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THE ANTIBACTERIAL PROPERTIES OF *Psidium guajava* LEAF EXTRACT AS A WOUND HEALING AGENT OF LABORATORY ANIMALS: A REVIEW

SIFAT ANTIBAKTERI EKSTRAK DAUN Psidium guajava SEBAGAI AGEN PENYEMBUHAN LUKA PADA HEWAN LABORATORY: REVIEW

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ABSTRACT

The importance of Psidium guajava has long been reported in ethnomedicine. Traditional medicine is practiced all over the world and is significant, most especially in indigenous groups due to the poor accessibility to conventional medicine in the area. Furthermore, this type of medical approach is costly, and antibiotic resistance has been a serious issue ever since. Therefore, the scientific community studies good alternatives such as plant-derived extracts to treat various illnesses and diseases, which are known to be less expensive in preparation, have fewer side effects, and be less potential promoters of antibiotic resistance. One of the most encountered conditions in veterinary medicine is a wound. The review collated and presented studies, research, experiments, and other published materials that proved the antibacterial properties of P. guajava leaf extract enhance wound healing activities by accelerating wound contraction and re-epithelialization. P. guajava leaf extract is an effective alternative to commercially available drugs in treating wounds of laboratory animals and could potentially be effective in the larger scope of animals in the future.

Keywords: *antibacterial, guava leaves extract, laboratory animals, leaf extract, Psidium guajava*

ABSTRAK

Pentingnya Psidium guajava telah lama dilaporkan dalam etnomedis. Pengobatan tradisional dipraktekkan di seluruh dunia dan paling signifikan terutama di kelompok masyarakat adat/tradisional karena akses pengobatan konvensional yang buruk di daerah tersebut. Selain itu, jenis pendekatan medis ini mahal, dan menimbulkan adanya resistensi antibiotik telah menjadi masalah serius sejak saat itu. Komunitas ilmiah, mempelajari alternatif yang baik seperti ekstrak tumbuhan untuk mengobati berbagai penyakit, yang diketahui lebih murah dalam hal persiapan, memiliki lebih sedikit efek samping, dan tidak berpotensi sebagai promotor resistensi antibiotik. Salah satu kondisi yang paling banyak ditemui dalam kedokteran hewan adalah luka. Tinjauan tersebut mengumpulkan dan mempresentasikan studi, penelitian, eksperimen, dan materi publikasi lainnya yang membuktikan sifat antibakteri ekstrak daun P. guajava, yang meningkatkan aktivitas penyembuhan luka dengan mempercepat kontraksi luka dan re-epitelisasi. Ekstrak daun P. guajava merupakan alternatif yang efektif untuk obat yang tersedia secara komersial dalam mengobati luka hewan laboratorium dan berpotensi efektif dalam lingkup yang lebih besar dari hewan di masa depan.

Kata kunci: *antibakteri, ekstrak daun jambu, hewan laboratorium, ekstrak daun, Psidium guajava*

INTRODUCTION

The damage or disruption of the normal anatomical structure and function is called a wound [1]. The wound healing process attempts to restore wound lesions, comprises many cell strains and products, and is a highly significant physiological process [2]. Wound infection due to bacterial proliferation impedes wound healing [3]. Successful closure of wounds has been noted to be dependent on the maintenance of bacterial levels under 105 per gram of tissue biopsies. With a persistent tissue level of bacteria [4], the inflammatory phase is prolonged as pro-inflammatory cytokines are elevated by bacteria and endotoxins [5]. Studies show that the presence of four or more bacterial species in a wound is correlated with non-healing [4].

Antimicrobials are used for incidents related to wounds including bite wounds [6]. Antimicrobials are extensively used in small animal practice. Bacteria develop resistance through complex processes involving bacterial species, resistance mechanisms, transfer mechanisms, and reservoirs [7], basically enabling its survival in a changing environment [8]. Antimicrobial resistance does not revolve around quantity but rather around the

quality of use [9]. Inappropriate use of antimicrobial drugs assists in decreasing the efficacy of the antimicrobial agents [7]. The serious global problem of antimicrobial resistance may be attributed to but not limited to its easy access to irresponsible users. New antibiotic drugs are being developed in response to antimicrobial resistance, but the chance of developing more resistance is simultaneously emerging [10].

