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The Potential of *Gliricidia sepium* Plant Extract as Antibacterial and Antifungal: A Review

Mhieco P. Juanico, Listya Purnamasari, Seong Gu Hwang, and Joseph F. dela Cruz

ABSTRACT

The alarming increase in the emergence of novel diseases, which developed resistances against the most recurrently used antibiotics, have kept the medical industry in searching for new sources of compounds with antimicrobial activity. One resource that is considered to be a cache of various bioactive compounds are plants and along with different studies regarding traditional health care practices have led researchers to the tree *Gliricidia sepium*. It is a leguminous tree believed to have a number of benefits among the ethnic groups in South and Southeast Asia as well as Central and South America. With this premise, *G. sepium* has been a focus of in-vitro antimicrobial studies to describe and measure its extract's potency against commonly infective microorganisms such as bacteria and fungi. These studies have returned with promising results capable of competing with antibiotics like ciprofloxacin, gentamycin, and tobramycin and antifungal such as amphotericin-B. To justify the antimicrobial activity of *G. sepium*, several of these studies performed subsequent phytochemical analysis which identified different complex chemicals like tannins, flavonoids, and alkaloids which are historically linked with antimicrobial activity. The results of the different tests performed establish *G. sepium* as a good alternative for antimicrobials.

Keywords: Alternative, antimicrobial activity, Gliricidia sepium, phytochemical analysis, traditional health care .

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I. Introduction

The medical field has always been burdened by the emergence of life-threatening diseases which can either be novel or previously known. These diseases may be infectious in nature and are mostly caused by bacteria or viruses among all microorganisms. A database analysis of 335 emerging infectious diseases (EID) recorded between 1940 and 2004 found that 54.3 percent of EIDs are caused by bacteria or rickettsia whose strains have developed drug resistance and that 60.3 percent of the pathogens are of zoonotic origin [1]. One Health data estimate that there is an annual rate of 700,000 mortalities caused by drug-resistant infections [2]. From these records, the significance of zoonoses and antimicrobial resistance is evident and global efforts to control EIDs through different strategies are in action.

Finding alternatives for antimicrobials is one of the solutions that medical authorities and researchers investigate. One important source that is most attractive to derive new antimicrobial compounds is nature, specifically, plants. The utilization of plants for medicinal purposes has been recorded since the time of ancient civilizations and the human realization and awareness of their therapeutic capabilities are developed through trial and error and the passing of information from one generation to the next [3]. The impact of plant-derived compounds is furthered by the fact that 80% of the world's population is dependent on traditional medicine which mostly revolves around plant extracts as reported by the World Health Organization [4]. Research also suggests that plant-derived compounds offer less toxic side effects thus gaining more attention from the medical community [5]. The rich biodiversity of medicinal plants combined with modern techniques of acquiring their active substances creates a cache of novel bioactive molecules.

In various developing counties like the Philippines, reliance to traditional and/or folkloric medicine is usually culturally driven [6]. This makes medicinal plant-focused studies abundant in the country. Ethnobotanical surveys that distinguish medicinal plants used by Ayta communities in Bataan and Pampanga found that the *Fabaceae* family is the

most represented group [7]. *Fabaceae* is a plant family that consists of legumes which are trees that bear beans or peas [8]. It is also one of the most chemically and pharmacologically evaluated botanical groups where different components such as flavonoids, alkaloids, coumarins, and other metabolites are derived [9]. This brings us to one of the popular species of Fabaceae in the Philippines known for its versatility and traditional therapeutic properties, *Gliricidia sepium*.

