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# GC-MS Analysis, Antidiarrhoeal, and *In Vitro* Antioxidant Activities of Ethanol Extract of *Blumea balsamifera* (L.) DC. Leaves from North Sumatra Province

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ABSTRACT

*Blumea balsamifera* leaves exhibit biological activities as antibacterial, antidiarrhoeal, and antioxidant agents. This study aims to identify chemical compounds with potential antibacterial, antidiarrheal, and antioxidant activities through *in vitro* methods. The study used GC-MS to determine the chemical components in *Blumea balsamifera* leaves and evaluated antidiarrhoeal activity through an antibacterial approach using the Kirby Bauer method against *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus cereus*, *Helicobacter pylori*, and *Escherichia coli* with extract concentrations of 0% (DMSO), 10%, 30%, 50%, 70%, and 100%. Antioxidant activity was assessed using the DPPH method. The results of the GC-MS analysis showed that the EtOH extract of *Blumea balsamifera* leaves contains isopropanol, azelaic acid (AZA), palmitic acid (PA), myristyl alcohol, (Z)-hexadec-11-en-1-ol, and hendecanoic acid. The 10-100% ethanol extract demonstrated antibacterial activity against diarrhoea-causing bacteria, while the antioxidant activity of the EtOH extract showed an $IC_{50}$ value of 117.560 ppm, and ascorbic acid had an $IC_{50}$ value of 3.358 ppm. The EtOH extract of *Blumea balsamifera* leaves demonstrated potential as an antibacterial, antidiarrhoeal, and antioxidant agent *in vitro*.

***Keywords*:** Antidiarrhoeal, Antibacterial, Antioxidant, *Blumea balsamifera.*

## Introduction

Diarrhoea is a digestive disorder characterized by fluid imbalance in the intestinal tract. It includes acute and chronic diarrhoea. Bacterial infection of the digestive system is the cause of acute diarrhoea, while chronic diarrhoea is due to pathogenic infection or intestinal inflammation.[1] Bacteria that can cause diarrhoea include *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus cereus*, *Helicobacter pylori*, and *Escherichia coli*, with symptoms of hypermotility triggered by excessive bacterial stimulation and inflammation that leads to prostaglandin release into the intestinal mucosa.[2] The mechanism of bacteria-induced diarrhoea can involve enterotoxins, and exotoxins causing enterocolitis[3,4] and gastroenteritis.[5]

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Additionally, bacterial infections can cause hypersecretion accompanied by oxidative damage, leading to glutathione depletion.[6] Therefore, an alternative approach to treating diarrhoea can be pursued through antibacterial and antioxidant activities, utilizing *Blumea balsamifera*. *Blumea balsamifera*, known as galunggung, is one of the plants traditionally used by the Karo ethnic group in North Sumatra, Indonesia, primarily for treating diarrhoea, hypertension, and menstrual disorders.[7] *Blumea balsamifera* (L.) DC. (Asteraceae), has long been utilised in traditional medicine in Southeast Asian countries[8,9] such as Indonesia, China, the Philippines, and Thailand. Indonesian population has used leaves of *B. balsamifera* to treat dermatitis, beriberi, rheumatism[10], sinusitis, and cough, as a diuretic[11], and to address diarrhoea, influenza, asthma, mouth ulcers, and angina pectoris[12], by boiling either fresh or dried leaves. The traditional use of *B. balsamifera* leaves is associated with the presence of chemical compounds like polysaccharides, terpenoids, flavonoids, essential oils, alkaloids, saponins[13], and phenolics, which are believed to possess pharmacological activities such as antibacterial, antidiarrhoeal, and antioxidant properties. The MeOH, chloroform fractions, and EtOAc extracts of *B. balsamifera* leaves have shown antibacterial activity against *B. cereus*, *S. aureus*, *S. pneumoniae*, *P. aeruginosa*[14,15,16], and *E.* coli.[15] The MeOH, EtOAc, n-hexane and EtOH extracts of *B. balsamifera* leaves have shown $IC_{50}$ values of 105.1 ppm[17], 23.68 ppm, 113.716 ppm[18] and 17.59 ppm[13], respectively against some pathogenic bacteria. This study aims to identify the chemical compounds in the EtOH extract of *B. balsamifera* leaves that have potential as antibacterial, antidiarrhoeal, and antioxidant agents, tested *in vitro*.