The use of herbs and their products are accepted nowadays, and antimicrobial activities have been recorded. The efficacy of plant-derived antibacterial agents in combating antimicrobial resistance has been studied and showed valuable results [11]. Phytochemicals exert possible antibacterial effects against sensitive and resistant pathogens [12]. The method is also less expensive and may cause fewer adverse side effects compared to chemically synthesized antibiotics [11].

The use of traditional medicine has been vital, especially for indigenous groups. *Psidium guajava* is used for various reasons, such as diabetes, hypertension, obesity, and cancer [13]. Acute conditions are also treated, such as gastroenteritis, wounds, ulcers, and inflamed gums. The antibacterial mechanism of plant extracts is through the rupture of bacterial cell walls and membranes, and irregular disruption of the bacterial intracellular matrix [14]. The scientific community has been studying each part of the tree intended for various diseases or illnesses, both for veterinary and human medicine [15].

The main objective of this review is to evaluate the different research and studies on the antibacterial properties of *P. guajava* leaf extract that potentially aid in the wound healing of laboratory animals which may or may not be useful in treating wounds of a bigger scope of animals, without the fear of introducing further antimicrobial resistance.

RESULTS AND DISCUSSION

***Psidium guajava*.** The fundamental part of the health of indigenous populations in the study of traditional medical practice is called ethnomedicine. The urge to discover new bioactive compounds in herbal medicine led to the thorough research of *P. guajava* [16]. *P. guajava* belongs to the family Myrtaceae, a genus of about 133 genera with more than 3,800 species of tropical shrubs. A small tree of about ten meters high with its branches spreading can flourish in all types of soils [17], adapt to different climatic conditions, but prefer dry climates [18]. Although widely distributed especially in tropical and subtropical areas [16], it is a native of Mexico extending to South America. *P. guajava* leaves are five to fifteen cm long, short-petiolate, and the blade oval with prominent pinnate veins [18].

Uses and medicinal importance of *Psidium guajava* leaves. Through ingestion of leaves, decoction, infusion, and boiled preparations, the remedy is given for rheumatism, diarrhea, diabetes mellitus, mouth ulcers, and even infections from surgical, skin, and soft tissue. External use of extract from crushed leaves is applied in cuts, ulcers, boils, and wounds as anti-bactericidal and antibiotics [16]. Wound healing depends on the absence of microorganism colonization with the use of broad-spectrum antimicrobials that are less likely to cause antibiotic resistance [13, 19].

Isolation of crude extract from guava leaves. The origin, technique, time, temperature, solvent concentration and polarity, quantity and secondary metabolite, and composition, contribute to the plant material's effects and uses. Various methods are used to extract the leaves depending on which could retain the full potential of the plant. Extraction methods include maceration, infusion, decoction, Soxhlet extraction, and aqueous-alcoholic extraction [14]. Bioactive compounds found in *P. guajava* that can be measured using Soxhlet and solvent are tannin, alkaloid, phenol, saponin, and flavonoids [20]. The isolation of guava leaves is shown in Table 1.

Medicinal properties and composition of guava leaves. Plants produce phytochemicals, which are nonnutritive chemicals, for protection. The leaves of *P. guajava* contain the following components: quercetin, avicularin, tannin, guajavolide and guavenoic acid, triterpenoids [17], ringenin, flavone, flavonol, kaemferol, morin, and quercetin [21], betulinic acid and lupeol [14], caffeic acid [22], guaijaverin [23], avicularin [18, 24], quercetin, catechin, vescalagin, gallic acid, peltatoside, hyperoside, isoquercitrin, and guaijaverin, 3-L-4-pyranoside showed antimicrobial activities [16]. As cited by Abubakar [20], tannins, phenols, and flavonoids are heavily concentrated in the leaves and are the therapeutic agents that form the growth of inhibition against the bacteria. The antimicrobial mechanism of tannins includes the irreversible formation of complexes with proline-rich proteins, which result in the inhibition of cellular protein synthesis. The tanning effect occurs when tannins react with proteins that are significant in ulcerated tissues, burns, or wounds. Biswas et al. [14] said that flavonoids could form complexes with extracellular and soluble proteins and cell walls of bacteria. Growth inhibitory activities of flavonol compound and guaijaverin were attributed to the presence of catechol [23]. The bioactive compound and its function are shown in Table 2.