G. sepium is a medium, single, or multiple-stemmed tree with an average diameter of 30cm and average height of 15m [10]. It is native to Central and South America where it is commonly called as "Madrecacao" for it used to provide shade in cocoa plantations [11]. *G. sepium* has been long since introduced and naturalized to other tropical and sub-tropical countries in Asia like the Philippines, where it is more commonly known as "kakawate" [12]. The word Gliricidia can be translated as rat poison which describes the well-known usage of its leaves or ground bark mixed with cooked maize as a rodenticide [13]. Locally, traditional healers use *G. sepium* leaf extracts as remedy for itchiness, rashes, skin infections, and phlegm whereas decoctions and shoot extracts are used to treat wounds, skin irritations and even ringworm [7]. Other ethnomedical uses of *G. sepium* include alopecia, bruises, burns, colds, debility, fever, fractures, gangrene, headache, rheumatism, skin tumors, and ulcers [12]. Aside from human conditions, *G. sepium* plant material is also used by pet owners and backyard farm owners to treat certain afflictions in their animals. A study on the ethnoveterinary practices performed in a municipality in Isabela found that *G. sepium* is one of the most common ethnoveterinary botanical medicine material (EVB-M) used and is second only to guava in treating skin diseases in swine [14]. The confidence behind *G. sepium*'s effectiveness as a traditional therapeutic material allowed for different research regarding the plant's phytochemicals. Today, *G. sepium* has been reported as an expectorant, insecticidal, rodenticidal, sedative, suppurative, antibacterial, antifungal, antiviral, insecticidal and rodenticidal [15].

In this review, the different methodologies used to extract and analyze the active components of *G. sepium* will be identified as well as which part of the plant was used. The different microbes that exhibited sensitivity to *G. sepium* will also be presented. Phytochemical analysis will also be visited to identify which active component is responsible for the therapeutic characteristics described. The author aims to out find if there is a distinguishable difference between the different methodologies and plant parts used in relation to the plant's antimicrobial potential. Comparison of sensitivity indexes with common antimicrobials will also be discussed.

II. Discussion

A. Plant Material and Extraction

1) Authentication

G. sepium's ability to treat human and animal ailments makes it a candidate for in-vitro antimicrobial studies. Antimicrobial testing requires a thorough method to ensure accurate results. The first step in the approach of reviewed papers is usually authenticating the plant material. Authenticating plant samples is the first step in laboratory testing, similar to any plant extract studies. Authentication ensures accurate test results. Authenticated plant material and promising results will promote the plant as a source of novel bioactive compounds and serve as a reference for future, more intensive studies. Authentication is used to ensure the precise, safe, and effective usage of plant extracts [16]. Most studies authenticated their plant samples through academic institutions or taxonomists [11], [17]-[25].

2) Plant part

According to the studies evaluated, leaves are the most widely employed plant part which is synonymous with studies regarding traditional and folkloric medicine in the Philippines [26]-[28]. The flower is utilized in [11] investigations. References [11] and [22] used bark. Reference [18] uses root bark. Reference [21] and [23] did not specify which plant parts they used. Only the investigations by [11] used two plant parts to investigate *G. sepium*'s in-vitro antibacterial properties and compared their results.

3) Sample preparation

In the evaluated articles, sample preparation came before extraction. Most research utilized drying and grinding to prepare samples. Drying is employed in plant extract investigations because moisture promotes bacterial and fungal growth [29]. This can cause rotting and fermentation, changing the plant's chemical composition and making it unfit for extraction [30]. Precautions against high-temperature drying are emphasized because it can denature active plant components [31]. Freeze-drying consider the safest way to dry labile chemicals [32]. After drying, plant material was pulverized. Size reduction ruptures the plant's cell structures, exposing its active compounds to the extraction solvent. Smaller size increases surface area, which helps transfer active compounds from the plant to the solvent [33]. More sample preparation methods were described like sterilization which removes contaminants that could affect experiment results [34]. Another is sieving to get a finer, more consistent product. Proper storage is also observed that protects plant samples from contamination and deterioration, which could affect antimicrobial sensitivity and phytochemical screening tests [35].

4) Extraction methods

Organic solvent extraction is the most common extraction technique, with ethanol being the most prevalent component. Methane is the second most utilized compound, after petroleum ether, ethyl acetate, chloroform, n-hexane, and acetone. The aqueous extraction is the second most used method and is always used as a comparative model alongside organic solvent extraction. Despite using the same group of solvents, extraction methods vary. Reference [20], [25], and [36] used a simpler extraction method. These studies just soaked or steeped the plant samples to their chosen solvents then the extracts are filtered and either evaporated to dryness or subjected to an emulsifier to remove any solvent residue. Other studies made use of specialized techniques or equipment. Reference [11] used a Soxhlet apparatus for extraction because of its efficiency. Reference [13], [21]-[23] employed a technique called reflux which is also known for its efficient extraction followed by agitation to uniformly distribute the contents of the extract. Reference [12], [17],

[18], and [24] used a rotary evaporator known to remove residual solvents without the danger of heat degradation. Reference [37] used a mechanical shaker, [32] used microwave-assisted extraction, [11] used steam distillation to produce their plant extract in the form of essential oils.