## Materials and Methods

*Plant collection and identification*

*Blumea balsamifera* (L.) DC. leaves were collected from the Binjai Plantation area, South Binjai District, Langkat Regency, North Sumatra Province, at coordinates 03°35'12.0"N-98°28'45.60"E in September 2023. The physical factors included a pH of 5, light intensity of 398 cd, and soil temperature of 30°C. Plant identification was conducted at the Herbarium Medanense, Department of Biology, Faculty of Natural Sciences, Universitas Sumatera Utara, Indonesia, with identification number 1396/MEDA/2023.

*Sample preparation*

A total of 8 kg of sorted and thoroughly washed leaves were dried at about 50°C for two weeks. Once dried, the simplicia was ground into a fine powder and weighed. The simplicia powder was macerated using ethanol (EtOH pro analysis, Smart Lab, Indonesia) for 5 days in a dark room. The crude extract was filtered through Whatman No. 1 filter paper, then the filtrate was evaporated to dryness using a rotary evaporator (Heidolph, Germany) at 40°C.

*Phytochemical screening*

The plant sample was screened for the presence of secondary metabolites, such as flavonoids, saponins, alkaloids, tannins, and steroids/triterpenoids, using standard methods. Alkaloid testing used Mayer, Dragendorff, and Bouchardat reagents. Flavonoid testing involved Mg, HCl, and amyl alcohol. Saponin testing used hot water. Tannin testing used $FeCl_3$, and steroid/triterpenoid testing used n-hexane, anhydrous acetic acid, and $H_2SO_4$.[19]

*In Vitro antidiarrhoeal activity testing*

Kirby Bauer method was used for *in vitro* antidiarrhoeal testing, employing diarrhoea-causing test bacteria specifically *Staphylococcus aureus* ATCC 6538, *Salmonella typhi* ATCC 14028, *Bacillus cereus* ATCC 11778, *Helicobacter pylori* ATCC 43504, and *Escherichia coli* ATCC 8739. The concentrations of EtOH extract of *B. balsamifera* leaves tested were 0% (DMSO), 10%, 30%, 50%, 70%, and 100%.

*Antioxidant activity testing*

The 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma, Amerika) method was used to test for the antioxidant activity. The EtOH extract of *B. balsamifera* leaves was tested at concentrations (10, 50, 100, 150, and 200 ppm). The DPPH $\lambda_{max}$ of 516 nm was measured using a UV-Vis spectrophotometer (Genesys 10s, United States). Ascorbic acid (used as a standard control). Ascorbic acid (10 mg) was dissolved in ethanol, and diluted with distilled water to a final volume of 100 mL to make a stock concentration of 100 ppm. It was then adjusted to 6.5 ppm, 7 ppm, 7.5 ppm, 8 ppm, and 8.5 ppm. The formula used to measure antioxidant activity was the percentage inhibition of DPPH radicals:

$$Inhibition\ (\%)\ \frac{(Ab-As)}{(Ab)} \text{ x } 100 \ ^{(20)}$$

Where; Ab = Absorbance Blanc, As = Absorbance Sample.

The $IC_{50}$ of DPPH free radicals was calculated from the linear regression plot (y = ax + b) using the formula:

$$IC50\ \frac{(50-b)}{(a)} \text{ x } 100 \ ^{(21)}$$

Where; **b** denotes the y-intercept and **a** is the regression line's slope.

*GC-MS analysis*

Chemical component identification was conducted through GC-MS analysis (GC Model SCION 436 and 456, Netherlands). Helium was used as the carrier gas and a capillary column under constant pressure, the filtered extract solution was injected into the GC-MS at a split ratio of 8:1 psi and a total flow rate of 1.2 mL/min. The operational temperatures were set to 280ºC and 140ºC, the injector to 250ºC, and the detector to 230ºC. Using a mass detector, eluted components were detected.[22]

*Statistical analysis*

The antibacterial activity of diarrhoea-causing bacteria was measured by determining the diameter of the inhibition zone around the disc. The EtOH extract of *B. balsamifera* leaves was chemically analyzed using GC-MS analysis, and the results of antioxidant activity were computed using $IC_{50}$ values.

