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Ethnopharmacology and scientific validation of antidiarrheal plants used by the Osing Tribe in Banyuwangi, Indonesia

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Abstract. *Cendekiawan KA, Kintoko, Yuliani S, Wardhani FA. 2026. Ethnopharmacology and scientific validation of antidiarrheal plants used by the Osing Tribe in Banyuwangi, Indonesia. Biodiversitas 27 (2): d270229. https://doi.org/10.13057/biodiv/d270229.* Indonesia has a rich heritage of medicinal plant use; however, much of this traditional knowledge, especially among indigenous communities such as the Osing people in East Java, remains undocumented. This study aimed to explore the antidiarrheal plants used by the Osing community through an ethnopharmacological survey and scientific validation. Data were collected through structured interviews with local informants in Banjar and Licin Villages, Licin Sub-district, and Banyuwangi District. Use value (UV) and fidelity level (FL) indices were calculated to assess the cultural significance and therapeutic relevance of each species. Phytochemical screening and liquid chromatography-mass spectrometry (LC-MS) were conducted to identify bioactive compounds. The most frequently reported species were *Psidium guajava* (UV = 2.44; FL = 64.7%) and *Piper betle* (UV = 1.11). LC-MS analysis confirmed the presence of major secondary metabolites, including quercetin and kaempferol, which are known for their pharmacological activity in the treatment of gastrointestinal disorders. Scientific validation in this study also incorporated in silico approaches, including Lipinski's Rule of five evaluation to assess drug-likeness, ADMET profiling to predict pharmacokinetic and toxicity parameters, and molecular docking to explore the binding interactions of identified phytoconstituents with antidiarrheal target proteins. Quercetin demonstrated strong binding affinity (ΔG = -9.72) and an acceptable RMSD value (1.83 Å). A TPC value of 0.2357 was obtained from a mixture containing 0.84 g of *P. guajava* and 0.18 g of *P. betle*. The antioxidant activity, measured as IC_{50}, was 7.51 µg/mL. These findings suggest that quercetin holds strong potential as a lead compound for antidiarrheal drug development. Overall, this study highlights the scientific value of traditional knowledge and encourages further investigation into plant-based treatments for gastrointestinal disorders.

Keywords: Antidiarrheal, chromatography, ethnopharmacology, phytochemical, spectroscopy

INTRODUCTION

Medicinal plants have long been a foundational element in Indonesian traditional medicine, serving not only as therapeutic agents but also as integral components of cultural identity and community healthcare systems. These plants have been valued for generations, deeply embedded in indigenous healing traditions that reflect a profound understanding of local biodiversity and its medicinal applications (Reyes-García 2010). Among Indonesia's culturally rich ethnic groups, the Osing people of Banyuwangi District, East Java, who predominantly inhabit the culturally significant Ijen Mountains, possess a particularly rich ethnobotanical heritage (Prasetyo et al. 2018). Although their spiritual and ritual customs have been extensively documented, systematic and detailed written records focusing specifically on their medicinal plant knowledge remain limited. The preservation of this knowledge is crucial, not only to safeguard the cultural legacy of the Osing people but also to explore its untapped potential in the discovery and development of novel therapeutic agents (Kusumo et al. 2023).

Ethnopharmacology offers comprehensive and structured framework for studying traditional medical practices within their cultural and ecological contexts. This interdisciplinary approach integrates ethnobotanical documentation, which captures indigenous knowledge and the practical use of plants, with rigorous pharmacological validation to scientifically substantiate the therapeutic claims associated with traditional remedies (Sumarni et al. 2019). While research into Indonesian ethnomedicine is progressively expanding, there remains a significant gap in focused studies on the medicinal practices of the Osing community. This study aims to address this gap by systematically documenting and scientifically analyzing the Osing people's traditional use of antidiarrheal plants. Such work not only supports the preservation of cultural heritage but also facilitates the identification and characterization of bioactive natural compounds with potential pharmacological relevance (Bhagawan et al. 2022).

Phytochemical screening was performed to identify major metabolite groups, including phenolics, tannins, alkaloids, terpenoids, and saponins. These qualitative tests help distinguish primary metabolites, which support plant growth, from secondary metabolites, which contribute to defense and stress adaptation. Many secondary metabolites are also known for important pharmacological properties, making them valuable in drug discovery (Farag et al. 2020). To further characterize the chemical composition, liquid chromatography-mass spectrometry (LC-MS) was used. This technique combines chromatographic separation with sensitive and specific mass detection, enabling accurate identification of compounds even at low concentrations. Because of its precision in analyzing complex herbal extracts, LC-MS is widely applied in phytochemical and pharmaceutical studies (Zhou 2005). Using this approach, the study produced detailed phytochemical profiles of the selected plant extracts and provided insight into their chemical diversity and potential bioactive compounds.

Building on the ethnobotanical findings, this study specifically concentrated on two species frequently cited by the Osing community for their antidiarrheal properties: *Piper betle* and *Psidium guajava*. Despite their widespread traditional use, detailed chemical data on these plants remain relatively scarce in the scientific literature. Methanolic extracts of these species were subjected to LC-MS analysis to identify their constituent compounds. The identified phytochemicals were then further evaluated using in silico molecular docking techniques. These computational simulations assessed the binding affinities and interactions, including hydrogen bonding, between the plant-derived compounds and protein targets implicated in diarrhea pathophysiology. This molecular-level investigation provides a predictive framework for understanding the mechanisms by which these traditional remedies may exert their therapeutic effects.

Therefore, this study aimed to explore the antidiarrheal plants used by the Osing community through an ethnopharmacological survey and scientific validation. This comprehensive methodology not only aids in preserving the valuable medicinal knowledge of the Osing people but also generates scientific evidence supporting the efficacy of their antidiarrheal remedies. Ultimately, the findings contribute to a deeper understanding of traditional plant-based treatments and hold promise for the development of innovative, plant-derived therapeutics with potential clinical applications in managing diarrheal diseases. This approach exemplifies how indigenous wisdom, when combined with cutting-edge scientific techniques, can accelerate the discovery of novel bioactive compounds while respecting and preserving cultural heritage.

MATERIALS AND METHODS

Study area

Fieldwork was conducted in two principal Osing Villages within the Ijen National Park: Licin and Banjar, Banyuwangi, East Java Province, Indonesia (Figure 1). These sites were intentionally chosen based on their deep-rooted commitment to Osing cultural practices and their ongoing engagement in the use and preservation of traditional ethnomedicinal knowledge. Licin Village, located in the Banyuwangi District (8°11’37.174″S, 114°16′3.445″E) and covers approximately 4.67 km². Situated at an elevation exceeding 785 m above sea level (m asl), it ranks among the highest permanently inhabited areas on the island of Java (BPS 2024). In the same district, Banjar Village (8°13′0.48000″S, 114°16′15.24000″E), with a total land area of about 9.00 km². It is widely known for its strong commitment to preserving ancestral healing traditions (BPS-Statistics of Banyuwangi District 2024).