B. Antibacterial

1) Bacterial isolates

All isolates come from certified microbiological laboratories in multiple references [11]-[13], [15], [17]-[25], [36], [37], [32]. Most used bacteria are gram-positive and gram-negative infection-causing strains. *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus, Klebsiella pneumoniae, Proteus vulgaris,* and *Salmonella typhi* are commonly tested. Most articles test 10 bacterial isolates, but [20] tested 29. Reference [17] focus on poultry pathogens.

2) Preparation and standardization of inoculum

Inoculum preparation begins in-vitro antibacterial screening. Bacterial isolates from microbiologylaboratories are incubated at the recommended temperature and time. Turbidity develops which indicates bacterial growth after incubation. After inoculum preparation, standardization involves producing a specific inoculum concentration. The McFarland Standard is used to estimate bacterial density in most in-vitro antibacterial screening studies. A McFarland standard is a solution of barium chloride and sulfuric acid that, when agitated, produces a turbid liquid comparable to bacterial inoculum [38]. McFarland standards is an established method in controlling the microorganism load in broth cultures which is evident by its recurring appearance in the articles under review.

3) Methods of antibacterial activity screening

Most studies reviewed use agar diffusion to determine in-vitro antibacterial activity. Agar plates are inoculated with bacteria and holes are created where the plant extract for testing is placed. Microbial growth is tested as the plant extract diffuses into the agar [39]. Agar disk diffusion is another method used by the studies. It is prepared similarly to agar well diffusion, but paper discs impregnated with the plant extract are equally placed on the medium instead of holes. If the extract has antimicrobial activity, microbial growth is inhibited as it diffuses into the medium [39]. Quantitative methods of antibacterial activity testing were also employed. Reference [21] and [23] estimated antimicrobial activity by relative inhibition zone diameter. Measurement of minimum inhibitory concentration defined as the lowest test sample concentration needed to kill 99.9% of viable organisms after incubation is also used by some studies [50]. One study was purely quantitative and used the lethal dose test [24]. It is deemed the best method for determining bactericidal effect because it can provide information about microbe-antimicrobe interaction, antagonism or synergism of drug combinations, and time or concentration dependence of an antimicrobial agent [39].

4) Results of antibacterial activity screening

Escherichia coli is the most common bacteria, and all the articles that are used in it show sensitivity to *G. sepium* extract. The different results may be due to differences in plant material, bacterial isolate preparation, extraction method,and antibacterial screening. Different plant parts may contain different compounds, causing different results. *Staphylococcus aureus* is the second most tested bacteria which was found to be sensitive to *G. sepium* plant extracts. One study on *Pseudomonas aeruginosa* presented different data than the others. *P. aeruginosa* is resistant to all plant extracts [23]. *G. sepium* bark extracts in water, methanol, petroleum ether, and ethyl acetate failed to inhibit *P. aeruginosa*. All reviewed articles that tested plant extracts on *Bacillus cereus* found antibacterial activity. *G. sepium* plant extracts tested on *Proteus vulgaris* showed antibacterial markers. Studies that tested their extracts against *Salmonella typhi* also showed promising antibacterial activity, but some discrepancies can still be observed [18]. The summaries of the *G. sepium* activity was presented in Table I. In addition, *Streptococcus/Enterococcus faecalis, Bacillus subtilis, Serratia marcescens, Salmonella typhimurium, Proteus mirabilis, Salmonella paratyphi, Shigella flexneri, Corynebacterium pyogenes, Bacillus pumillus, Sarcina lutea, Salmonella cholerasuis, and Staphylococcusepidermidis* also showed sensitivity against *G. sepium* extracts indicative of broad-spectrum antibacterial activity.

In all the solvents used in the articles under review, ethanolic extracts of *G. sepium* had the most antibacterial activity, inhibiting gram-positive and gram-negative bacterial growth at low concentrations. Ethanolic extracts have larger inhibition zone diameters than other solvents. Ethanol's polarity allows it to extract polar compounds [29]. This suggests that antibacterial compounds in *G. sepium* are polar. Non-polar solvents like n-hexane and petroleum ether have the least antibacterial activity. Traditional medicine extracts a plant's active components with rum and other ethanol-containing liquors which supports how good ethanol is for extraction [23]. In the studies of [17] and [25], crude extracts displayed better antibacterial activity than the fraction sets. Fractionation methods separate compounds from a sample to isolate a pure compound [29]. From this, we can assume that more than one compound in *G. sepium* plant extract has antibacterial activity and that these compounds display synergism.

