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Phytochemical Screening and Total Flavonoid Content of Annona squamosal and Centella asiatica Ethanolic Extracts for Diabetic Wound Patch

Pemeriksaan Fitokimia dan Kandungan Flavonoid Total Ekstrak Etanol Annona squamosal dan Centella asiatica untuk Plester Luka Diabetes

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Abstract

Diabetic wounds are one of the chronic complications of diabetes mellitus, characterized by delayed healing and a high risk of bacterial infection. The development of natural product-based pharmaceutical preparations is considered a promising alternative approach due to their relative safety and wide availability. Soursop leaves (*Annona squamosa* L.) and gotu kola leaves (*Centella asiatica* L.) are known to contain various secondary metabolites that contribute to antibacterial activity and wound healing. This study aimed to perform phytochemical screening and determine the total flavonoid content of ethanolic extracts of soursop leaves and gotu kola leaves as a scientific basis for the development of an antibacterial patch for diabetic wound management. Extraction was conducted using maceration with 96% ethanol. Qualitative phytochemical screening was performed to identify flavonoids, phenolics, tannins, saponins, steroids, and alkaloids. Total flavonoid content was determined using UV–Vis spectrophotometry with quercetin as the reference standard at a maximum wavelength of 427 nm. The results showed that the extraction yields of soursop leaf and gotu kola leaf ethanolic extracts were 14.64% and 14.72%, respectively. Both extracts contained flavonoids, phenolics, tannins, steroids, and alkaloids, while saponins were detected only in the gotu kola leaf extract. The total flavonoid contents of soursop leaf and gotu kola leaf extracts were 1.5% and 1.12%, respectively. In conclusion, ethanolic extracts of soursop leaves and gotu kola leaves possess phytochemical profiles that support antibacterial and wound-healing activities, indicating their potential as active ingredients in antibacterial patch formulations for diabetic wounds.

Abstrak

Luka diabetes merupakan salah satu komplikasi kronis diabetes melitus, yang ditandai dengan penyembuhan yang lambat dan risiko infeksi bakteri yang tinggi. Pengembangan sediaan farmasi berbasis produk alami dianggap sebagai pendekatan alternatif yang menjanjikan karena relatif aman dan tersedia secara luas. Daun sirsak (Annona squamosa L.) dan daun gotu kola (Centella asiatica L.) diketahui mengandung berbagai metabolit sekunder yang berkontribusi pada aktivitas antibakteri dan penyembuhan luka. Studi ini bertujuan untuk melakukan skrining fitokimia dan menentukan kandungan flavonoid total dari ekstrak etanol daun sirsak dan daun gotu kola sebagai dasar ilmiah untuk pengembangan plester antibakteri untuk manajemen luka diabetes. Ekstraksi dilakukan menggunakan maserasi dengan etanol 96%. Skrining fitokimia kualitatif dilakukan untuk mengidentifikasi flavonoid, fenolik, tanin, saponin, steroid, dan alkaloid. Kandungan flavonoid total ditentukan menggunakan spektrofotometri UV-Vis dengan kuersetin sebagai standar referensi pada panjang gelombang maksimum 427 nm. Hasil penelitian menunjukkan bahwa rendemen ekstraksi etanol daun sirsak dan daun gotu kola masing-masing sebesar 14,64% dan 14,72%. Kedua ekstrak tersebut mengandung flavonoid, fenolik, tanin, steroid, dan alkaloid, sedangkan saponin hanya terdeteksi pada ekstrak daun gotu kola. Kandungan flavonoid total ekstrak daun sirsak dan daun gotu kola masing-masing sebesar 1,5% dan 1,12%. Kesimpulannya, ekstrak etanol daun sirsak dan daun gotu kola memiliki profil fitokimia yang mendukung aktivitas antibakteri dan penyembuhan luka, menunjukkan potensinya sebagai bahan aktif dalam formulasi plester antibakteri untuk luka diabetes.

