANOVA) on the zones of inhibition of the treatments.

Source	df	SS	MS	F (Comp.)	F (Tab.)
Treatments	7	528.70	75.52	82.39**	2.66 ($p<0.05$) 4.03 ($p<0.01$)
Error	16	14.67	0.92		
Total	23				
Note. ** significant at 0.05 and 0.01 levels of significance.					

One-way analysis of variance (Table 3) revealed a highly significant difference among treatment means at 0.05 and 0.01 levels of significance with a computed F value of 82.39.

The comparison between treatment means revealed that P. guajava leaf extract with a mean Z.I. of 23.00 + 1.000 was the most significantly effective treatment against wound bacteria. On the other hand, among the plant extracts, M. oleifera leaf extract with a mean Z.I. of 9.33 + 1.15 was the least effective in inhibiting wound bacteria. Distilled water did not inhibit wound bacterial growth based on the 6mm agar well.

The broad antibacterial activity of plant extracts may be due to the presence of secondary metabolites from the plants (alkaloids, flavonoids, tannins, and saponins). Previous studies have demonstrated that tannins have antibacterial activity because of their ability to infer bacterial protein synthesis (Akiyama et al., 2001; Sanches et al., 2005; Min et al., 2008). Usman and Osuji (2007) posited that tannins had been commonly used topically

to superficial wounds. Consequently, it is possible that the extract' tannins and other phenolics in plants were responsible for these broad antibacterial activities. On the other hand, flavonoids and saponins can inhibit bacterial growth by forming complexes with extracellular and soluble proteins and bacterial cell walls (Min et al., 2008). Accordingly, the antibacterial activity of P. guajava was consistent with Roy et al.'s (2006) research, which revealed that P. guajava contains several chemical compounds with antibacterial, antimycobacterial, cytotoxic, antidiarrheal, antimalarial, antihyperglycemic, and antioxidant properties. Begum et al. (2004) reported the presence of over twenty chemicals in the leaves of P. guajava. Tannins are present in the aqueous leaf extract, whereas the ethanolic extract is rich in anthocyanins, alkaloids, flavonoids, tannins, and steroids/terpenoids.

Antibiotic Activity Index

The antibiotic activity of the six (6) selected ethanolic plant extracts were compared with the three (3) antibiotics, namely: Amikacin 30 mcg, Erythromycin 15 mcg, and Penicillin 10 U. As shown in Table 4, the mean zones of inhibition for the plant extracts were greatest in Psidium guajava Leaf Extract with a mean of 16.32 mm and least in Lantana camara Leaf Extract with a mean of inhibition of 6.12 mm. The zone of inhibition for the antibiotics was greatest in Amikacin 30 mcg with a mean of 21.60 mm, followed by Erythromycin 15 mcg with a mean of 20.63 mm, and with Penicillin 10 U with a mean of 6.70 mm.

Table 4. Zones of inhibition of the plant extracts and antibiotics on wound bacteria.

Treatments	Mean Zone Of Inhibition (mm)
Caesalpinia sappan Bark Extract	9.77 ± 0.37
Jatropha curcas Leaf Extract	7.05 ± 1.41
Lantana camara Leaf Extract	6.12 ± 0.10
Mimosa pudica Leaf Extract	7.90 ± 0.45
Moringa oleifera Leaf Extract	6.85 ± 0.68
Psidium guajava Leaf Extract	16.32 ± 1.96
Amikacin 30 mcg	21.60 ± 0.53
Erythromycin 15 mcg	20.63 ± 2.71
Penicillin 10 U	6.70 ± 1.13
Note. Values are mean ± standard deviation.	

Meanwhile, the antibiotic activity index of the plant extracts (Table 5), when compared with Amikacin 30 mcg, ranged from 0.76 to 0.32, with the highest activity observed in Psidium guajava leaf extract, the least A.I. was observed in Jatropha curcas leaf extract and Moringa oleifera leaf extract.The antibiotic activity index of the plant extracts, when compared with Erythromycin 15 mcg, ranged from 0.79 to 0.30, with the highest activity observed in Psidium guajava leaf extract and the least A.I. was observed in *Lantana camara* leaf extract. The antibiotic activity index of the plant extracts, when compared with Penicillin 10 U, ranged from 2.43 to 0.91, with the highest activity observed in *Psidium guajava* leaf extract and the least A.I. was observed in *Lantana camara leaf* extract.

Table 5. Antibiotic activity index (A.I.) of the plant extracts when compared with antibiotics

Plant Extracts	Antibiotics		
	Amikacin 30 mcg	Erythromycin 15mcg	Penicillin 10 U
Caesalpinia sappan Bark Extract	0.45	0.47	1.46
Jatropha curcas Leaf Extract	0.32	0.34	1.05
Lantana camara Leaf Extract	0.28	0.30	0.91
Mimosa pudica Leaf Extract	0.37	0.38	1.18
Moringa oleifera Leaf Extract	0.32	0.33	1.02
Psidium guajava Leaf Extract	0.76	0.79	2.43
Note. Activity index (A.I.) = Mean of zone of inhibition of the extract / Zone of inhibition obtained for standard antibiotic drug.			

The antibiotic activity index reading of 1.00 means that the plant extract has the same antibiotic activity compared to a specific antibiotic. The study results showed that when the A.I. of the plant extracts were compared with Amikacin 30 mcg and Erythromycin 15 mcg, they had A.I. values lesser than 1.00, which means that they have lower A.I. than Amikacin 30 mcg and Erythromycin 15 mcg. However, the A.I. of the plant extracts, when compared with Penicillin 10 U, showed remarkable results. Among the six plant extracts, only *Lantana camara* leaf extract had an A.I. value lower than 1. This means that the five plant extracts have higher antibiotic activity than Penicillin 10 U in inhibiting the growth of wound bacteria.

The antibacterial activity of *Psidium guajava* suggests that it has a broad variety of therapeutic uses. Numerous components of the plant have been used in traditional medicine to treat various diseases, including wounds, gastroenteritis, vomiting, dysentery, malaria, diarrhea, coughs, sore throats (Biswas et al., 2013).

CONCLUSIONS

The phytochemical components, namely alkaloids, saponins, flavonoids, and tannins, were present in the ethanolic plant extracts. Anthraquinones were absent in the ethanolic extract of all plants evaluated. More so, *Caesalpinia sappan* Linn., *Jatropha curcas* Linn. , *Lantana camara* Linn., *Mimosa pudica* Linn., *Moringa oleifera* Lam., and *Psidium guajava Linn.* were effective in inhibiting the growth of wound bacteria when compared with betadine solution. All the six plant extracts exhibited antibacterial activity, and that *Psidium guajava* Linn. was the most effective plant extract, which is more effective than

betadine in inhibiting wound bacteria. Further, all the plant extracts have a lower antibiotic activity index when compared with Amikacin 30mcg and Erythromycin 15 mcg. However, except for *Lantana camara* extract, the five plant extracts have remarkably higher antibiotic activity index when compared with Penicillin 10 U. The findings of this research corroborate the traditional uses of the plants tested and indicate that some of the plant extracts contain antibacterial components that might be used as antimicrobial agents.

On that note, the researchers recommended to determine the bioactive substances from the plant extracts; to carry out more pharmacological evaluation on *Psidium guajava* to maximize its use as an antimicrobial agent; bacteria from the wound of hamster be isolated and identified; test the selected plants on in vivo wound healing activity; and test other plants for their phytochemicals that can be isolated and purified and employed in the formulation of novel antimicrobial agents for the treatment of wounds antibacterial activity on wound bacteria.

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