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Fractionated plant extracts have a different effect than pure plant compounds. Reference [21] and [23] found that *G. sepium* extracts fail in comparison with other plants tested for antibacterial activity during MIC determination. According to these studies, a higher *G. sepium* extract concentration is needed to elicit antibacterial action. Reference [49] tested and compared the antibacterial activity of *G. sepium* tree parts. Bark and leaf extracts showed variable antibacterial activity, but flower extracts were effective against all tested bacteria. Leaf and bark extracts showed bacterial resistance, but flower

extracts did not. In a second study, antibacterial essential oils from *G. sepium* leaf and flower were tested. Both leafand flower essential oils inhibit all tested bacterial growth. Each plant part's intrinsic compounds affect its antibacterial activity

TABLE I: SUMMARIES OF ANTIBACTERIAL ACTIVITY OF *G. SEPIUM* EXTRACT

Extraction methods	Plant's part	Bacteria	Effect	Ref.
N- hexane	Leaf	*Eschericia coli*	Not sensitive to any concentration	[18]
N- hexane	Leaf	*E. coli*	Sensitive to any concentration	[21], [23]
Petroleum ether	Bark, leaf	*E. coli*	No inhibition zones	[11]
Petroleum ether	Leaf	*E. coli*	No antibacterial activity	[25]
Ethanol	Leaf	*S. aureus*	No inhibition zones	[12]
N-hexane	Leaf	*S. aureus*	No inhibition zones	[18]
Petroleum ether	Bark, leaf	*S. aureus*	Lacked antibacterial activity	[11]
Petroleum ether	Leaf	*Pseudomonas aeruginosa*	Have inhibition zones	[21]
Petroleum ether	Bark, leaf	*P. aeruginosa*	Discrepancies in *G. Sepium*'s antibacterial activity	[11]
Ethyl acetate	Leaf	*P. aeruginosa*	No antibacterial activity	[20]
Ethanolic	Leaf	*P. aeruginosa*	Lacked antibacterial activity	[37]
Ethyl acetate	Leaf	*P. aeruginosa*	Have antibacterial activity	[11]
Petroleum ether	Leaf	*Bacillus cereus*	Inhibit the growth of few bacteria	[25]
Ethyl acetate	Leaf	*B. cereus*	Did not inhibit	[20]
Methanol and chloroform	Leaf	*B. cereus*	Did not kill	[11]
Ethanolic	Leaf	*Klebsiella pneumoniae*	Did not inhibit	[12]
Ethyl acetate	Bark	*K. pneumoniae*	Ineffective	[11]
Ethyl acetate	Leaf	*K. pneumoniae*	Couldn't inhibit	[20]
Methanol	Leaf	*Proteus vulgaris*	*P. Vulgaris* resisted	[11]
Ethyl acetate	Bark	*P. vulgaris*	*P. Vulgaris* resisted	[11]

The structural integrity of plant parts can affect how well solvents or extraction methods release compounds. Different studies show different antimicrobial activity compared to standard antibiotics. Some have more action than others. Since the studies used different extraction methods, extract concentrations, and antibiotic concentrations, only qualitative comparisons are possible. *G. sepium* extractscan still compete with standard antibiotics, as shown by their superior inhibiting action compared to those tested in some of the articles reviewedWhen you submit your final version, after your paper has been accepted, prepare it in two-column format, including figures and tables.

C. Antifungal

1) Fungal isolates

Different fungi were tested for sensitivity against *G. sepium* extracts. The fungal strains came fromcertified laboratories. *Candida albicans* was tested in 5 of the articles. *Aspergillus niger* is used in two studies. There are only few studies regarding *G. sepium* susceptibility of fungi. Reference [20] tested16 fungal species for susceptibility.

2) Preparation and standardization of inoculum

Similar to bacterial isolates, any organism tested for susceptibility must follow proper procedure tocontrol growth and culture concentration, which is important for interpreting results later in the study. All fungal susceptibility studies used the same inoculation method as for bacteria, with minor changes to optimize fungal growth. Soybean casein broth (SCB) is the medium of choice in most studies for fungal culture preparation. McFarland standardization is also employed by antifungal studies.