## Results and Discussion

Based on GC-MS analysis, the EtOH extract of *B. balsamifera* leaves was found to contain natural compounds such as isopropanol, azelaic acid (AZA), palmitic acid (PA), myristyl alcohol, (z)-hexadec-11-en-1-ol, and hendecanoic acid (Table 1). These compounds have potential antibacterial, antioxidant and anti-inflammatory effects. Isopropanol is a compound used as a disinfectant and antiseptic. Isopropanol can disrupt the cell membrane integrity of *Staphylococcus aureus* (MRSA).[23] Azelaic acid or AZA is a group of carboxylic acid compounds formed in response to stress in plants. AZA exhibits antibacterial activity by blocking protein synthesis.[24] AZA also exhibits antityrosinase and antimicrobial activities by reducing free radicals, decreasing melanocyte activity, inhibiting melasma growth, and halting macular hyperpigmentation.[25] Furthermore, AZA has been applied in acne treatment and has been developed in the cosmetic and pharmacological fields.[24] Topically, AZA has demonstrated effectiveness in treating rosacea, acne vulgaris, and a variety of hyperpigmentation conditions. AZA has proven its ability to reduce the inflammatory cytokine cascade (IL-1, IL-6, and TNF-α), by blocking the activity of transcription nuclear factor. Additionally, research has shown that AZA's antibacterial activity is associated with altering the intracellular pH of bacteria.[26] Palmitic acid (PA) is a saturated fatty acid that functions as an energy source and plays a role in the structure and function of cell membranes.[27] Palmitic acid can bind to cell membranes and alter cellular mechanisms, making it potentially antibacterial. The mode of action of palmitic acid (PA) includes rupturing the cell walls of both gram-positive and gram-negative bacteria and interfering with oxidative phosphorylation, the chain of electron transport, and enzymes on membranes that control the production of energy in bacteria. This results in changes to membrane integrity, leading to cell permeability, growth inhibition, cell lysis, and cell death.[28] In addition to its antibacterial properties, palmitic acid also has antioxidant activity, donating hydrogen to free radicals and reducing them to non-reactive species.[29]

Myristyl alcohol, or 1-tetradecanol (1-TD), is a saturated straight-chain fatty alcohol that functions as an antibacterial agent. Previous research has shown that 73.4% of myristyl alcohol is present in the fruit of *Cymbocarpum erythraeum*, which has the potential to inhibit 24 types of bacteria.[30] Additionally, myristyl alcohol has anti-inflammatory properties by preventing *H. pylori*-infected gastric epithelial cells from producing IL-8. MIC levels of 0.5-1 mg of myristyl alcohol can inhibit the growth of *H. pylori.*[31] The compound 11-hexadecen-1-ol, (z)- functions as an insecticide and is a terpene derivative. Approximately 40% of 11-hexadecen-1-ol, (z)- was found in *Crithmum maritimum.*[32] This differs from previous studies, which reported that GC-MS analysis of *B. balsamifera* leaves revealed compounds such as borneol, caryophyllene, camphor, jasmoline, dimethyl ether ($C_{10}H_{12}O_3$), L-borneol, -aminoethanol hydrogen sulfate (ester), and camphor.[33] The variation in secondary metabolites in the same plant species can be influenced by several factors, including geographical location, soil nutrient availability, physical factors (temperature, pH, humidity, light intensity), genetics, environmental stress, climate, and altitude.[34] The compounds azelaic acid (AZA), palmitic acid (PA), and hexadecanoic acid belong to the group of carboxylic acid compounds. Carboxylic acid compounds are natural chemical components derived from plants[35], because they function in the respiration process, photosynthesis, maintaining oxidation-reduction balance, maintaining cell homeostasis[36], cell membrane formation, and hormone synthesis (phytohormones).[37] The Asteraceae and Dryopteridaceae families are groups of plants that contain a lot of carboxylic acid.[35] Carboxylic acid has pharmacological benefits used as an antioxidant, antiseptic and anti-inflammatory.[36] More than 450 drugs containing carboxylic acid groups have been traded, such as non-steroidal anti-inflammatory drugs, antibiotics, anticoagulants and cholesterol-lowering drugs.[38] Compounds with carboxylic groups found in sembung leaves can be used as drug candidates.