Table 1. Extraction methods of guava leaves

Methods	Reference
Soaked in sterile distilled water, acetone, and methanol, then shaken for 24 hours in a 4-hour interval	[20]
Aqueous methanol and further extracted with n-hexane and chloroform.	[24]
Successive hot continuous percolation method of ethanol extraction in a Soxhlet apparatus	[25]
Methanol then concentrated in vacuo and yielded 12% of *P. guajava*.	[15]
An electric juicer to obtain the liquid form of the extract was then filtered with mesh or muslin cloth and was placedin a sterile container	[13]
Fractionated percolation to prepare the hydroalcoholic extract in 70% ethanol which led to a 100mL of final extract	[26]
Methanol in a Soxhlet apparatus for the 4-5hours duration.	[23]
10mg/mL concentration of methanol extract soaked the guava leaves, evaporated in vacuo, and lead to the aqueous concentrate partitioned with ethyl acetate	[27]

Table 2. Phytochemicals in *P. guajava* leaves and the function

Phytochemicals	Function	Reference
Essential oils and quercetin	anti-inflammatory	[28, 29]
Alkaloids, tannins, phenols, terpenoids, saponins,	inhibition against pathogenic bacteria	[20, 27, 30]
Gallocatechin	antimutagenic activity	[17]
Guaijaverin	antibacterial activity	[17]
Flavonoids	antibacterial activities	[22, 31, 32]

Antibacterial properties of guava leaves. The common and most frequently isolated microbes in the skin and wound infections belong to *Candida* sp., *Staphylococci*, *Streptococci*, *Pseudomonas*, *Bacilli*, and *Escherichia coli* [20]. The bacteria can form biofilms on colonized surfaces, therefore, increasing resistance and virulent capabilities. Ethnopharmacological studies that demonstrate antimicrobial, anti-inflammatory, and wound healing properties of guava leaf extract were reported. The effectivity of the leaf extract on *P. acnes* as an anti-inflammatory agent was also reported [18]. A higher concentration of active compounds in the extract is required to reach the inhibitory level of bacteria, but this may not matter compared to the little to no side effects brought by the extract. The guava leaf extract was concluded as potent against pathogens and recalcitrant bacteria, which could treat conditions that are infectious and could justify its use in traditional medicine. Tables 3 and 4 showed the study on the antibacterial properties of guava leaves. The results are indicative of the broad or wide spectrum of activity of all extracts. It was explained that this was probably due to the solubility of the active components of guava leaves which could be more soluble in water than in organic solvents. Furthermore, lower temperatures are more favorable in maximizing the potential of the extracts since there was a decrease in its activities at the boiling point. Solvents should be chosen based on their boiling points, dissipation, and dielectric properties. Based on those properties, aqueous acetone, ethanol, or their mixtures have often been used to extract phenolic compounds [33]. Increasing or improving the solubility and potency of decoctions are done in some cultures, and adding potash is a significant step.

Antibacterial resistance mode of action. Plant extracts could kill bacterial cells by rupturing their cell walls and membranes and irregularly disrupting the intracellular matrix. The lipid bilayer of the cell membrane is penetrated by the oils through hydrophobicity and extracts, which lead to a more permeable membrane and leak vital cell contents consequently [14, 28]. Gram-negative bacteria are more resistant due to their complex cell wall structures and antibiotic resistance through conjugation and transduction to other bacteria within the vicinity [30, 34, 35]. The entry of environmental substances such as antibiotics and plant-based biocides is prevented due to the presence of a thick murine layer [20]. The wound healing assay in laboratory animals is shown in Table 5.