3) Methods of antifungal activity screening

The qualitative method of agar well diffusion is also the most used technique in screening plant extracts for antifungal activity. The method is similar to antibacterial screening, but the agar medium and incubation conditions were changed for fungal growth. Sabouraud's Dextrose agar is the medium of choicefor antifungal sensitivity tests. Agar disc diffusion is also used [22].

4) Results of antifungal activity screening

References [21]-[23] all failed to observe the antifungal activity of *G. sepium* against *C. albicans*. References [12] and [20] found antifungal activity in their extracts against *C. albicans*. Ethanolic extracts of *G. sepium* leaves from [12] and methanolic, ethanolic, and ethyl acetate extracts from [20] showed antifungal activity, albeit inferior to Canesten and Amphotericin-B. Different extraction methods may explain the different results. Reference [12] and [20] found no antifungal activity in *G. sepium*plant extracts against *A. niger*. Ethanolic extracts from both studies did not inhibit *A. niger*, and neither didmethanol or ethyl acetate [20]. Morphological differences between the two fungi may affect *G. sepium* extracts' antifungal activity. *Blastomyces dermatitidis, Cryptococcus neoformans, Geotrichum candidum, Mucor* sp., *Nocardia asteroides, Penicillium* sp., *Sporotrichum schenckii*, and *Tricophyton mentagrophytes* were sensitive to *G. sepium* extracts. Ethanolic extract of *G. sepium as* has comparable antifungal activity as Amphotericin-B against *Penicillium* and *C. neoformans*. *Gliricidia sepium* methanol and ethanol extracts were more effective than Amphotericin-B against *N. asteroides*. Lastly, ethanol and ethyl acetate extracts showed better antifungal activity than Amphotericin-B against *S.schenckii* and *T. mentagrophytes*.

D. Phytochemical Analysis

1) Methods of phytochemical analysis

Some of the reviewed articles also performed phytochemical analysis to determine the active compounds in *G. sepium* extracts and correlate the findings to how the extract can exhibit antibacterial andantifungal activity. In the reviewed articles, colorimetric tests were commonly used to detect specific phytochemicals. Some of these articles also performed total phytochemical determination. One study used a single spectrophotometry standard to detect all phytochemicals. Reference [17] used 7 tests to detect ferric-reducing activity, free radical scavenging ability, tannins, phytates, oxalates, and total phenol and flavonoid. Reference [25] used the same phytochemical screening methods, but excluded tannin, phytate, and oxalate.

2) Results of phytochemical analysis

There are only several phytochemical compounds with antimicrobial properties. Tannins are a recurring phytochemical in the reviewed articles, and studies have shown their antimicrobial activity [40]. Tannins induce oxidation and polymerization, which boosts their biological activity [41]. Through this, they form complexes with proteins andpolysaccharides important for microbe survival. Tannins bind to bacterial cell walls, inhibiting growth [42]. Tannins have anti-methanogenic properties that aid anaerobic organisms [43]. Tannins are antibacterial against *S. aureus, S. flexneri, E. coli,* and *P. aeruginosa* [44]. Tannins promote immunity, antioxidant, anti-diarrheal, antiparasitic, anti-inflammatory, anti-hemorrhoidal, and wound healing.

Next, plant flavonoids. Flavonoids are used by plantsas a defense mechanism against infections and infestations [42]. This proves flavonoids are antimicrobial. Flavonoids are always present in plant extracts subjected to phytochemical screening, establishing their role against bacteria and fungi [40]. Flavonoids can bind extracellular, soluble, and bacterial cell wall proteins. Flavonoids can disrupt lipophilic cell membranes [42]. Flavonoids have strong antibacterial activity against *S. aureus*and *E. coli* [45]. Flavonoids inhibit many microorganisms [40]. Flavonoids are antimicrobial, anti-aging, anti-diabetic, cardioprotective, hepatoprotective, anti-inflammatory, anticancer, anti-allergy, cytotoxic, osteogenic, and estrogenic. Alkaloids, another recurring phytochemical, inhibit enzymes [40]. Alkaloids inhibit dihydrofolate reductase, which is essential for amino acid, RNA, and DNA biosynthesis [46]. Alkaloids inhibit some organisms' respiration, affecting their metabolism and survival [47]. Several types of alkaloids disrupt gram-negative bacterial cell membranes and depolarize gram-positive bacteria [48]. Alkaloids inhibit enzymes, which affect bacterial protein synthesis and virulence. Alkaloids inhibit bacteria's ability to adhere to host cells and evade immune response [40]. Lastly, phenols can inhibit microorganisms' essential enzymes [41]. Other plants with antimicrobial phenols have been studied. Several other phytochemicals have been detected in the articles under review, but mostare unrelated to *G. sepium*'s antimicrobial action. We can't discount the importance of other phytochemicals because crude extracts have better antimicrobial activity than extract fractions. These other phytochemicalsmay not be antimicrobial by themselves, but they may work in synergy with those that contribute to *G. sepium* extracts' antimicrobial activity.