**Table 1:** GC-MS analysis results of EtOH extract of *Blumea balsamifera*

| No. | RT (min) | Peak Name | Compound Nature | Molecule Formula | Molecular Weight (g/mol) | Amount | Ref. |
|---|---|---|---|---|---|---|---|
| 1 | 1.518 | Isopropyl Alcohol | Isopropanol | $C_3H_8O$ | 60.10 | 7.871 | Antivirus[39] |
| 2 | 34.253 | Nonanedioic Acid | Azelaic Acid | $C_{10}H_{18}O_4$ | 202.25 | 2.091 | Antimicrobial, antioxidant, antityrosinase [24] |
| 3 | 35.321 | n-Hexadecanoic Acid | Palmitic Acid | $C_{16}H_{32}O_2$ | 256.42 | 1.848 | Antibacterial [27] |
| 4 | 37.567 | Cis-9-Tetradecen-1-ol | Myristyl Alcohol | $C_{14}H_{28}O$ | 212.37 | 0.525 | Antibacterial, anti-inflammatory [30] |
| 5 | 37.694 | 11-Hexadecen-1-ol, (z) | (Z)-Hexadec-11-en-1-ol | $C_{16}H_{32}O$ | 240.42 | 0.368 | Antibacterial [40] |
| 6 | 39.233 | Undecanoic Acid, Hydroxy-1 | Hendecanoic Acid | $C_{11}H_{22}O_2$ | 186.29 | 0.571 | Antibacterial [41] |

A total of 8 kg of fresh *Blumea balsamifera* leaves yielded 750 g of simplicia powder and 61.32 g of thick crude extract. The screening of secondary metabolite groups present in the simplicia and ethanol extract of *B. balsamifera* leaves aimed to identify the phytochemical compounds with antibacterial, antidiarrhoeal, and antioxidant potential, is shown in Table 2. Simplicia and the EtOH extract of *B. balsamifera* leaves contain secondary metabolites, including flavonoids, alkaloids, saponins, steroids/triterpenoids, and tannins.

**Table 2:** Phytochemical constituents of Simplicia and *Blume balsamifera* (L.) DC. Leaf Extract

| **Secondary Metabolites** | ***Blumea balsamifera*** | |
|---|---|---|
| | **Simplicia** | **Extract** |
| Flavonoids | + | + |
| Alkaloids | + | + |
| Saponins | + | + |
| Tannins | + | + |
| Steroids/triterpenoids | + | + |

Key: (+) Positive contain secondary metabolites

The potential of this plant as an antibacterial, antidiarrhoeal, antioxidant, and other pharmacological effects is closely related to the presence of secondary metabolites. Previous studies indicate that *B. balsamifera* contains flavonoids, sesquiterpenes, essential oils, and sesquiterpenoid esters, which are thought to have biological effects such as antibacterial, anticancer, antifungal, and anti-inflammatory properties.[42] Previous research reported that the EtOH and ethyl acetate extracts of *B. balsamifera* leaves contained tannins, steroids, phenolics, flavonoids, and saponins.[32] The leaves of *B. balsamifera* can be used as an alternative treatment option. The saponin compounds, which contain esterified trisaccharide groups in *B. balsamifera*, exhibit strong antimicrobial activity. Saponins generally cause pore formation and loss of membrane integrity, inducing permeabilisation and affecting membrane fluidity. Terpenoid compounds with aldehyde groups can interact with proteins incorporated in the membrane, altering membrane conformation and function.[43] The presence of flavonoid compounds indicates potential antidiarrhoeal properties through antibacterial, anti-inflammatory, and antioxidant activities. Flavonoids' anti-inflammatory activity as an antidiarrhoeal drug involves decreasing abdominal cavity capillary permeability and inflammatory cytokine levels, such as IL-6, IL-12, and TNF-$\alpha$.[1] As antioxidants, flavonoids play a role in neutralising free radicals by donating hydrogen atoms, which helps mitigate oxidative stress in the intestinal fluids and mucosa, thereby preventing intestinal inflammation.[44] As antibacterial agents, flavonoids bind to bacterial membrane phospholipids, disrupting proton motive force and metabolism. Additionally, prenyl group-containing flavonoids with hydrophobic substituents inhibit the formation of biofilms and the function of bacterial membranes.[45]The results of the *in vitro* antidiarrhoeal test were conducted by evaluating the antibacterial activity against diarrhoea-causing bacteria, as shown in Table 3 and Figures 1 and 2. The EtOH extract of *B. balsamifera* leaves

demonstrated strong antibacterial potential against diarrhoea-causing microorganisms,