Ijen National Park’s montane ecosystem strongly shapes the physical and ecological conditions of nearby villages. The area has a tropical highland climate, with temperatures of 7-18°C and clear wet and dry seasons, creating suitable conditions for many medicinal plants used in Osing traditional medicine. Vegetation includes sub-montane and montane forests, alpine grasslands, and agricultural terraces, where medicinal plants are often cultivated with crops such as cabbage and potatoes. Fertile volcanic soils further support plant growth and therapeutic diversity. Its steep terrain, valleys, and volcanic ridges enhance ecological richness and endemism, which are closely reflected in the Osing community’s ethnobotanical knowledge and careful harvesting practice (Putri et al. 2022).

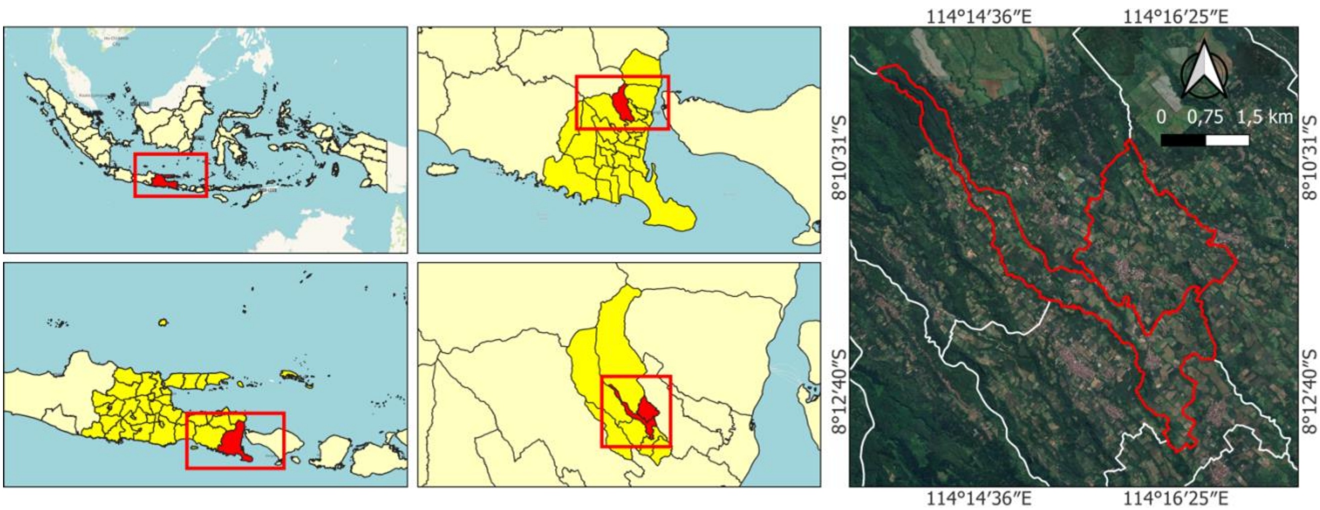


Figure 1. The study area map shows the location of Osing Tribe in Banjar Village, and Licin Village, Licin Sub-district, Banyuwangi District, East Java Province, Indonesia

Ethics and legal considerations

This study received ethical approval from the Health Research Ethics Commission of the Faculty of Health Sciences at Universitas Dr. Soebandi Jember, Indonesia (Approval No. 569/KEPK/XI/2024). The research adhered to the seven core ethical principles promoted by the World Health Organization, which emphasize the study's scientific and social value, the fair distribution of risks and benefits, risk reduction, avoidance of exploitation, and the safeguarding of participants' privacy, confidentiality, and autonomy through informed consent. Written informed consent was obtained from the village leadership, and verbal consent was secured from all individual participants before data collection.

Ethnopharmacology study

An ethnopharmacological study employing a cross-sectional design and purposive snowball sampling was undertaken from November to December 2024 in Banjar Village, located in the Licin Sub-district of Banyuwangi District. The data collection process adhered to rigorously established ethnographic methodologies, incorporating participant observation, semi-structured interviews, and systematic documentation of indigenous medicinal knowledge. A purposive sampling approach was used to select 34 participants from the Osing ethnic group, with inclusion criteria of age (≥40 years), long-term residency, and demonstrated expertise in traditional herbal practices. Data were gathered using structured questionnaires and semi-structured interview protocols.

Informant recruitment was conducted using a snowball sampling method, starting with recommendations from respected local figures known for their in-depth knowledge of ethnomedicine, including *dukun* (traditional healers) and *dukun bayi* (traditional midwives), who are culturally recognized as key custodians of medicinal knowledge. This sampling technique aligns with methodologies employed in earlier ethnobotanical research within the Osing community, thereby enhancing both the cultural relevance and the credibility of the findings (Jadid et al. 2020; Nugraha et al. 2024). Botanical specimens were collected during fieldwork and initially identified through observational data and informant input. Preliminary identification was guided by existing ethnomedicinal literature pertinent to the study region. For taxonomic verification, plant species and their corresponding families were cross-referenced and validated using the World Flora Online database (https://wfoplantlist.org/).

Quantitative analysis of ethnomedicinal data

To evaluate traditional medicinal knowledge, a series of ethnobotanical indices were utilized, specifically the Use Value and Fidelity Level. These analytical tools facilitated the assessment of the relative indigenous knowledge and the degree of consensus regarding the utilization of medicinal plant species within the target community.

Use Value (UV)

The Use Value (UV) index was used to assess the importance of each medicinal plant species in respondents' reports for treating diarrhoea and pain. This metric helps pinpoint plants with notable therapeutic benefits and broad cultural usage (Hu et al. 2020; Phillips 1993).

$$\text{Use Value (UV)} = \sum U / N$$

Where, ∑U: number of use-reports cited for a species by all respondents, N: total number of respondents.

In this context, UV denotes the Use Value of a given plant species, where U represents the total number of citations or mentions by respondents, and N signifies the overall number of participants interviewed. A higher UV score reflects frequent citation, indicating greater cultural and medicinal relevance, whereas a UV approaching zero implies minimal usage and limited significance within the local ethnopharmacological framework.

Fidelity Level (FL)

The Fidelity Level (FL) index assesses the degree of specificity with which a particular plant species is used to address specific health conditions, such as diarrhea and pain. Derived from the level of agreement among respondents, this metric illustrates the extent to which a given species is preferentially employed for a defined therapeutic application (Friedman et al. 1986; Leonti 2022).

$$\text{Fidelity Level (FL)} = (Np / N) \times 100$$

Where, Np: number of respondents who cited the use of a plant for a specific purpose, N: total number of respondents.

In this context, Nu refers to the total number of respondents who acknowledged all known medicinal uses of a particular species across various pharmacological contexts, including diarrhea treatment. Meanwhile, Np indicates the number of respondents who specifically identified using that species to address a distinct diarrhea condition. A higher Fidelity Level (FL) value indicates greater consensus among respondents on the intended use of the species, underscoring its significant ethnomedical relevance for the specific ailment. These indices were used to determine the cultural significance and specific use of each medicinal plant cited by respondents.

Scientific validation

Sample preparation

Fresh samples of guava and betel leaves were washed, chopped, and air-dried at 40-50°C. The dried material was ground to a fine powder using a blender and passed through a B-30 sieve. Extraction was carried out by maceration using 70% methanol for 72 hours at room temperature with intermittent shaking. The extracts were filtered and concentrated using a rotary evaporator under reduced pressure. The resulting crude extracts were stored at 4°C for further analysis (Farag et al. 2020).

