VARIOUS SOLVENT EXTRACTS OF *Ipomoea pes-caprae*: A PROMISING SOURCE OF NATURAL BIOACTIVE COMPOUNDS COMPARE WITH VITAMIN C

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ABSTRACT

This research investigates the phytochemical composition, antioxidant activity, and antibacterial efficacy of *Ipomoea pes-caprae* extracts collected from Pantai Talang Siring Pamekasan, Madura. Phytochemical analysis revealed the presence of alkaloids, flavonoids, phenolic compounds, terpenoids, tannins, and saponins in the extracts, indicating their rich bioactive profile. Antioxidant activity assessed via the DPPH assay demonstrated concentration-dependent scavenging of free radicals, with methanol and ethanol extracts exhibiting superior activity compared to the aqueous extract. Antibacterial assays against *Escherichia coli* and *Staphylococcus aureus* revealed significant inhibitory effects, with methanol and ethanol extracts displaying greater potency than the aqueous extract. These findings underscore the potential of *Ipomoea pes-caprae* as a source of natural antioxidants and antimicrobial agents, warranting further investigation into its pharmacological applications and therapeutic potential.

Key words: antigenotoxicity, biochemical, GC-MS analysis, micronucleus, gracilaria.

مستخلص أوراق أوراق التقييم المناعي لأوراق النيم ضد المناعة غير المحددة لسمكة البلطي المصابة ببكتيريا *A. hydrophila*

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المستخلص

يبحث هذا البحث في التركيب الكيميائي النباتي، ونشاط مضادات الأكسدة، والفعالية المضادة للبكتيريا لمستخلصات Ipomoea pes-caprae التي تم جمعها من بانتاي تالانج سيرينج باميكاسان، مادورا .كشف التحليل الكيميائي النباتي عن وجود القلويدات والفلافونويدات والمركبات الفينولية والتيربينويدات والعفص والصابونين في المستخلصات، مما يشير إلى أنها غنية بالنشاط الحيوي .أظهر نشاط مضادات الأكسدة الذي تم تقييمه عبر اختبار DPPH مسح الجذور الحرة المعتمد على التركيز، حيث أظهرت مستخلصات الميثانول والإيثانول نشاطًا فائقًا مقارنة بالمستخلص المائي .كشفت الاختبارات المضادة للبكتيريا ضد الإشريكية القولونية والمكورات العنقودية الذهبية عن تأثيرات مثبطة كبيرة، حيث أظهرت مستخلصات الميثانول والإيثانول فعالية أكبر من المستخلص المائي .تؤكد هذه النتائج على إمكانات *Ipomoea pes-caprae* كمصدر لمضادات الأكسدة الطبيعية والعوامل المضادة للميكروبات، مما يستدعي إجراء مزيد من البحث في تطبيقاته الدوائية وإمكاناته العلاجية.

الكلمات المفتاحية: نشاط مضاد للجراثيم؛ النشاط المضاد للأكسدة؛ فحص .DPPH إيبوموبيا بيس كابراي؛ الملف الكيميائي النباتي

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INTRODUCTION

Ipomoea pes-caprae, commonly known as beach morning-glory or railroad vine, is a versatile plant renowned for its adaptability to coastal environments, salt tolerance, and remarkable bioaccumulation capabilities (10. Belonging to the Convolvulaceae family, *Ipomoea pes-caprae* thrives in tropical and subtropical regions across Asia, America, Africa, and Australia (2). The plant has garnered significant attention in scientific research due to its diverse biological activities and medicinal properties. Research studies have revealed the pharmacological potential of *Ipomoea pes-caprae* extracts, attributing its therapeutic effects to various bioactive compounds. Phenolic compounds found in the plant have been associated with hypotensive activity, contributing to its traditional use in treating hypertension (8). Additionally, lipophilic glycosides, including pentasaccharides, have shown promise as potential antineoplastic agents, highlighting the plant's potential in cancer research (30) *Ipomoea pes-caprae* has also been investigated for its antimicrobial properties, demonstrating efficacy against bacterial and fungal strains (25). Furthermore, studies have explored its anti-inflammatory properties, suggesting its potential as a traditional herbal medicine for treating inflammation-related conditions (15). Notably, xylose-containing oligosaccharides present in the plant have shown inhibitory effects on multidrug resistance in bacteria, providing new avenues for combating antibiotic resistance (10). Moreover, *Ipomoea pes-caprae* has been recognized for its ability to bioaccumulate metal ions from aqueous solutions, indicating its potential application in environmental remediation (33).. Recent discoveries of novel compounds from *Ipomoea pes-caprae* exhibiting collagenase inhibitory activity further underscore the plant's pharmaceutical relevance (39). *Ipomoea pes-caprae* emerges as a promising subject for further research in pharmacology and traditional medicine due to its diverse biological activities and medicinal properties. This study aims to comprehensively investigate the phytochemical composition, antioxidant activity, and antibacterial efficacy of *Ipomoea pes-caprae* extracts collected from Pantai Talang Siring Pamekasan, Madura. By elucidating the bioactive constituents and pharmacological properties of the plant extracts, this research seeks to contribute to the understanding of the medicinal potential of *Ipomoea pes-caprae* and its possible applications in pharmacology and traditional medicine. Additionally, this study aims to provide scientific evidence to support the traditional uses of *Ipomoea pes-caprae* in treating various ailments, thereby validating its therapeutic significance and promoting its conservation and sustainable utilization.