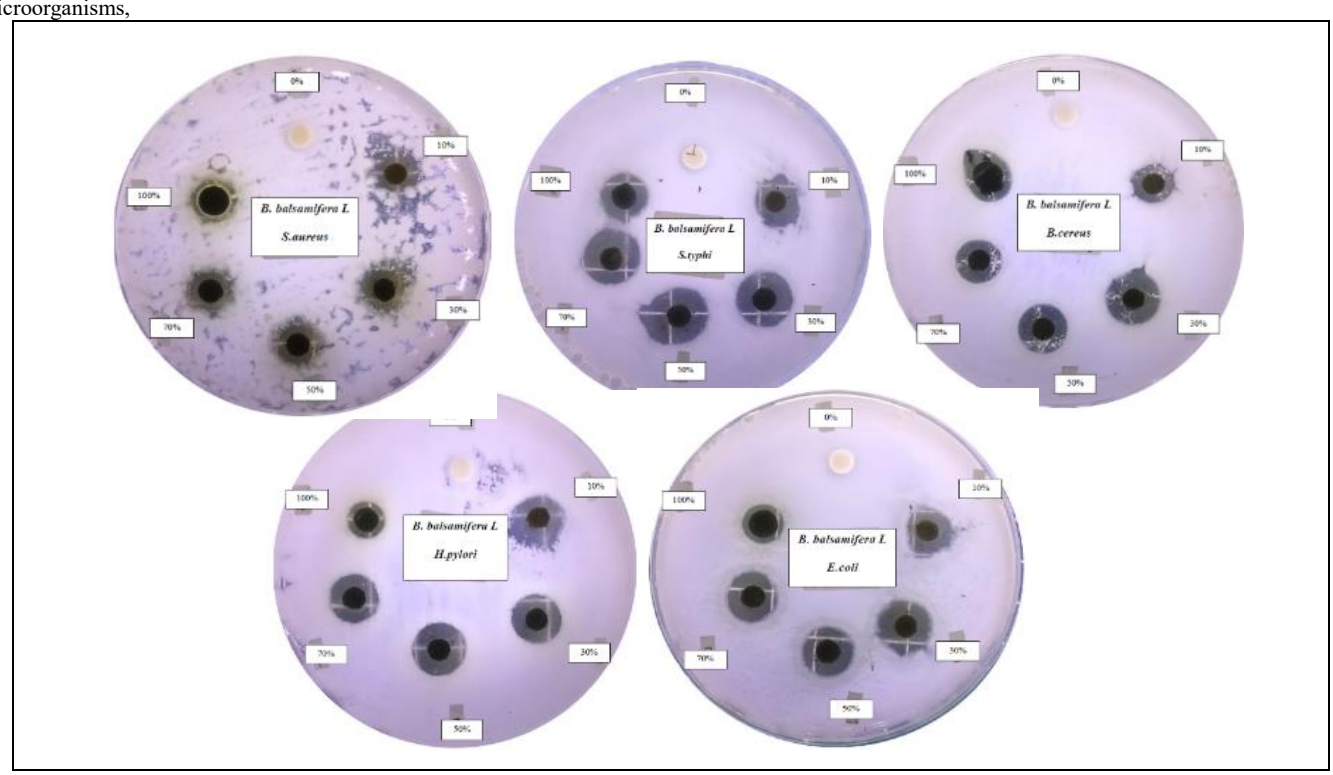


**Figure 1:** Antibacterial activity of EtOH extract of *Blumea balsamifera* showing the diameter of inhibition zone

as indicated by the inhibition zone diameter at each concentration (10-100%), with values exceeding 10 mm for all tested bacteria. Several variables, including the antibacterial agent, bacterial type, extract concentration, and extract diffusion power, can influence the antibacterial activity of a compound. Alkaloids, flavonoids, steroids, and cardiac glycosides identified in *B. balsamifera* leaves have been shown in previous studies to have broad antibacterial action against both Gram-positive and Gram-negative bacteria.[18] The MIC of *B. balsamifera* essential oil at 150 ppm inhibited *Bacillus cereus*, *S. aureus*, and *Candida albicans*. Additionally, the ethyl acetate extract of *B. balsamifera* leaves exhibited antibacterial activity against *Pseudomonas aeruginosa* (9.0 mm) and *Staphylococcus aureus* (9.2 mm). Secondary metabolites including tannins, alkaloids, flavonoids, saponins, and steroids/triterpenoids have been shown to have antibacterial effects.[46]

**Table 3:** *In Vitro* Antidiarrheal Activity of EtOH extract of *Blumea balsamifera*

| Concentration (%) | Inhibition Zone Diameter (mm) | | | | |
|---|---|---|---|---|---|
| | ***Staphylococcus aureus*** | ***Salmonella typhi*** | ***Bacillus cereus*** | ***Helicobacter pylori*** | ***Escherichia coli*** |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 11.30±0.53 | 14.4±3.80 | 14.51±2.66 | 14.46±4.72 | 11.35±1.10 |
| 30 | 12.35±0.90 | 16.41±0.59 | 15.93±1.13 | 14.90±1.91 | 13.26±1.38 |
| 50 | 12.63±0.83 | 15.38±0.87 | 14.70±1.60 | 14.20±1.26 | 12.63±0.43 |