Phytochemical screening

Phytochemical screening of guava and betel leaf extracts was conducted using standard qualitative methods adapted from Farag et al. (2020) to identify major classes of secondary metabolites. Alkaloid detection was performed using Dragendorff's and Mayer's reagents; the appearance of a reddish-brown precipitate indicated a positive result for alkaloids. Flavonoids were identified by adding magnesium powder to the extract, followed by concentrated hydrochloric acid; the development of a pink-to-red color confirmed their presence (Farag et al. 2020). Tannins were detected by adding either 1% ferric chloride ($FeCl_3$) or 1% lead acetate to the extract; a dark green or yellow precipitate, respectively, indicated their presence (Farag et al. 2020). Saponins were tested by shaking the extract with hot water and observing foam formation; stable foam that persisted for more than 10 seconds was considered a positive indication of saponins (Farag et al. 2020). Finally, triterpenoids and steroids were detected using the Liebermann-Burchard test, in which the extract was treated with acetic anhydride and concentrated sulfuric acid; the appearance of a reddish-purple color suggested triterpenoids, while green or blue indicated the presence of steroids (Farag et al. 2020). These tests provided preliminary chemical evidence supporting the traditional use of the selected plant extracts as antidiarrheals.

Identification of phytochemical profile by LC-MS

Identification of the compounds in guava and betel leaves was performed using liquid chromatography-Mass Spectrometry (LC-MS). Each sample was tested four times to ensure consistent results. The mobile phases were water and a small amount of formic acid, and the other contained acetonitrile and formic acid. The liquids were mixed in different amounts over time to separate the chemicals. The flow rate was kept at 0.2 mL/min. The chemical compounds were detected by electrospray ionization in positive-ion mode, targeting particles with mass-to-charge ratios between 50 and 1200. The machine was set to 35°C-60°C, and nitrogen gas was used to ionize the solvent. The energy used to break down the chemicals varied from 4 to 60 eV. A software called MassLynx was used to analyze the results. Important chemicals, such as quercetin and kaempferol, were identified by comparing their properties with known standards and databases. When possible, known samples of these chemicals were used to confirm their identity.

Ligand preparation

Molecular docking analyses elucidate the compatibility of various molecular structures, such as a drug and an enzyme or protein. This technique is primarily employed to predict the interaction between a protein (an enzyme) and a small molecule (a ligand) (Hakiki et al. 2024). The molecular tethering process commences with the preparation of the test ligand. Initially, the structure of the test compound is opened in MarvinSketch, where it undergoes preparation, including 2D cleaning and protonation, before being saved in the MRV format. Subsequently, the MRV file is reopened for conformation analysis. During this process, multiple bond energy values are generated, and the lowest is selected and saved in MOL2 format. In the preparation of ligands, 12 test compounds were utilized. The ligands were drawn using MarvinSketch version 24.1.0, followed by geometry optimization and protonation at pH 7.4, with the results saved in MRV format. A conformational search was then conducted, and the results were saved as PDB and MOL2 files.

Protein preparation

To predict interaction between bioactive compounds and protein targets involved in diarrheal pathophysiology, molecular docking was performed using AutoDock Tools 1.5.7 and AutoDock 4.2.6 (The Scripps Research Institute, USA). The 3D structures of target proteins were retrieved from the Protein Data Bank (PDB) (https://www.rcsb.org) using the appropriate PDB IDs for diarrhea-related biological targets, such as enterotoxins or ion channel regulators. For example, one key receptor used in this study was 5FBH, a known structure of a bacterial toxin subunit (Ihsan et al. 2024).

Validation of docking method

To ensure the reliability of the molecular docking protocol, validation was performed by re-docking native ligands into their original binding sites on six target receptors. The co-crystallized ligands were removed from the receptor structures, which were re-docked using AutoDock Tools 1.5.7, and the predicted poses were compared to the crystallographic conformations. Docking accuracy was evaluated using Root Mean Square Deviation (RMSD) values, with < 2.0 Å considered acceptable for reliable reproduction of experimental binding conformations (Ruswanto et al. 2020). In this study, all validation RMSD values were below 2.0 Å, confirming the robustness of the docking protocol. The Genetic Algorithm (GA) settings were optimized by increasing the number of runs to 100, which enhances the likelihood of identifying the most stable and biologically relevant binding poses. At the same time, other parameters were kept at their default settings (Kelutur et al. 2020).

Analysis of docking results

From running, the results are reported as binding energy and RMSD values. A good RMSD value is <2 Å. A binding energy value is considered good if the value is lower. Receptors and ligands that have been docked are converted to PDB format and then analyzed using Molegro Molecul Viewer (MMV) software, and the interactions are visualized in 2D and 3D (Ihsan et al. 2024).

Screening ligand-based drug likeness (drug scan)

Drug screening is performed on ligands with low binding energy and strong interactions with the target enzyme. Drug observation analysis is carried out with respect to the rule of good medicine (Lipinski's rule of five) (Ihsan et al. 2024).

Total phenolic content

The total phenolic content of extracts was determined using the Folin-Ciocalteu reagent. A 2.5 mL aliquot of the sample solution (20,000 mg/L) was transferred into a 10 mL volumetric flask. Then, 500 µL of Folin-Ciocalteu reagent was added, and the mixture was shaken for 1 minute to ensure homogeneity. After 6 minutes of incubation, 4.0 mL of 7.5% w/v sodium carbonate (Na_2CO_3) solution was added, followed by shaking for 1 minute and the addition of aquadest to the mark. The mixture was homogenized by shaking and incubated at room temperature for 30 minutes. The absorbance was measured at 760 nm using a spectrophotometer.

Antioxidant activity assay

The antioxidant activity of the extract was tested using the DPPH radical-scavenging assay. DPPH stock solution (0.2 mM) was made by dissolving 3.945 mg of DPPH in 50 mL of water. Then, a working solution (0.1 mM) was prepared by mixing 2 mL of the stock solution with 2 mL of water and incubating for 2 hours at room temperature in the dark. The absorbance of the DPPH solution was measured at about 1.00±0.05 before use. The extract was prepared as a 20,000 ppm stock solution in methanol and diluted with water to obtain final concentrations of 5,000, 4,000, 3,000, 2,000, and 1,000 µg/mL for the test. Ascorbic acid was used as a standard and prepared at concentrations of 50, 40, 30, 20, 15, and 10 µg/mL by diluting it in water. The test was done in a 96-well microplate, and absorbance was measured at 517 nm using a microplate reader. All tests were performed three times, and antioxidant activity was shown as percentage inhibition compared to the control. The IC_{50} value, which is the concentration needed to stop 50% of DPPH radicals, was found from the dose-response relationship for both extracts and ascorbic acid.