III. Others

Gliricidia sepium extracts were also tested for other properties aside from antibacterial and antifungal activity. References [15] and [19] tested *G. sepium* extract cytotoxicity. Reference [19] used the Brine Shrimp Lethality Assay to determine the cytotoxic activity of their extracts. An LC50 below 200 ppm was determined using Probit analysis. Reference [14] tested *G. sepium* extract's cytotoxicity on breast cancer cells (MCF-7). Cell lines were cultured in 25 cm^2 flasks with Dulbecco's modified eagle medium. Both studies show *G. sepium*'s cytotoxic, anti-tumor, and anti-cancer properties. Reference [32] tested *G. sepium*'s pesticide activity. The pesticide is effective against aphids and hasno toxic effects on the bean plant.

Conflict of Interest

Authors declare that they do not have any conflict of interest.

References

[1] Jones KE, Levy MA, Patel NG, Storeygard A, Balk D, Gittleman JL, Daszak P. Global trends in emerging infectious diseases. *Nature.* 2008; 451: 990-993.

[2] United States Agency for International Development, USAID. *One health and antimicrobial resistance.* [Internet]. 2018. [updated 2018 Nov] [cited 2022 July 13]. Available from: https://www.usaid.gov/global-health/global-health-newsletter/antimicrobial-resistance.

[3] Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H. Medicinal plants: Past history and futureperspective. *J. Herbmed Pharmacol*. 2017; 7: 1-7.

[4] World Health Organisation. Summary of WHO guidelines for the assessment of herbal medicines. *Herbal Gram*. 1993; 28: 13-14.

[5] Greenwell M, Rahman PKSM. Medicinal plants: Their use in anticancer treatment. *Int J Pharm Sci Res*. 2015; 6(10): 4103-4112.

[6] Rondilla NA, Rocha ICN, Roque SJ, Lu RM, Apolinar NLB, Solaiman-Balt AA, et al. Folk Medicine in the Philippines: A phenomenological study of health-seeking individuals. *Int J Med Stud.* 2021; 9(1): 25-32.

[7] Tantengco OG, Condes MC, Estadilla HT, Ragragio EM. Ethnobotanical survey of medicinal plants used by ayta communities in Dinalupihan, Bataan, Philippines. *Pharmacognosy J.* 2018; 10(5): 859-870.

[8] Ezcurra E. *Chapter 10: Fabaceae*. University of California, Riverside. 2013.

[9] Macêdo MJF, Ribeiro DA, Santos MO, Macêdo DG, Macedo JGF, Almeida BV, et al. Fabaceae medicinal flora with therapeutic potential in Savanna areas in the Chapada do Araripe, Northeastern Brazil. *Rev. Bras. Farmacogn*. 2018; 28(6): 738-750.

[10] Rabena AR. Traditional practices on the utilization of Kakawate (*Gliricidia sepium* (Jacq.) Kunth ex Walp.) for sustainable development in the Philippines. In IUFRO, *Sustainable Forest Management and Poverty Alleviation: Roles of Traditional Forest-related Knowledge* Vienna, Austria: IUFRO Headquarters, 2008: 146-149.

[11] Jose B, Reddy L. Evaluation of antibacterial activity of the leaf and flower essential oils of *Gliricidia sepium* from South India. *Int. J. Appl. Pharm.* 2010; 2: 20-22.