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disease with increasing global prevalence and remains a major public health problem [1]. One of its most serious complications is diabetic wound or diabetic foot ulcer (DFU), which is characterized by delayed healing, prolonged inflammation, and high risk of infection. Diabetic wounds are closely associated with impaired tissue perfusion, peripheral neuropathy, and microbial infection [1]. These factors contribute to a prolonged wound-healing process and significantly increase the risk of lower-extremity amputation, thereby reducing the patient's quality of life and increasing healthcare costs [2].

In recent years, the development of natural product-based pharmaceutical preparations has become a promising approach due to their broad pharmacological activities and relatively minimal side effects compared to conventional synthetic agents [3]. Traditional medicinal plants have been utilized for generations in wound care, and many have been scientifically investigated for their healing potential through in vitro, in vivo, and clinical studies [4]. Several medicinal plants have been reported to show beneficial activity in the management of gangrenous wounds, including *Annona reticulata*, *Carica papaya*, *Aloe vera*, *Centella asiatica*, *Punica granatum*, and *Anredera cordifolia* [4]. These findings support the idea that medicinal plants may serve as important sources of bioactive compounds for the development of modern wound care products, particularly for chronic and difficult-to-heal wounds such as diabetic ulcers [4,3].

Among various medicinal plants, soursop (*Annona squamosa* L.) and gotu kola (*Centella asiatica* L.) are considered potential candidates due to their reported pharmacological properties related to wound healing [3,5,6]. According to previous studies, gotu kola has been reported to be effective for grade IIa wounds, while soursop was effective for grade IIb wounds, with a reported healing percentage of 100% 3. These results suggest that both plants may be explored as alternative therapeutic materials for wound healing applications [3].

Soursop leaves (*Annona squamosa* L.) have been reported to possess antibacterial and wound-healing activity, which may be associated with their phytochemical constituents, including flavonoids and phenolic compounds [5,7]. The presence of antibacterial activity is particularly important for diabetic wounds because infection is one of the most common causes of delayed healing and wound deterioration [1,4]. In addition, antioxidant activity contributes to wound healing by reducing oxidative stress that may damage cells and prolong inflammation, thereby interfering with tissue regeneration and wound closure [1,7]. A previous study also confirmed that *A. squamosa* leaves exhibited antibacterial and wound-healing activities, supporting their potential development in topical wound therapy [5].

Gotu kola (*Centella asiatica* L.) is a traditional medicinal plant that promotes wound healing by stimulating fibroblast proliferation and collagen synthesis, which are crucial for extracellular matrix formation and tissue repair during the proliferation phase. Experimental excision wound models have also confirmed the wound-healing potential of *C. asiatica* extract, supporting its development as a topical active ingredient, particularly for chronic wounds with impaired tissue repair [6].

Flavonoids are major phenolic compounds that support wound healing through antioxidant, anti-inflammatory, and antibacterial activities [7]. These effects help reduce oxidative damage, regulate inflammation in chronic wounds, and control infection, which is important in diabetic ulcer management [5,7]. Therefore, phytochemical screening and total flavonoid content determination are essential for the preliminary evaluation of wound-healing plant extracts [7].

Based on the above considerations, this study focuses on phytochemical screening and the determination of total flavonoid content of ethanolic extracts of soursop leaves (*Annona squamosa* L.) and gotu kola leaves (*Centella asiatica* L.) as candidate active ingredients for the development of an antibacterial patch for diabetic wound treatment [7].

METHODS

Materials

The materials used in this study were dried soursop leaves (*Annona squamosa* L.) and dried gotu kola leaves (*Centella asiatica* L.), 96% ethanol, methanol, concentrated hydrochloric acid (HCl), magnesium powder, 10% aluminum chloride ($AlCl_3$) solution, and quercetin as a reference standard.

Research Procedure

Extraction Procedure: Extraction was carried out using the maceration method with 96% ethanol for 72 h under periodic stirring. The filtrate was then concentrated using a rotary evaporator to obtain a viscous crude extract [8].

Phytochemical Screening: Qualitative phytochemical screening was performed using standard color reaction tests to identify the presence of flavonoids, phenolics, tannins, saponins, steroids, and alkaloids [9].

Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined using UV–Vis spectrophotometry with quercetin as the reference standard at a maximum wavelength range of 400–450 nm. The results were expressed as percentage of quercetin equivalent (% w/w) [10].

a. **Preparation of Quercetin Standard Solutions**

   Quercetin powder (50 mg) was dissolved in 96% ethanol and diluted to 50 mL to obtain a 1000 ppm stock solution (Stock 1). Stock 2 (100 ppm) was prepared by transferring 5 mL of Stock 1 and diluting with 96% ethanol to 50 mL. Standard series solutions were prepared by pipetting 5, 6, 7, 8, and 9 mL of Stock 2 into separate 50 mL volumetric flasks and diluting with 96% ethanol to obtain concentrations of 10, 12, 14, 16, and 18 ppm, respectively.

b. **Determination of Maximum Wavelength**

   The UV–Vis spectrophotometer was set to scan in the range of 400–450 nm using 96% ethanol as blank. A 10 ppm quercetin solution was then measured to determine the maximum wavelength.

c. **Construction of the Quercetin Calibration Curve**

   Each standard solution (10–18 ppm) was pipetted (1 mL) and mixed with 1 mL of 2% $AlCl_3$ and 1 mL of 120 mM potassium acetate, followed by incubation for 30–60 min. The absorbance was then measured using UV–Vis spectrophotometry to obtain the calibration curve.

d. **Preparation of Sample Solutions**

Each extract sample (75 mg) was dissolved in ethanol and diluted to 50 mL to obtain a sample concentration of 1500 ppm.

e. Measurement of Total Flavonoid Content

One milliliter of the sample solution was mixed with 1 mL of 2% $AlCl_3$ and 1 mL of 120 mM potassium acetate, followed by incubation for 30–60 min. The absorbance was measured using UV–Vis spectrophotometry, and total flavonoid content was calculated using the linear regression equation obtained from the quercetin calibration curve

RESULT AND DISCUSSION

Soursop leaves (*Annona squamosa* L.) and gotu kola leaves (*Centella asiatica* L.) are known to contain various phytochemical constituents with potential antibacterial, antioxidant, and wound-healing activities [6]. The maceration method using ethanol was selected due to its ability to extract a wide range of phytochemical compounds efficiently while maintaining the structural integrity of bioactive molecules. Flavonoids are phenolic compounds that are soluble in 70% ethanol; therefore, the maceration approach applied in this study was considered appropriate for extracting flavonoid-rich fractions [11].

A. Extraction Yield

Table 1. Summarizes the extraction yield

Material	Soursop leaf extract (*A. squamosa* L.)	Gotu kola leaf extract (*C. asiatica* L.)
Sample weight	1 kg	0.5 kg
Extract weight	146.41 g	73.6 g
Yield (%)	14.64%	14.72%

The extraction yields of ethanolic extracts of Annona squamosa and Centella asiatica were 14.64% and 14.72%, respectively. These findings indicate that 96% ethanol effectively extracted polar and semi-polar phytochemical constituents.

B. Phytochemical Screening

Tabel 2. Qualitative phytochemical screening results

Material	Soursop leaf extract (*A. squamosa* L.)	Gotu kola leaf extract (*C. asiatica* L.)
Saponins	Not detected (−)	Detected (+)
Phenolics	Detected (+)	Detected (+)
Tannins	Detected (+)	Detected (+)
Flavonoids	Detected (+)	Detected (+)
Steroids	Detected (+)	Detected (+)
Alkaloids	Moderate (+2)	Weak (+1)

Notes: (−) not detected; (+) detected. Alkaloid intensity: (+1) weak reaction, (+2) moderate reaction, (+3) strong reaction.

Phytochemical screening demonstrated that both extracts contained flavonoids, phenolics, tannins, steroids, and alkaloids. Saponins were detected only in Centella asiatica extract.