RESULTS AND DISCUSSION

Demographic characteristics of respondents

This ethnopharmacological study documents the traditional use of medicinal plants by the Osing people in Banjar and Licin Village, Licin Sub-district, Banyuwangi District, with a specific focus on antidiarrheal treatments. The selection of this site was strategic due to the high representation of Osing communities that continue to preserve indigenous health practices. Based on Table 1, 34 respondents contributed valuable knowledge shaped by experience and cultural transmission. The gender composition of the respondents was relatively balanced, with a slight predominance of males (52.94%) over females (47.06%). This almost even distribution of male and female respondents differs from several ethnomedicinal studies in other regions in Indonesia, where male respondents typically predominate due to prevailing sociocultural structures (Azis et al. 2020; Febriyanti et al. 2024). The relatively even gender representation observed in this study may reflect a more collaborative role between Osing men and women in the preservation, practice, and transmission of ethnomedicinal knowledge.

The respondents represented a broad age range, with the majority aged 21-60. Specifically, 17.65% were aged 21-30, 20.59% were aged 31-40, 39.85% were aged 41-50, and 23.53% were aged 51-60. Importantly, individuals aged 61-80 also made substantial contributions to the body of ethnomedicinal knowledge, underscoring the continued relevance of traditional practices among older community members. However, the notably low representation of respondents aged 80 and above (2.94%) raises concerns regarding the intergenerational transmission of this knowledge. Without deliberate efforts to transfer such expertise to younger generations, the gradual loss of elder knowledge holders may pose a significant risk to the preservation of ethnomedicinal heritage (Sujarwo et al. 2014; Ouma 2022).

Regarding educational attainment, half of the respondents (50.00%) had completed only elementary education. Other participants reported having completed junior high school (5.88%), senior high school (26.47%), or university education (5.88%), while 11.76% of respondents were illiterate. These findings suggest that traditional medicinal knowledge is not limited to older or non-literate individuals but is maintained across diverse educational backgrounds, highlighting the coexistence of formal and informal knowledge systems within the community (Oktavia et al. 2022).

Diversity of medicinal plant species used in the treatment of diarrhea

This study identified nine medicinal plant species traditionally used by the Osing community for the treatment of inflammation and pain. Comprehensive ethnomedicinal data pertaining to these species are systematically organized and presented in Table 2, which encompasses essential ethnobotanical indicators such as Use Value and Fidelity Level, along with each plant's scientific name, botanical family, local designation, traditional therapeutic uses, specific plant parts employed, preparation techniques, and modes of administration. The documented diversity reflects the depth of Osing's ethnopharmacological knowledge and underscores the community's extensive reliance on plant-based remedies to manage inflammatory disorders and pain-related health issues.

Table 1. Demographic characteristics of the respondents participating in the ethnomedicinal survey

Parameter	Category	Informant numbers	Percentage (%)
Gender	Male	18	52.94
	Female	16	47.06
Age	21 to 30 years old	6	17.65
	31 to 40 years old	7	20.59
	41 to 50 years old	8	23.53
	51 to 60 years old	6	17.65
	61 to 70 years old	5	14.71
	71 to 80 years old	1	2.94
	81 to 90 years old	1	2.94
Education level	No formal education	4	11.76
	Elementary school	17	50.00
	Junior high school	2	5.88
	Senior high school	9	26.47
	University	2	5.88

Table 2. Medicinal plants used by the Osing tribe for diarrhea treatment

Familia / Species	Name of plant	Part used	Preparation	Administration	UV	FL
Myrtaceae						
Psidium guajava L.	Guava	Leaf	Raw, boiled	Orally	2.44	64.7%
Piperaceae						
Piper betle L.	Betel	Leaf	Decoction	Orally, topically	1.11	29.4%
Musaceae						
Musa × *paradisiaca* L.	*Pisang klutuk*	Fruit	Raw	Orally	0.6	17.6%
Musa × *sapientum* L.	*Plantain*	Fruit	Raw	Orally	0.33	8.82%
Zingiberaceae						
Curcuma longa L.	Turmeric	Rhizome	Decoction	Orally	0.44	11.7%
Rosaceae						
Rubus allegheniensis Porter	*Calingan*	Leaf, fruit	Raw	Orally	0.33	8.82%
Potentilla arguta Pursh	*Grunggung*	Leaf	Crush, boiled	Orally, topically	0.11	2.94%
Acoraceae						
Acorus calamus L.	*Dringu*	Leaf, fruit	Crush	Topically	0.11	2.94%
Lauraceae						
Litsea cubeba (Lour.) Pers.	*Kranggean*	Leaf, fruit	Decoction	Orally	0.11	2.94%

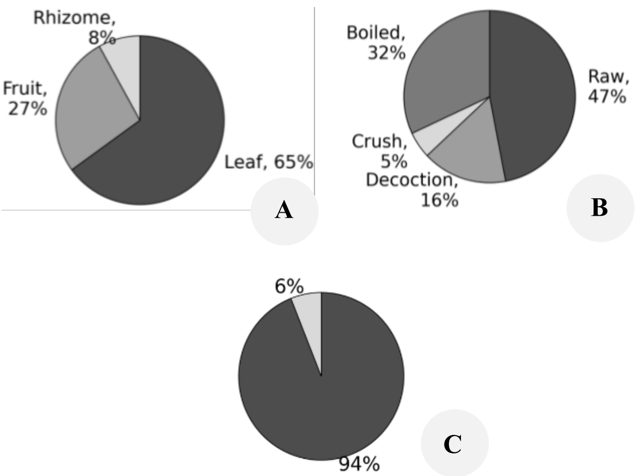


Figure 2. A. Plant part uses, B. Preparation methods, C. Administration routes employed by the Osing community for treating diarrhea

Table 2 showed that the Osing community used seven botanical families to treat diarrhea. Among these, Musaceae and Rosaceae were the most frequently represented, each comprising two species. These were followed by Myrtaceae, Lauraceae, Zingiberaceae, Piperaceae, and Acoraceae, each represented by a single species. As depicted in Figure 2, different plant parts were reported to be used by the Osing community to manage diarrhea. Leaves were the most commonly used part, with 32 instances (94.12%), followed by fruits, cited in 13 cases (38.24%), and rhizomes, which were mentioned in 4 cases (11.76%). The widespread use of leaves is consistent with previous ethnomedicinal research, which suggests that leaves are often preferred for their accessibility, renewability, and high levels of bioactive secondary metabolites such as flavonoids, alkaloids, and tannins (Bhagawan et al. 2022).

The notable use of fruits and rhizomes may also reflect their role as storage sites for secondary metabolites with recognized antidiarrheal effects. Moreover, reliance on leaves and other above-ground plant parts supports sustainable harvesting practices by avoiding the uprooting of whole plants. Such practices are vital for conserving local biodiversity, especially in ecologically fragile areas like the Ijen highlands, where many of these medicinal species are found.

The variety of preparation techniques reflects the community's extensive ethnopharmacological knowledge tailored to the specific therapeutic uses of each plant species (Figure 2.B). Regarding routes of administration (Figure 2.C), the majority of remedies were delivered orally (94%), highlighting the systemic approach commonly employed in traditional treatments for internal inflammatory ailments. In contrast, topical applications were documented in eight instances and were generally used to alleviate localized symptoms, such as bloating.

Based on ethnopharmacological interviews, nine plant species were identified as being used by the Osing people for the treatment of diarrhea. Table 2 lists these plants along with their species and botanical families. Guava (*P. guajava*) and betel (*P. betle*) were cited most frequently.