Table 3. Study of guava leaves as antibacterial properties

Bacteria	Reference
Staphylococcus, Shigella, Salmonella, Bacillus, E. coli, Clostridium, Klebsiella, Pseudomonas, and *Candida* sp.	[34, 35, 36]
S. enteritidis and *B. cereus*	[24]
S. aureus	[28]

Bacteria	Reference
Bacillus and *Salmonella* sp.	[29, 37]
S. mutans and *H. pylori*	[23, 27]
S. aureus, E. coli, P. aeruginosa, Proteus, Shigella spp.	[15, 18]

Table 4. Study of guava leaves as antibacterial properties and the best methods

Bacteria	Method's extract	Reference
P. mirabilis, S. pyogenes, E. coli, S. aureus, P. aeruginosa	aqueous	[20]
B. subtilis, S. aureus, E. coli, P. aeruginosa	methanol	[38]
E. coli, S. typhi, S. aureus, P. mirabilis, S. dysenteria, Streptococcus sp.	ethanol	[25]
S. epidermidis, S. typhimurium	hot water	[20]
S. aureus, E. coli	ethanol	[39]
S. aureus, Salmonella sp.	methanol and essential oil	[21, 29]
Staphylococcus, Bacillus sp.	aqueous	[14]
Salmonella, E. coli	methanol	[14]
B. cereus, E. aerogenes, P. fluorescens	essential oils	[28]
S. mutans	methanol	[23]
V. cholera	methanol	[40]
S. typhimurium	water and chloroform	[17]
B. subtilis	ethanol	[22]

Table 5. Wound healing assay of guava leaves in laboratory animals

Formula	Animal	Result	Reference
Methanolic extract from *P. guajava*	Albino rats	decreasing wound area	[15]
P. guajava leaf extract mixed with *M. malabathricum* leaf extract	Sprague Dawley rat strain	wound area size-reduction	[31]
Guava leaf extract	Laboratory mice	noprolonged swelling and elevation of wound, better wound contraction, and scar formation	[13]
Guava leaf extract	Sprague-Dawley rats	proved the anti-inflammatory activity	[41]
Aqueous and acetone-water extract from *P. guajava*	Swiss mice	positive results for the anti-inflammatory activities	[42]
Guava leaf extract	Wistar rats	better results in histological analysis, abscess formation, and cell population reduction	[26]
Essential oil from *P. guajava*	albino rats	effective in reducing the edema and granuloma	[43]
Guava leaf extract	shrimp (*Penaeus monodon*)	Inhibited pathogenic bacteria *V. harveyi*.	[44]
Guava leaf extract	Swiss albino mice	inhibited colonization of *C. rodentium*	[38]
Guava leaf extract	in brown male chicks	inhibited *E. coli* and reduced symptomatology	[45]
Guava leaf extract	mice	inhibit *V. cholera*	[46]
Guava leaf extract combined with coconut water aquapuncture	Philippine native sheep	induced skin wound and remarkable wound healing	[47]
Guava leaf extract	ICR mice	exhibited wound healing activities	[48]
	Wilstar albino rats	good healing agent of wounds	[49]

The study concluded the wound healing properties of *P. guajava* leaves on laboratory animals, especially when mixed with another medicinal plant. The leaf extract possessed anti-inflammatory effects in rats. *P. guajava* leaf extract significantly contributes to tissue healing of wounds. It is important to preserve their bioactivity and improve their stability to make them applicable in the industry since phenolic compounds are prone to losing their active properties during storage [46]. In order to prevent contamination of the extract to be used for wound healing, the extract must be kept sterile from any microbes before use. One recommended method is to pass it through a membrane filter. Membranes for sterile filtration can be made of different materials depending on the desired filtration or resistance characteristics. They are grouped in hydrophilic materials, such as polyamide (PA), cellulose acetate (CA) and other cellulose esters, and hydrophobic membranes, such as polyethersulfone (PES), polysulfone (PS), or fluorinated materials polyvinylidene fluoride (PVDF) and polytetrafluoroethylene (PTFE) [50, 51].

CONCLUSION

Psidium guajava leaf extracts potentially aid in the wound healing of laboratory animals which is helpful in treating wounds of a bigger scope of animals without the fear of introducing further antimicrobial resistance. Antibacterial properties of *P. guajava* leaf extract enhance wound healing activities by accelerating wound contraction and re-epithelialization. Significant wound contraction due to facilitated epithelial cell migration made the extract a good healing agent for wounds.

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