MATERIAIS AND METHODS

Plant collection and extract preparation

Ipomoea pes-caprae specimens were meticulously collected from Pantai Talang Siring Pamekasan, Madura, Indonesia, during the months of October to November 2023. This collection period was chosen to coincide with the plant's peak growth and flowering stages, ensuring optimal phytochemical content. A team comprising local people conducted the collection, adhering to ethical guidelines and regulations regarding wild plant harvesting. Upon arrival at the collection site, specimens exhibiting typical morphological features of *Ipomoea pes-caprae* were carefully selected. Emphasis was placed on choosing healthy individuals free from signs of disease or physical damage. Leaves, stems, and flowers were harvested, ensuring a representative sampling of the plant's aerial parts. Following collection, the specimens were promptly transported to the laboratory for further processing. Upon arrival, they were first washed thoroughly with distilled water to remove any adhering debris or contaminants. Subsequently, the cleaned plant material was air-dried under shade to preserve the integrity of phytochemicals and prevent enzymatic degradation. Once dried, the plant material was finely ground into a powder using a mortar and pestle. The powdered material was then subjected to extraction to obtain bioactive compounds. Aqueous, methanol, and ethanol extracts were prepared separately using the maceration method. In this process, the powdered plant material was soaked in the respective solvents for a predetermined duration, typically 24 to 48 hours, to facilitate maximum extraction of phytoconstituents.

After the extraction period, the resulting mixtures were filtered using Whatman filter paper to remove solid residues and obtain clear filtrates. These crude extracts were then concentrated under reduced pressure using a rotary evaporator or by air-drying at low temperature. The resulting concentrated extracts were stored in tightly sealed containers at appropriate temperatures until further analysis.

Yield and phytochemical analysis

To determine the presence of various phytochemical compounds in the extracts of *Ipomoea pes-caprae*, a comprehensive qualitative analysis was conducted using standard chemical tests. These tests provide initial insights into the diversity of bioactive constituents present in the plant material. The qualitative analysis using published methods [9,10] focused on detecting major classes of phytochemicals, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, and terpenoids.

Antioxidant assay

Natural antioxidants are widely distributed in food and medicinal plants (13). The antioxidant assay was performed using published methods [11] with concentration modification of 100, 200, and 500 ppm. Test samples were prepared by diluting the extracts of *Ipomoea pes-caprae* in appropriate solvents. Ascorbic acid (Vitamin C) was used as a positive control. Equal volumes of DPPH (2,2-diphenyl-1-picrylhydrazyl) solution and test samples were mixed and incubated in the dark for 30 minutes. Absorbance was measured spectrophotometrically at 517 nm. Percentage inhibition of DPPH radicals was calculated based on the decrease in absorbance. Results were expressed as the percentage inhibition of DPPH radicals by the test samples at concentrations of 100, 200, and 500 ppm.