The ethnomedicinal practices of the Osing people are deeply rooted in their agricultural way of life and spiritual beliefs. The high citation rates for *Curcuma longa* not only indicate its perceived medicinal value but also its integration into local culinary and ritual practices. Knowledge is mainly passed down orally by elder women within the community, a pattern also seen in Javanese and Balinese ethnopharmacology (Nitayadnya et al. 2025). Although this oral transmission system is culturally rich, it highlights the urgent need for documentation to prevent the loss of knowledge due to modernisation.

The study employed quantitative ethnobotanical indices—Use Value (UV) and Fidelity Level (FL)—to assess the significance and specificity of medicinal plant use. The UV indicates the importance of each plant species based on the number of mentions across respondents. To determine the importance of each plant, a Use Value (UV) was calculated for each species (Table 2).

P. guajava showed the highest Use Value (UV = 0.72), highlighting its central role in Osing ethnomedicine in the management of diarrhea. This high UV may be due to its year-round availability and the perceived effectiveness of leaf decoctions, which are commonly passed down through intergenerational knowledge transfer. The prominence of this species is similar to findings among the Baduy tribe (UV = 0.68; Rahayu et al. 2018) and the Dayak community (UV = 0.65; Syamsudin et al. 2020), indicating that *P. guajava* remains highly valued across various ethnolinguistic groups in Indonesia. These findings imply that guava and betel are not only frequently mentioned but also regarded as effective by the Osing community. A comparable pattern was observed by Mustofa and Rahmawati (2018), who reported UV and FL values of 3.44 and 2.08, respectively, in plants used by traditional healers in Sulawesi (Sholikha and Arini 2023). The findings align with earlier studies in other Osing-populated areas such as Singojuruh and Kabat Sub-districts, which also reported the use of *P. guajava* (guava) leaves to treat diarrhea. These consistencies reinforce the importance of local plant knowledge as a cultural and therapeutic asset (Zaman et al. 2021).

Fidelity level of medicinal plant species

Table 2 shows medicinal plants identified through Fidelity Level (FL) analysis that the Osing community uses to treat inflammation and pain. The FL values for the documented species ranged from 1.11% to 100%, indicating varying levels of agreement among respondents regarding their specific therapeutic uses. *P. guajava* had the highest FL for diarrhoea (FL: 64.74%), followed by *P. betle*, which also showed a relatively high FL value (FL: 29.4%) for the same condition. The prominence of these plants in treating diarrhoea in the study area is likely driven by their widespread use in traditional Indonesian medicine to manage various health problems (Wiart 2007; Arozal et al. 2020). These values reflect both the cultural importance and therapeutic reputation of these plants. Ethnopharmacology not only examines the medicinal potential of plant species but also considers the socio-cultural context in which this knowledge is practiced and transmitted (Laily et al. 2015). The Osing people's reliance on guava and betel illustrates a well-established empirical tradition, rooted in generations of observation and experience (Bhagawan et al. 2022).

Phytochemical screening

The phytochemical screening of guava and betel leaf extracts supported the ethnobotanical data. Both were found to contain alkaloids, flavonoids, tannins, and steroids, all of which are known for their pharmacological activities (Amat-Ur-rasool et al. 2020). Flavonoids and tannins, in particular, are widely recognised for their antidiarrhoeal properties due to their astringent and anti-secretory effects. Previous studies also confirm the antioxidant properties of guava and betel leaves, further enhancing their therapeutic potential (Kumar et al. 2021). Phytochemical screening of guava and betel leaf extracts revealed the presence of alkaloids, flavonoids, tannins, and steroids in both samples, while saponins were not detected (Table 3).

To support and verify the preliminary findings, LC-MS analysis was conducted to detect specific secondary metabolites. The results confirmed the outcomes of the phytochemical screening, particularly through the identification of flavonoid compounds such as quercetin and kaempferol (Kurnia et al. 2021). These bioactive constituents are well documented in the scientific literature for their therapeutic roles in gastrointestinal health. Further LC-MS analysis reinforced the phytochemical screening results by detecting specific secondary metabolites. The chromatograms showed strong peaks corresponding to quercetin and kaempferol in both guava and betel leaf extracts (Figure 3). To further confirm these findings, additional LC-MS analysis was performed to identify specific secondary metabolites. LC-MS analysis revealed 15 major phytoconstituents, mainly flavonoids (e.g., quercetin, kaempferol) and tannins, both known for their antimicrobial and antidiarrheal properties. Docking analysis showed that quercetin exhibited the highest binding affinity (−9.4 kcal/mol) for the intestinal chloride channel protein CFTR, suggesting a potential mechanism for reducing secretory diarrhoea.

The results were consistent with the phytochemical screening, particularly with the identification of flavonoids such as quercetin and kaempferol (Kurnia et al. 2021). These compounds have been widely studied for their effects on the gastrointestinal tract. Quercetin is known to reduce intestinal motility and secretion, while kaempferol has demonstrated anti-inflammatory and spasmolytic activity (Sholikha and Arini 2023). According to Fratiwi and Yolanda (2015), guava leaves with high quercetin content significantly reduced the incidence of diarrhea. Kurnia et al. 2020 emphasized the importance of further exploring quercetin's potential using chemotaxonomic approaches. The identification of these compounds in both guava and betel supports the hypothesis that these plants have pharmacological efficacy against diarrheal disorders.

Table 3. Phytochemical constituents of guava and betel leaf extracts

Phytochemical test	Reagent	Result	
		Guava leaf	Betel leaf
Alkaloid	Mayer	+	+
	Bouchardat	+	+
	Dragendorff	+	+
Flavonoid	Powder of Mg, add some amyl alcohol	+	+
	HCl in concentrated	+	+
Tannin	$FeCl_3$	+	+
Saponin	Shake hot water vertically	-	-
Triterpenoid or steroid	Liebermann - Bouchardat	+	+