| 70 | 12.01±0.98 | 14.16±0.84 | 13.91±1.29 | 13.25±1.01 | 13.23±1.53 |
| 100 | 10.61±0.33 | 12.38±0.51 | 15.21±2.14 | 10.75±0.61 | 10.95±0.78 |

Phenolic compounds (flavonoids, tannins) exhibit antimicrobial activity through mechanisms such as inhibition of enzymes, adhesion binding, binding of protein, lack of substrate, complexation of metal ions, membrane damage, and DNA interaction.[15] Additionally, the antimicrobial potential of plant secondary metabolites may be influenced by factors such as target cell features (bacteria/fungi, Gram-positive/Gram-negative bacteria), environmental conditions for antimicrobial action, concentration, temperature, and pH.[47] The antioxidant test aimed to determine the inhibitory capacity (% inhibition) of the EtOH extract of *B. balsamifera* leaves against DPPH and to calculate the $IC_{50}$ value. The results of the antioxidant activity test are shown in Table 4 and Figure 3 and 4 illustrates that the percentage of inhibition increases as the concentration of the EtOH extract of *B. balsamifera* leaves increases. Based on the results of the antioxidant activity test, the EtOH extract of *B. balsamifera* leaves showed a moderate $IC_{50}$ value of 117.560 ppm, which is similar to previous findings where the n-hexane extract of *B. balsamifera* leaves showed an $IC_{50}$ of 113.716 ppm.[18] The MeOH extract of *B. balsamifera*

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leaves had an $IC_{50}$ of 105.1 ppm.[18] This is due to the presence of secondary metabolites including flavonoids, in the EtOH extract which can serve as antioxidants. The pharmacological effects of flavonoids include antibacterial, anti-inflammatory, and antioxidant effects.