Antibacterial assay

The preparation of bacteria for the antibacterial assay involved several steps to ensure consistency and accuracy in the evaluation of antibacterial activity (15). Firstly, bacterial strains of *Escherichia coli* and *Staphylococcus aureus* were obtained from a culture collection or freshly isolated from clinical specimens. These strains were maintained on agar slants or in freeze-dried form at appropriate temperatures (-70°C or below) until required for experimentation.To initiate the antibacterial assay, the bacterial strains were subcultured onto nutrient agar plates and incubated at 37°C for 24 hours to obtain well-isolated colonies. Colonies were then suspended in sterile saline solution (0.85% NaCl) and adjusted to a turbidity corresponding to the 0.5 McFarland standard, ensuring a standardized bacterial inoculum. The nutrient agar plates were prepared by pouring sterile nutrient agar medium into petri dishes and allowed to solidify. Once solidified, the agar surface was evenly spread with the standardized bacterial suspension using a sterile cotton swab, ensuring uniform distribution of the inoculum across the agar surface. Sterile filter paper discs (6 mm diameter) were impregnated with various concentrations of the test samples (extracts of *Ipomoea pes-caprae*) dissolved in appropriate solvents (aqueous, methanol, or ethanol). As a positive control, discs containing known antibacterial agents such as ampicillin or chloramphenicol were also prepared. The prepared discs were then aseptically placed onto the surface of the inoculated agar plates using sterile forceps. Care was taken to ensure adequate spacing between discs to prevent overlapping inhibition zones. Following disc placement, the plates were incubated at 37°C for 24 hours to allow bacterial growth and diffusion of the test substances into the agar medium. After incubation, the plates were examined for the presence of clear zones of inhibition around the discs, indicating the antibacterial activity of the test samples against the respective bacterial strains.The diameter of the inhibition zones was measured using a calibrated ruler, and the results were recorded for further analysis. The antibacterial activity of the test samples was assessed based on the size of the inhibition zones observed and compared with that of the positive control.

RESUITS AND DISCUSSION

Phytochemical result: Phytochemicals are naturally occurring compounds found in plants. They're not considered essential nutrients like vitamins and minerals, but research suggests they have potential health benefits. These compounds contribute to the color, flavor, and disease resistance of plants.

Table 1. phytochemical profile for *Ipomoea pes-caprae* during the research

Phytochemical	Qualitative Result
Alkaloids	**Present**
Flavonoids	**Present**
Phenolic Compounds	**Present**
Terpenoids	**Present**
Tannins	**Present**
Saponins	**Present**

The phytochemical analysis of *Ipomoea pes-caprae* extracts revealed the presence of diverse bioactive compounds, which are known to contribute to the plant's medicinal properties. The qualitative and quantitative assessments conducted provided valuable insights into the phytochemical composition of the plant material. Alkaloids, nitrogen-containing compounds known for their pharmacological activities, were identified in all three extracts (aqueous, methanol, and ethanol) of *Ipomoea pes-caprae*. These compounds are recognized for their potential antipyretic, analgesic, and antimicrobial properties, which could contribute to the therapeutic effects of this plant (2,13). Alkaloids have been extensively studied for their diverse pharmacological properties, including antibacterial, antiproliferative, antioxidant, and antiviral effects (7 , 42) The presence of alkaloids in *Ipomoea pes-caprae* aligns with the broader understanding of alkaloids in medicinal plants and their significant roles in providing various health benefits (8,12,28). Furthermore, the study of *Ipomoea pes-caprae* has also revealed the presence of collagenase inhibitory quinic acid esters, which can play a role in processes like wound healing and tumor invasion [8]. Additionally, the plant has been investigated for its potential hypotensive activity, with flavonoids like quercetin derivatives being highlighted for their anti-hypertensive properties (3). The bioaccumulation of metals and metalloids in *Ipomoea pes-caprae* has also been studied, indicating the plant's ability to accumulate certain elements from the environment (19). Flavonoids and phenolic compounds, known for their antioxidant and anti-inflammatory properties, were detected in significant levels in all extracts of *Ipomoea pes-caprae*. Higher concentrations were observed in methanol and ethanol extracts compared to the aqueous extract, indicating that these solvents are more effective for extracting these beneficial compounds from the plant, potentially enhancing their antioxidant capabilities (2,20). The antioxidant and anti-inflammatory properties of flavonoids and phenolic compounds have been extensively researched in various plant species, emphasizing their significance in providing health benefits and therapeutic effects (17, 21). The presence of these compounds in *Ipomoea pes-caprae* aligns with the broader understanding of the role of secondary metabolites in plants and their potential contributions to human health [20]. Furthermore, the bioactivity of *Ipomoea pes-caprae* has been associated with its phytochemical composition, including flavonoids and phenolic compounds, which have been linked to various pharmacological activities (2, 20). The efficient extraction of these compounds from the plant using methanol and ethanol solvents further supports the traditional uses of *Ipomoea pes-caprae* in natural medicine and highlights its potential as a source of antioxidant and anti-inflammatory agents (2, 20). Terpenoids, a diverse group of secondary metabolites with various biological activities, were identified in all extracts of *Ipomoea pes-caprae*, albeit at relatively lower concentrations compared to other phytochemicals. The presence of terpenoids in the plant extracts suggests their potential contribution to the pharmacological effects of *Ipomoea pes-caprae* (24, 44). Terpenoids have been extensively studied for their structural diversity and biological activities, showcasing their importance in plant biochemistry and potential therapeutic applications (16, 42) The detection of terpenoids in *Ipomoea pes-caprae* aligns with the broader understanding of these compounds as essential components of plant secondary metabolism, with implications for ecological interactions and human health (27,28)While terpenoids may be present in lower concentrations compared to other phytochemicals in the plant, their biological activities, including antimicrobial, anti-inflammatory, and cytotoxic properties, highlight their potential significance in the overall pharmacological profile of *Ipomoea pes-caprae* (11, 20). Moreover, the structural and pharmacological diversity of terpenoids,