Note: "+": indicates presence; "–": indicates absence

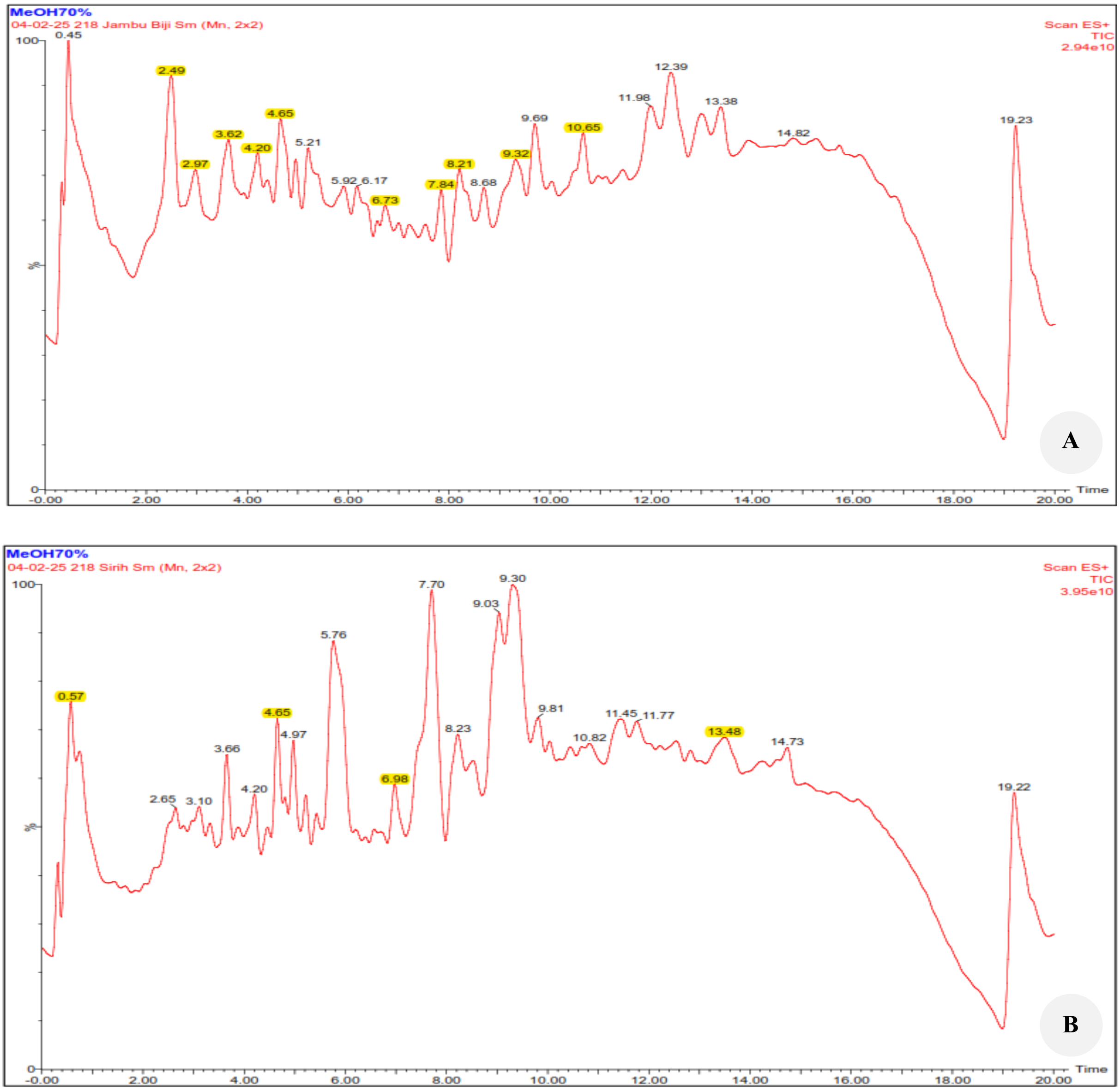


Figure 3. LC-MS chromatogram highlighting secondary metabolite profile: A. Guava leaf extract, B. Betel leaf extract

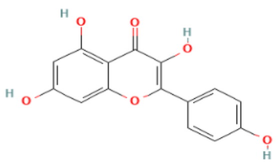
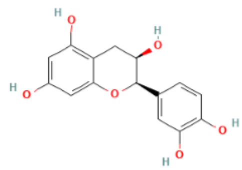
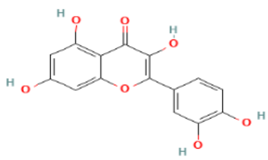
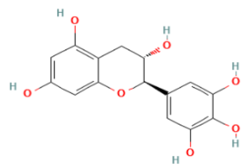
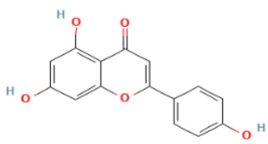
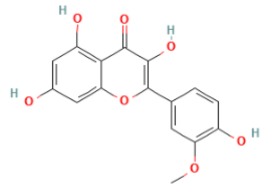
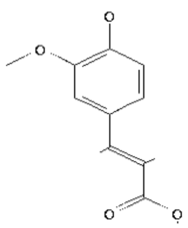
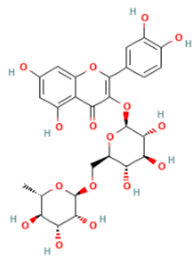
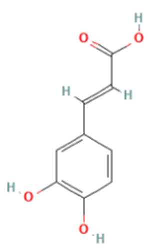
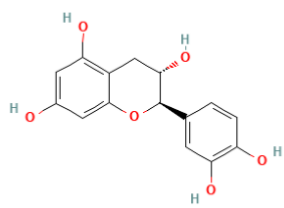
Subsequently, LC-MS spectra were analyzed using MassLynx software to identify the composition and classify the compounds corresponding to each peak. The results are presented in Table 4. Quercetin is known to reduce intestinal motility and secretion, while kaempferol has demonstrated anti-inflammatory and spasmolytic activity (Sholikha and Arini 2023). According to Fratiwi and Yolanda (2015), guava leaves with high quercetin content significantly reduced the incidence of diarrhea. Kurnia et al. 2020 emphasized the importance of further exploring quercetin's potential using chemotaxonomic approaches. The identification of these compounds in both guava and betel supports the hypothesis that these plants have pharmacological efficacy against diarrheal disorders.

This integration of traditional knowledge and modern scientific analysis demonstrates the potential of ethnopharmacology to bridge cultural practices and evidence-based medicine. The findings also underscore the importance of preserving local knowledge, which not only holds cultural value but may also contribute to the discovery of novel therapeutic agents. However, this study has limitations. The relatively small sample size and the absence of in vitro or in vivo pharmacological tests limit conclusions about efficacy. Future research should incorporate bioassays, toxicological evaluations, and, if possible, formulation studies to determine the dosage, safety, and effectiveness of these plant-based remedies.

The Osing community's use of guava and betel leaves for diarrhea treatment is both culturally embedded and scientifically plausible. The high UV and FL values, coupled with the presence of relevant secondary metabolites, support the use of these plants as natural antidiarrheal agents. These results highlight the significance of safeguarding indigenous knowledge and encourage further research. *P. betle* and *P.*

guajava, which are widely recognized to be the main focus in modern metabolomics studies. The chromatographic study identified 12 bioactive components. These findings reinforce the pharmacological potential of *P. betle* and *P. guajava*, which have long been empirically utilized in traditional medicine to address various pathological conditions. Flavonoids exhibit significant antioxidative capacity, as shown in phytochemical analysis (Effiong et al. 2024). The presence of alkaloids and phenolic compounds supports validated antimicrobial potential, while terpenoids, such as andrographolide, show promising hepatoprotective properties. The complexity of these secondary metabolite profiles suggests a wide range of biological activities, making *P. betle* and *P. guajava* potential sources for the development of natural material-based drugs (Iberahim et al. 2015).