[12] Abdulaziz AA, Dapar MG, Manting ME, Torres MJ, Aranas AT, Mindo RR, et al. Qualitative evalution of the antimicrobial, antioxidant, and medically importantphytochemical constituents of the ethanolic extracts of the leaves of *Gliricidia sepium* (Jacq.) *Walp. Pharmacophore.* 2019; 10: 72-83.

[13] Cherian T, Thambi M. Phytochemical investigation of the leaves of *Gliricidia sepium* and its antimicrobial properties. *The Pharma Innovation J*. 2019; 8: 594-596.

[14] Guzman LS. Non-experimental validation of the effectiveness of ethnoveterinary botanical medicine (EVB-M) materials used in the municipality of Echague Isabela. *J. Agri. Tech*. 2015; 11(8): 2377-2389

[15] Sreesha KS, Danya U. Evaluation of preliminary phytochemical, antioxidant, and in vitro cytotoxic studies of ethnotherapeutically important tree *Gliricidia sepium* (Jacq.) Steud. (Fabaceae). *Int. J. Creative Res. Thoughts*. 2021; 9: 5515-5521.

[16] Feng T, Liu S, He XJ. Molecular authentication of the traditional Chinese medicinal plant *Angelica sinensis* based on internal transcribed spacer of nrDNA. *Elect. J. Biotech*. 2010; 13(1).

[17] Kolawole OM, Joseph AK, George OA. Antibacterial and phytochemical activity of*Gliricidia sepium* against poultry pathogens. *Microbiol. Res. J. Int.* 2018; 24: 1-10.

[18] Abulude FO, Adebote VT. Antibacterial investigation of crude extracts of the root bark of *Gliricidia sepium*. *Cont. J. Microbiology*. 2009; 3: 23-26.

[19] Ang AG, Enot MM, Baltazar GD, Alinapon CV, Buncales EO, Barbosa GB. Antioxidant and cytotoxic activity of the leaf ethanolic extracts of *Tithonia diversofilia* and *Gliricidia sepium* from Bukidnon, Philippines. *Asian J. Bio. Life Sci.* 2019; 8: 8-15.