as evidenced by their presence in marine invertebrates, soft corals, and plant endophytic fungi, underscores the wide range of sources and bioactivities associated with these compounds (2,,14, 24). The identification of terpenoids in *Ipomoea pes-caprae* extracts adds to the growing body of research on the bioactive compounds present in this plant species and their potential implications for drug discovery and natural medicine 2 , 34).

Antioxidant Activity

Antioxidant activity refers to the ability of certain substances to neutralize or counteract the harmful effects of free radicals in the body. Free radicals are highly reactive molecules that can cause damage to cells, proteins, and DNA, leading to oxidative stress and potentially contributing to various chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders. Antioxidants work by donating electrons to stabilize free radicals, thereby preventing them from causing oxidative damage.

Table 2. Antioxidant activity data obtained using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay for *Ipomoea pes-caprae* extracts compared with vitamin C at concentrations of 100, 200, and 500 ppm in aqueous, methanol, and ethanol solvents

Sample	Concentration (ppm)	Antioxidant Activity (% Inhibition)
Vitamin C	**100**	**65.2**
	200	**78.6**
	500	**91.3**
Ipomoea pes-caprae	**100**	**42.7**
(Aqueous Extract)	**200**	**58.3**
	500	**72.9**
Ipomoea pes-caprae	**100**	**55.1**
(Methanol Extract)	**200**	**68.4**
	500	**81.2**
Ipomoea pes-caprae	**100**	**48.6**
(Ethanol Extract)	**200**	**63.8**
	500	**76.5**

The assessment of antioxidant activity in *Ipomoea pes-caprae* extracts using the DPPH assay provided valuable insights into the potential health-promoting properties of the plant. The results indicated varying degrees of antioxidant activity across different concentrations and solvent extract. Alkaloids, nitrogen-containing compounds known for their pharmacological activities, were identified in all three extracts (aqueous, methanol, and ethanol) of *Ipomoea pes-caprae*. These compounds are recognized for their potential antipyretic, analgesic, and antimicrobial properties, which could contribute to the therapeutic effects of this plant (2,9). Alkaloids have been extensively studied for their diverse pharmacological properties, including antibacterial, antiproliferative, antioxidant, and antiviral effects (7 , 42)The presence of alkaloids in *Ipomoea pes-caprae* aligns with the broader understanding of alkaloids in medicinal plants and their significant roles in providing various health benefits (8,17,18). Additionally, the plant has been investigated for its potential hypotensive activity, with flavonoids like quercetin derivatives being highlighted for their anti-hypertensive properties (8).. Flavonoids and phenolic compounds, known for their antioxidant and anti-inflammatory properties, were detected in significant levels in all extracts of *Ipomoea pes-caprae*. Higher concentrations were observed in methanol and ethanol extracts compared to the aqueous extract, indicating that these solvents are more effective for extracting these beneficial compounds from the plant, potentially enhancing their antioxidant capabilities (2,20). The antioxidant and anti-inflammatory properties of flavonoids and phenolic compounds have been extensively researched in various plant species, emphasizing their significance in providing health benefits and therapeutic effects (17,21). The presence of these compounds in *Ipomoea pes-caprae* aligns with the broader understanding of the role of secondary metabolites in plants and their potential contributions to human health (20). Furthermore, the bioactivity of *Ipomoea pes-caprae* has been associated with its phytochemical composition, including flavonoids and phenolic compounds, which

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Antibacterial Activity

Antibacterial activity refers to the ability of a substance to inhibit the growth of bacteria or kill bacteria outright. This activity is crucial in combating bacterial infections and maintaining overall health.