Table 4. Structures of test ligands from guava leaves (*Psidium guajava*)

Compound	**2D structure**	**Compound**	**2D structure**
Kaempferol Structure: $C_{15}H_{10}O_6$ Retention time (minutes): 9.3 Molecular weight (g/mol): 286.23		Epicathecin Structure: $C_{15}H_{14}O_6$ Retention time (minutes): 2.97 Molecular weight (g/mol): 290.26	
Quercetin Structure: $C_{21}H_{20}O_{12}$ Retention time (minutes): 4.65 Molecular weight (g/mol): 464.37		Gallocatechin Structure: $C_{15}H_{14}O_7$ Retention time (minutes): 3.62 Molecular weight (g/mol): 306.26	
Apigenin Structure: $C_{15}H_{10}O_5$ Retention time (minutes): 7.84 Molecular weight (g/mol): 270.23		Isorhamnetin Structure: $C_{16}H_{12}O_7$ Retention time (minutes): 8.21 Molecular weight (g/mol): 316.26	
Ferulic acid Structure: $C_{10}H_{10}O_4$ Retention time (minutes): 10.65 Molecular weight (g/mol): 194.18		Rutin Structure: $C_{27}H_{30}O_{16}$ Retention time (minutes): 6.73 Molecular weight (g/mol): 610.51	
Caffeic Acid Structure: $C_9H_8O_4$ Retention time (minutes): 4.2 Molecular weight (g/mol): 180.15		Cathecin Structure: $C_{15}H_{14}O_6$ Retention time (minutes): 2.49 Molecular weight (g/mol): 290.46	

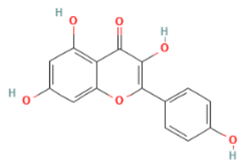
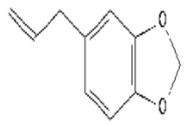
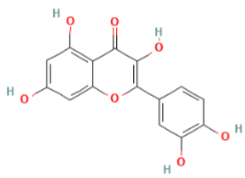
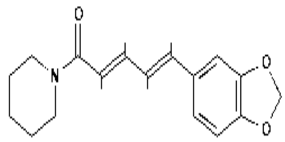
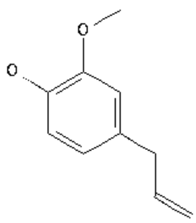
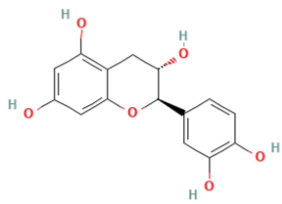
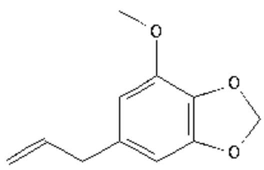
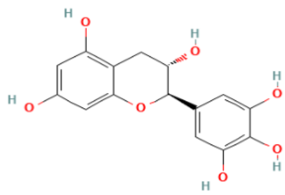
Ligand preparation

The test ligands used in this in silico test are compounds from guava leaf plants (*P. guajava*) and betel leaf (*P. betle*). Molecular tethering simulations were performed on the 12 compounds in Table 4 to determine their interactions with 6 receptors. The goal of the molecular docking simulation is to identify the most suitable ligand candidate by assessing the affinity between the receptor's conformation and the ligand (Salsabila et al. 2023).

Protein preparation

After preparing the test ligand, we can proceed with preparing the receptor. Drug receptors are specific macromolecules (e.g., lipoproteins, nucleic acids) that bind ligands (drugs, hormones, neurotransmitters) to trigger chemical signals in cells, thereby producing effects (Salsabila et al. 2023). The structures of macromolecules are deposited in the Protein Data Bank (PDB) website at http://www.rcsb.org. The Protein Data Bank (PDB) is a repository of experimental data on the three-dimensional structure of macromolecules. Then, open the file in the MOLEgro to separate the native ligand from the protein. Then save it in a working folder in PDB format. After that, the preparation was performed by removing water molecules in Discovery Studio.

Table 5. Structure of test ligands from betel leaf (*Piper betle*)

Compound	2D Structure	Compound	2D Structure
Kaempferol Structure: $C_{15}H_{10}O_6$ Retention time (minutes): 6.17 Molecular weight (g/mol): 286.23		Safrol Structure: $C_{10}H_{10}O_2$ Retention time (minutes): 7.70 Molecular weight (g/mol): 162.18	
Quercetin Structure: $C_{21}H_{20}O_{12}$ Retention time (minutes): 4.65 Molecular weight (g/mol): 464.37		Piperin Structure: $C_{17}H_{19}NO_3$ Retention time (minutes): 13.48 Molecular weight (g/mol): 285.33	
Eugenol Structure: $C_{10}H_{12}O_2$ Retention time (minutes): 0.57 Molecular weight (g/mol): 164.2		Cathecin Structure: $C_{15}H_{14}O_6$ Retention time (minutes): 2.65 Molecular weight (g/mol): 290.46	
Myristicin Structure: $C_{11}H_{12}O_3$ Retention time (minutes): 5.76 Molecular weight (g/mol): 192.21		Gallocatechin Structure: $C_{15}H_{14}O_7$ Retention time (minutes): 9.81 Molecular weight (g/mol): 306.26	

Removing water molecules aims to reduce computational load and thereby shorten the simulation time. After removing water molecules, hydrogen atoms are added to adjust the docking environment to match the body's pH. The receptors used in this study were six receptors including Regeneration and signaling in the intestinal epithelium EGFR (PDB code id: 5WB7), Inflammation and apoptosis regulation PI3K/Akt/NF-κB (PDB code id: 3O96), The receptors used in this study were six receptors including Regeneration and signaling in the intestinal epithelium EGFR (PDB code id: 5WB7), Inflammation and apoptosis regulation PI3K/Akt/NF-κB (PDB code id: 3O96), Pro-inflammatory mediators TNF and NF-κB (PDB code id: 3ALQ), Electrolyte balance and antidiarrheal effects Na+/K+-ATPase (PDB code id: 3B8E), Regulation of intestinal transit in Opioid receptor (PDB code id: 4DKL), Bowel motility in Muscarinic M3 receptor (PDB code id: 4DAJ) based on investigation of diarrhea in GeneCards. The basis for the assessment is the Root Mean Square Deviation (RMSD) value. The method is considered valid if the RMSD is <2 Å. The smaller the error in the calculation results, the more accurate the calculation; in addition, the lower the RMSD value, the more stable the protein (Kaharudin et al. 2022).

Table 6 shows the results for 6 native ligands. The above native ligand meets the docking method's validation criteria, with an RMSD < 2 Å. However, the native ligand 3ALQ does not meet the requirements or has an RMSD value of 31.96 Å. Table 5 presents the lists of test ligands that meet the Lipinski constraints, which are then subjected to virtual screening to obtain the binding energies of each test ligand by docking them to the prepared protein targets.

Based on Table 8, Binding energy is a measure of the drug's ability to bind to the receptor. The smaller the binding energy value, the higher the affinity between receptors and ligands, and vice versa. The greater the binding energy value, the lower the affinity between receptors (Shofi 2021). Based on the analysis, Quercetin is the most effective test ligand, binding to multiple receptors with the lowest binding energy value.

Total phenolic content

The total phenolic content was analyzed using gallic acid as the phenolic standard, yielding a regression coefficient of 0.9879 (Figure 4). It indicates a linear relationship between concentration and absorbance in the assay. The total phenolic content of a mixture of guava leaf (*P. guajava*) and betel leaf (*P. betle*) extracts, with a composition of 0.84 g of guava leaves and 0.16 g of betel leaves, was 0.2357 mg GAE/g. These results suggest that the combination of guava and betel leaves exhibits a synergistic effect due to interactions among their phenolic compounds.