C. Total Flavonoid Content

Table 3. Total flavonoid content of the ethanolic extracts of soursop leaves and gotu kola leaves

Parameter	Soursop leaf extract (*A. squamosa* L.)	Gotu kola leaf extract (*C. asiatica* L.)
Sample weight (mg)	75	75
Absorbance	0.2788	0.2095
Concentration (mg/L)	229.852	169.741
Total flavonoid content (%)	1.50	1.12

The total flavonoid contents of Annona squamosa and Centella asiatica extracts were 1.50% and 1.12%, respectively. The higher flavonoid level observed in Annona squamosa may contribute to its stronger antioxidant and antibacterial activities.

DISCUSSION

A. Extraction Yield

The extraction yield is closely related to the amount of bioactive compounds obtained from the raw material. A higher yield indicates a greater amount of extracted constituents, suggesting better extraction efficiency. In this study, the extraction yields were considered satisfactory because both extracts showed yields above 10% [12]. The extraction yields of soursop leaf extract and gotu kola leaf extract were 14.64% and 14.72%, respectively, indicating that 96% ethanol was effective in extracting polar and semi-polar secondary metabolites [13].

B. Phytochemical Screening

Qualitative phytochemical screening demonstrated that both extracts contained flavonoids, phenolics, tannins, steroids, and alkaloids, while saponins were detected only in the gotu kola leaf extract. These secondary metabolites play important roles in antibacterial activity and wound healing processes [14]. Annona squamosa leaves have been reported to contain various phytochemicals, including glycosides, phenolics, flavonoids, alkaloids, and phytosterols, and acetogenins such as squamocin and annonacin are known to exhibit strong antibacterial and antioxidant activities [13]. Meanwhile, *Centella asiatica* is rich in triterpenoids (asiaticoside, madecassoside, and asiatic acid) as well as flavonoids, which may synergistically contribute to skin regeneration and wound healing [1].

C. Total Flavonoid Content

Since the soursop leaf extract was confirmed to contain flavonoids, total flavonoid content (TFC) was determined based on the formation of a complex compound with $AlCl_3$. This reaction occurs due to the interaction between the C-4 keto group and the adjacent hydroxyl group at C-3 or C-5, resulting in a bathochromic shift toward the visible region, which produces a yellow color in the solution. Quercetin was used as the standard reference for TFC determination because it represents a common flavonoid compound [7]. The results showed that the total flavonoid content of soursop leaf extract (1.50%) was higher than that of gotu kola leaf extract (1.12%). Several related studies

reported similar findings, supporting the results obtained in this research. For comparison, a mulberry leaf extract patch formulated using a PVP–EC polymer matrix showed a yield of 12% and a flavonoid content of 1.8% [15].

Flavonoids act as strong antioxidants by scavenging free radicals, inhibiting lipid peroxidation, and supporting tissue regeneration. The higher flavonoid content in soursop extract compared to gotu kola may explain its stronger antibacterial potential [16]. However, gotu kola contains higher levels of triterpenoids and therefore may provide superior effects in skin regeneration.

A combination of both extracts is expected to provide a synergistic effect between the antibacterial activity of soursop and the tissue-healing activity of gotu kola. This combined extract profile supports synergistic mechanisms, where soursop phenolics and flavonoids may inhibit wound-infecting microbes such as Staphylococcus aureus and Escherichia coli in an animal wound model [5], while gotu kola saponins may stimulate fibroblast proliferation and enhance collagen synthesis, thereby accelerating epithelialization [6,16].

CONCLUSION

The ethanolic extracts of soursop leaves and gotu kola leaves contained secondary metabolites that support antibacterial activity and wound healing. The total flavonoid contents of 1.50% (soursop) and 1.12% (gotu kola) indicate promising pharmacological potential. Therefore, both extracts have potential to be developed as active ingredients in antibacterial patch formulations for diabetic wound treatment.

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AUTHOR CONTRIBUTION STATEMENT

J contributed to conceptualization, data collection, data analysis, and drafting of the manuscript. **YE** contributed to study design, supervision, data interpretation, critical revision of the manuscript, and served as the corresponding author. **US** contributed to methodology development and data validation and contributed to data analysis and manuscript review.

CONFLICT OF INTEREST DECLARATION

The authors declare no conflict of interest

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