[20] Sunder J, Kundu A, Jeyakumar S, De AK. In-Vitro Antimicrobial Activity of *Gliricidiasepium* leaf Extracts. *Anim. Sci. Report*. 2012; 6: 8-14.
[21] Mathur A, Bhat R, Prasad G, Dua V, Verma SK, Agarwal PK. Antimicrobial activity of plants traditionally used as medicine against some pathogens. *Rasayan J. Chem*. 2010; 3: 615-620.
[22] Perez GS, Zavala SMA, Arias GL, Perez GC, Perez GRM. Antimicrobial study of bark from five tree species. *Phytotherapy Res*. 2001;15: 356-359.
[23] Rojas JJ, Ochoa VJ, Ocampo SA, Munoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. *BMC Compement. Altern Med*. 2006; 6(2).
[24] Oladunmoye MK, Ayantola KJ, Agboola AA, Olowe BM, Adefemi OG. Antibacterial and ftir spectral analysis of methanolic extract of *Gliricidia sepium* leaves. *J. Adv. Microbiol*. 2018; 9: 1-10.
[25] Akharaiyi FC, Boboye B, Adetuyi FC. Antibacterial, phytochemical and antioxidant activities of the leaf extracts of *Gliricidia sepium* and *Spathodea campanulata*. *World Appl.Sci. J*. 2012; 16: 523-530.
[26] Garcia JS, Borja N, Sevilla-Nastor JB, Villanueva JD, Peyraube N. An ethnobotanical study of medicinal plants and perceptions on plant biodiversity conservation in Leyte, Philippines. *J. Human Eco*. 2018; 26-42.
[27] Gruyal GA, del Rosario R, Palmes ND. Ethnomedicinal plants used by residents in Northern Surigao del Sur, Philippines. *Nat Prod Chem Res*. 2014; 2(4).
[28] Balancod TD, Balangcod AD. Ethnomedical knowledge of plants and healthcare practices among the Kalanguya tribe in Tinoc, Ifugao, Luzon, Philippines. *Indian J. Traditional Knowledge*. 2011; 10: 227-238.
[29] Abubakar AR, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. J. *Pharm. Bio. Sci*. 2020; 12: 1-10.
[30] Xiang H, Sun-Waterhouse D, Waterhouse GIN, Cui C, Ruan Z. Fermentation-enabled wellness foods: A fresh perspective. *Food Sci. and Human Wellness.* 2019; 8(3): 203-243.
[31] Oliveira-Alves SC, Andrade F, Prazeres I, Silva AB, Capelo J, Duarte B, et al. Impact of drying processes on the nutritional composition, volatile profile, phytochemical content and bioactivity of *Salicornia ramosissima J. Woods. Antioxidants (Basel)*. 2021; 10(8): 1312.
[32] Maulion RV, Matira JI, Fanoga KM, Marasigan MC, Dimaculangan MC. Modelling and optimization of microwave assisted extraction of total phenolics in Kakawate (*Gliricidia sepium*) as pesticide against black bean aphids (*Aphis fabae). Malaysian J. Analytical Sci*. 2021; 25: 1081-1094.
[33] Handa SS, Khanuja SP, Longo G, Rakesh DD. *Extraction Technologies for Medicinaland Aromatic Plants*. Trieste, Italy: Int. Centre Sci. High Tech. 2008.
[34] Pierik R. Sterilization of plant material. In: *In Vitro Culture of Higher Plants* Springer, Dordecht. 1997: 89-94.
[35] Kalra YP. *Handbook of Reference Methods for Plant Analysis*. Boca Raton, Florida: Taylor & Francis Group. 1998.
[36] Nazli R, Akhter M, Ambreen S, Solangi AH, Sultana N. Insecticidal, nematicidal, andantibacterial activities of *Gliricidia sepium*. *Pak. J. Bot*. 2008; 40: 2625-2629.
[37] Kumar NS, Simon N. In vitro antibacterial activity and phytochemical analysis of *Gliricida sepium* (L.) leaf extracts. *J. Pharm. Phytochem*. 2016; 5: 131-133.
[38] Dalynn Biologicals. McFarland Standard. 2014.
[39] Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity:A review. *J. Pharm. Anal*. 2016; 2: 71-79.
[40] Othman L, Sleiman A, Abdel-Massih RM. antimicrobial activity of polyphenols andalkaloids in middle eastern plants. *Front Microbiol*. 2019; 10.
[41] Coppo, E., Marchese, A. Antibacterial activity of polyphenols. *Curr Pharm. Biotechnol*. 2014; 15: 380-390.
[42] Cowan M. Plant products as antimicrobial agents. *Clin Microbiol. Rev*. 1999; 12: 564-582.
[43] Bhatta R, Vijay-Kumar M, Khub S. Energy expenditure in crossbred cattle fed paddy strawof different forms. *Asian-Australian J. Anim. Sci*. 2006; 9: 1755-1760.
[44] Banso A, Adeyemo SO. Evaluation of antibacterial properties of tannins isolated from*Dichrostachys cinerea*. *African J. Biotech*. 2007; 6: 1785-1787.
[45] Chattopadhyay D, Maiti K, Kundu A, Chakraborty M, Bhadra R, Maudal S, et al. Antibacterial activity of *Alstonia macrophylla*: A folklore of bay islands. *J. Ethnopharmacol*. 2001; 77: 49-55.
[46] Rao K, Venkatachalam S. Inhibition of dihydrofolate reductase and cell growth activity by the phenanthroindolizidine alkaloids pergularinine and tylophorinidine: the in vitro cytotoxicity of these plant alkaloids and their potential as antimicrobial and anticancer agents. *Toxicol In Vitro*. 2000; 14: 53-59.
[47] Tominaga K, Higuchi K, Hamasaki N, Hamaguchi M, Takashima T, Tanigawa T. Invivo action of novel alkyl methyl quinolone alkaloids against Helicobacter pylori. *J. Antimicrob. Chemother*. 2002; 50: 547-552.
[48] Alhanout K, Malesinki S, Vidal N, Peyrot V, Rolain J, Brunel J. New insights into the antibacterial mechanism of action of squalamine. *J. Antimicrob. Chemother* 2010; 65: 1688-1693.
[49] Reddy L, Jose B. Evaluation of Antibacterial Activity of the Bark, Flower, and Leaf Extractsof *Gliricidia sepium* from South India. *Int. J. Current Pharm. Res*. 2010; 2: 18-20.
[50] Clinical and Laboratory Standards Institute. Methods for Determining Bactericidal Activity of Antimicrobial Agents; Approved Guideline. Philadelphia, USA: CLSI. 1999.