Antioxidant activity assay

The antioxidant activity of the extract was evaluated using the DPPH radical-scavenging assay. In Table 9, the extract demonstrated a concentration-dependent increase in free radical scavenging activity, with percentage inhibition values ranging from 45.53% at 1,000 µg/mL to 63.94% at 5,000 µg/mL. The highest inhibition was observed at the maximum tested concentration (5,000 µg/mL), indicating strong antioxidant potential. The calculated IC_{50} value for the extracts was 7.51 µg/mL, corresponding to the concentration required to inhibit 50% of DPPH radicals. In comparison, Table 10 shows that the ascorbic acid standard exhibited significantly higher scavenging activity, with inhibition values ranging from 72.15% to 82.24% across concentrations of 10-50 µg/mL, and an IC_{50} of 0.66 µg/mL. These results confirm that, while extracts have notable antioxidant capacity, their activity is lower than that of the reference antioxidant, ascorbic acid.

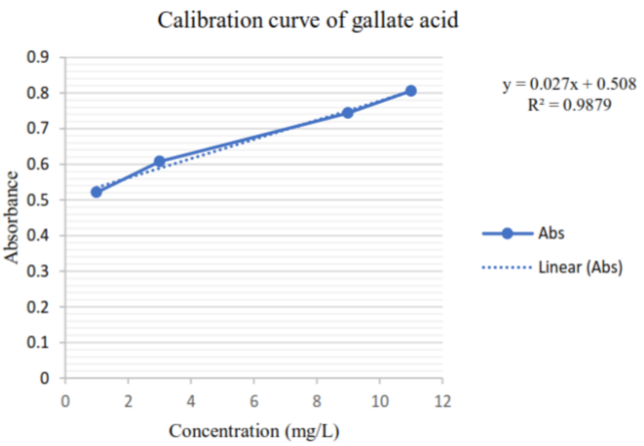


Figure 4. Calibration curve of gallic acid

Table 6. Redocking results

Receptors	Native ligand	Run	Binding energy (kkal/mol)	Rmsd (Å)
EGFR	5WB7	77	-10,08	1,02
PI3K/Akt/NF-κB	3O96	30	-9,57	0,60
TNF & NF-κB	3ALQ	11	-2,78	31,96
Na+/K+-ATPase	3B8E	35	-7,47	3,25
Opioid receptor	4DKL	26	-2,53	1,83
Muscarinic M3 receptor	4DAJ	35	-9,01	0,55

Table 7. Lipinski results for each compound

Compound	Molecular weight (<500g/mol)	Hydrogen donor (<5)	Hydrogen acceptor (<10)	Log p (<5)
Kaempferol	286,239	4	6	2,2824
Quercetin	302,238	5	7	1,988
Safrol	162,182	0	3	3,4559
Isorhamnetin	316,269	7	8	0,7426
Cathecin	290,271	5	6	1,5461
Apigenin	270,230	3	3	-0,6719
Piperin	285,332	2	2	-0,8208
Caffeic acid	180,155	1	1	0,111
Ferulic acid	224,211	1	1	0,4659
Epicathecin	290,266	0	2	2,2166
Gallocathecin	306,263	3	3	1,6916
Myristicin	192,211	2	5	2,3767
Rutin	610,512	3	3	0,211
Eugenol	164,211	2	5	0,4759

Table 8. Molecular tethering results of native ligand BHF with test ligands

Compounds	Binding energy (kkal/mol)					
	5WB7	3O96	3ALQ	3B8E	4DKL	4DAJ
Safrol	-8,08	-6,64	Doesn't meet the criteria	-6,47	-4,67	83,91
Kaempferol	-7,54	-7,18		-6,41	-4,88	95,38
Quercetin	-9,72	-6,92		-6,87	-5,46	150,38
Isorhamnetin	-3,65	-6,58		-5,64	-5,14	194,49
Cathecin	-7,69	-7,6		-7,23	-5,29	113,27
Apigenin	-3,19	-2,58		-3,22	-2,92	-1,46
Piperin	-3,36	-4,41		-4,22	-3,77	-1,41
Caffeic acid	-4,23	-3,50		-5,87	-3,50	186,5
Ferulic acid	-2,7	-4.31		-2,44	-2,49	-2,25
Epicathecin	5,08	-4,20		-4,96	-3,73	5,79
Gallocathecin	6,88	-5,65		-6,05	-4,99	31,4
Myristicin	-9,4	-7,37		-5,48	-5,39	199,33
Rutin	-2,58	-2,75		-2,49	-2,25	22,78
Eugenol	-4,41	-3,75		-3,73	5,79	14,68

Table 9. Calculation of the inhibition ratio of the extract

Final concentration (ug/mL)	Log concentration	Inhibition ratio average (%) ± SD	CV	IC_{50}
5000	3.698970004	63.93514293 ± 2.273350786	3.555714	
4000	3.602059991	58.06502009 ± 2.617414543	4.50773	
3000	3.477121255	54.78792993 ± 1.793258161	3.27309	7.509908
2000	3.301029996	52.28911793 ± 0.594929873	1.13777	
1000	3.000000000	45.53279781 ± 2.10214848	4.616779	

Table 10. Calculation of the inhibition ratio of ascorbic acid as a standard

Final concentration (ug/mL)	Log concentration	Inhibition ratio average (%) ± SD	CV	IC_{50}
50	1.698970004	82.23595 ± 0.153953	0.187209	
40	1.602059991	80.91973 ± 0.397092	0.490723	
30	1.477121255	77.2968 ± 1.559085	2.017011	0.655133
20	1.301029996	74.44903 ± 0.551931	0.741354	
10	1.000000000	72.14588 ± 0.826189	1.145165	

In conclusion, this study successfully documented and scientifically validated the Osing community's traditional knowledge of antidiarrheal medicinal plants in Banjar and Licin Villages, Banyuwangi District, East Java. Ethnopharmacological analysis identified *P. guajava* and *P. betle* as the most culturally significant species for the treatment of diarrhoea, supported by high Use Value and Fidelity Level indices. Phytochemical screening and LC-MS profiling confirmed the presence of important secondary metabolites, particularly flavonoids such as quercetin and kaempferol, which are widely recognized for their gastrointestinal and antimicrobial activities. In silico molecular docking further demonstrated that quercetin exhibited the strongest binding affinity for diarrhea-related protein targets, suggesting a potential mechanism for reducing intestinal secretion and inflammation. In addition, the extract mixture demonstrated notable antioxidant capacity (IC_{50} value of 7.51 µg/mL) and measurable total phenolic content, suggesting synergistic bioactivity between guava and betel extracts. Overall, integrating ethnobotanical documentation with chemical profiling and computational validation underscores the scientific relevance of Osing traditional medicine and supports the potential of quercetin as a promising lead compound for plant-based antidiarrheal drug development. Future research should focus on in vitro and in vivo pharmacological evaluation, toxicity assessment, and formulation standardization to further confirm efficacy and ensure safe therapeutic application, while also promoting the conservation of indigenous knowledge and sustainable use of medicinal plant resources.

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