Table 3. Antibacterial activity data for *Ipomoea pes-caprae* extracts against two common bacterial strains, *Escherichia coli* and *Staphylococcus aureus*, using the disc diffusion method

Sample	Bacterial Strain	Zone of Inhibition (mm)
Aqueous Extract	*Escherichia coli*	12.5
	Staphylococcus aureus	10.2
Methanol Extract	*Escherichia coli*	15.8
	Staphylococcus aureus	14.3
Ethanol Extract	*Escherichia coli*	13.2
	Staphylococcus aureus	11.5

The antibacterial analysis conducted on *Ipomoea pes-caprae* extracts against *Escherichia coli* and *Staphylococcus aureus* sheds light on their potential as natural antimicrobial agents. The results unveil varying levels of antibacterial activity, attributed to the presence of bioactive compounds within the extracts. Investigation into the susceptibility of *Escherichia coli* and

Staphylococcus aureus to *Ipomoea pes-caprae* extracts by Putri (36), illustrates that *Escherichia coli* generally exhibits higher susceptibility compared to *Staphylococcus aureus*, as evidenced by larger zones of inhibition around discs impregnated with the extracts (3). Moreover, the study highlights that the antibacterial activity varies among different solvent extracts, with methanol and ethanol extracts demonstrating superior antibacterial activity compared to the aqueous extract against both bacterial strains. This suggests that methanol and ethanol serve as more effective solvents for extracting antibacterial compounds from *Ipomoea pes-caprae*. These findings align with the ongoing quest for alternative antimicrobial agents to address escalating concerns of drug resistance in pathogenic bacteria, as emphasized by Abo-El-Sooud (1). The imperative for novel drug sources to combat therapeutic failure underscores the significance of exploring natural sources, such as plant extracts, for their antibacterial properties. Additionally, research by Chaieb (9), on the antibacterial activity of clove essential oil against various pathogenic bacteria provides pertinent insights into the antimicrobial potential of plant extracts. This bolsters the notion that plant extracts can demonstrate varying antibacterial effects against different bacterial strains, as evidenced in the case of *Ipomoea pes-caprae* extracts. Putri[36] study furnishes robust evidence of the distinct susceptibility of *Escherichia coli* and *Staphylococcus aureus* to *Ipomoea pes-caprae* extracts, with methanol and ethanol extracts showcasing superior antibacterial activity compared to aqueous extracts. This underscores the promise of plant extracts as reservoirs of effective antibacterial compounds, aligning with the imperative to explore alternative antimicrobial agents in light of mounting antibiotic resistance. The concentration-dependent response observed in the antibacterial activity of *Ipomoea pes-caprae* extracts, as discussed by Arif **(5)**, substantiates the potential dose-response relationship. Furthermore, the comparison with standard antibacterial agents, as highlighted by Frey and Meyers [40], underscores the viability of these extracts as natural alternatives for addressing bacterial infections. The antibacterial activity observed in this study aligns with previous reports in the literature. Nuskiya (29). investigated the antimicrobial, antioxidant, and secondary metabolite content of *Ipomoea pes-caprae* leaves, corroborating the plant's bioactive properties. Additionally, Pereda-Miranda [4] characterized lipophilic pentasaccharides from *Ipomoea pes-caprae*, providing further insights into the plant's chemical composition enhancing its antibacterial efficacy. These studies collectively confirm the documented antimicrobial potential of *Ipomoea pes-caprae* and the presence of bioactive compounds contributing to its antibacterial properties.

CONCLUSION

In conclusion, our study investigated the phytochemical composition, antioxidant activity, and antibacterial efficacy of *Ipomoea pes-caprae* extracts. The results revealed a rich array of bioactive compounds present in the plant extracts, including alkaloids, flavonoids, phenolic compounds, terpenoids, tannins, and saponins. These extracts exhibited significant antioxidant activity, scavenging free radicals in a concentration-dependent manner. Moreover, the extracts demonstrated notable antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, suggesting their potential as natural antimicrobial agents. Overall, our findings underscore the pharmacological potential of *Ipomoea pes-caprae* as a source of natural antioxidants and antimicrobial compounds. Further research is warranted to isolate and characterize individual bioactive constituents and elucidate their mechanisms of action. These efforts may lead to the development of novel therapeutic agents derived from *Ipomoea pes-caprae*, contributing to the advancement of natural medicine and the conservation of traditional knowledge associated with coastal plant species.

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