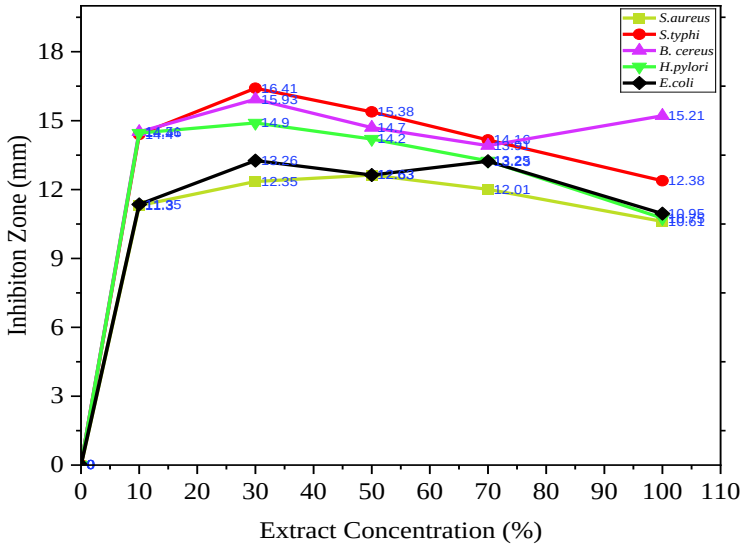
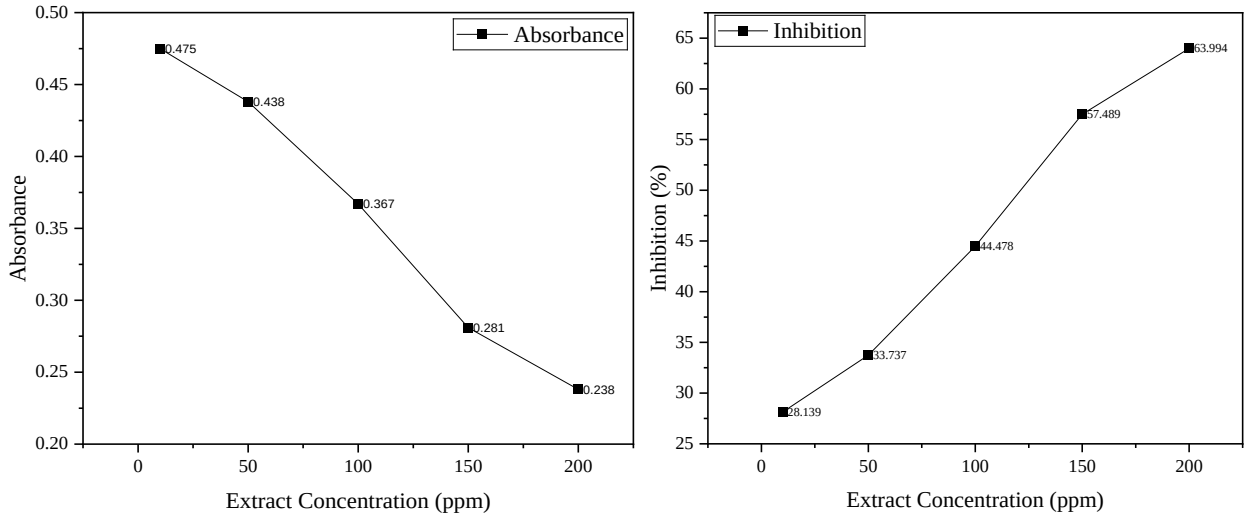



**Figure 2:** Inhibition Zone Diameter Graph for Ethanol Extract of *Blumea balsamifera* (L.) DC



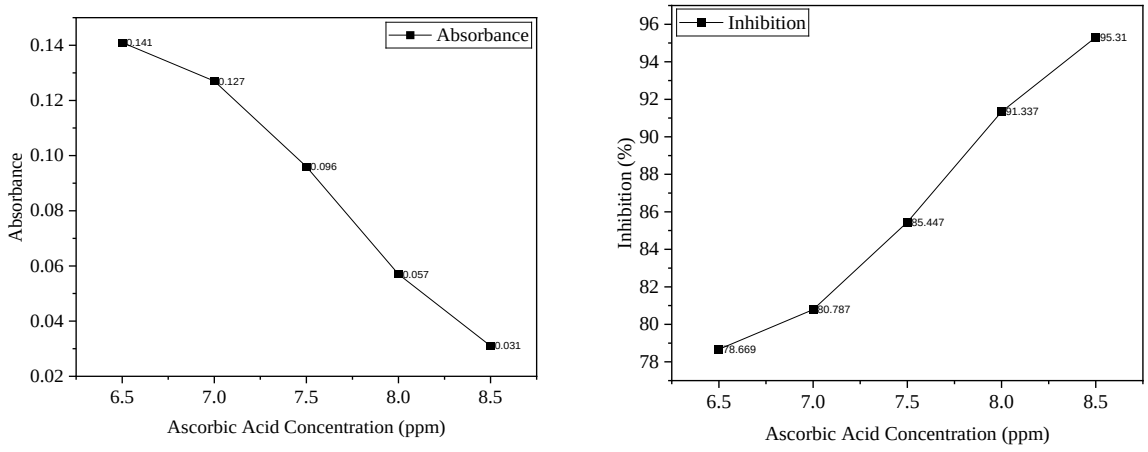



**Figure 3:** Absorbance and Inhibition Percentage of Ethanol Extract from *Blumea balsamifera* (L.) DC. and Ascorbic Acid

**Table 4:** Antioxidant Activity of *Blumea balsamifera* (L.) DC

| Concentration (ppm) | Blanc Absorbance | Sample Absorbance | % Inhibition | Regression Equations | $IC_{50}$ (ppm) |
|---|---|---|---|---|---|
| *Blumea balsamifera* leaf extract | | | | | |
| 10 | 0.661 | 0.475 | 28.139 | Y= 0.2205 X + 24.078 | 117.560 |
| 50 | 0.661 | 0.438 | 33.737 | | |
| 100 | 0.661 | 0.367 | 44.478 | | |
| 150 | 0.661 | 0.281 | 57.489 | | |
| 200 | 0.661 | 0.238 | 63.994 | | |
| Ascorbic Acid | | | | | |
| 6.5 | 0.661 | 0.141 | 78.669 | Y = 8.7664 X + 20.562 | 3.358 |
| 7 | 0.661 | 0.127 | 80.787 | | |
| 7.5 | 0.661 | 0.096 | 85.447 | | |
| 8 | 0.661 | 0.057 | 91.337 | | |
| 8.5 | 0.661 | 0.031 | 95.310 | | |

The antioxidant mechanism of flavonoids involves chelating reactive oxygen species and inhibiting enzymes that produce superoxide anions, preventing the development of alkoxyl and peroxyl radicals[48,49], and inhibiting lipid peroxidation.[50] The high tetradecanal (myristylaldehyde) content in the essential oil from mature *B. balsamifera* leaves contributes to strong anti-radical activity.[51]

Ascorbic acid exhibits very high antioxidant activity.[52] Monodehydroascorbate is produced using ascorbic acid as a single equivalent donor. The antioxidant activity of ascorbic acid includes the non-enzymatic reduction of superoxide, hydroxyl, alkoxyl, peroxyl and other radicals.[53]

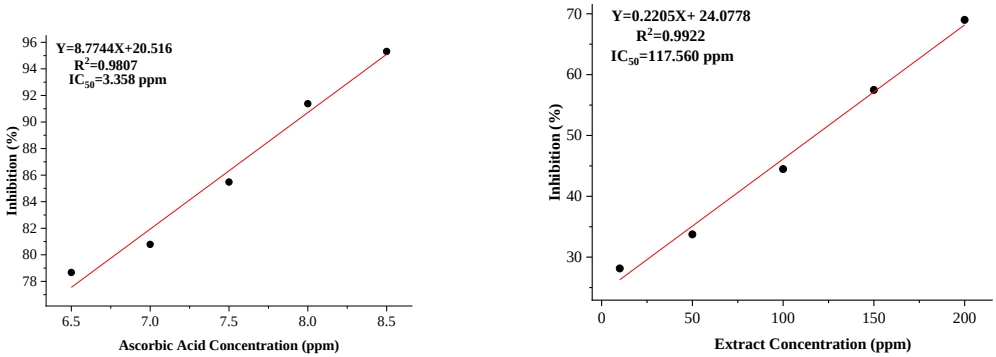



**Figure 4:** Regression Curve of Ethanol Extract from *B. balsamifera* (L) DC and Ascorbic Acid

## Conclusion

The use of *B. balsamifera* leaves as an antidiarrhoeal medicine by ethnic groups in North Sumatra, Indonesia may have been through antibacterial activity against diarrhoea-causing microorganisms, and antioxidant activity in the moderate category. The *in vitro* antidiarrhoeal activity of the extract may be related to the presence of chemical compounds revealed by GC-MS analysis. EtOH extracts from *B. balsamifera* leaves have the potential as a candidate for diarrhoea medicine. Further research is needed for the treatment of acute diarrhoea and chronic diarrhoea.

## Conflict of Interest

The authors declare no conflicts of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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