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EVALUATION OF THE PHYTOCHEMICAL COMPOSITION AND ANTIBACTERIAL POTENTIAL OF ETHANOLIC AND AQUEOUS *BRYOPHYLLUM PINNATUM* (MIRACLE LEAF) EXTRACTS AGAINST FOOD-BORNE PATHOGENS

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Abstract

Bryophyllum pinnatum is used in the treatment of different ailments. The present study investigated the phytochemical composition of *Bryophyllum pinnatum* and evaluated its antibacterial activities against some selected food-borne pathogens. Extracts were obtained using ethanol, and water as solvents and the phytochemical composition was qualitatively determined using conventional methods. The antibacterial activities were done using the tetrazolium microplate assay method and agar well diffusion method. Seven bioactive phytochemical compounds were identified in the extracts. Alkaloids and flavonoids (+++) were the dominant compounds in the ethanolic extracts while tannin (+++) was dominant in the aqueous extracts. The extracts exhibited significant antibacterial activity against some selected food-borne pathogens. The results of this study show that *Bryophyllum pinnatum* possesses some antibacterial properties and can be used to prevent and treat infections caused by sensitive bacteria.

Keywords: Extract; Phytochemical Composition; Inhibitory Concentration; Bactericidal Concentration.

Introduction

Using plants and parts to treat and control disease is as ancient as mankind. Among the innumerable plant types on earth that cut across different continents and peoples, some are selected thanks to their potential to alleviate maladies in humans and are, as such, graded as medicinal plants. Some plants have wide ethnobotanical applications and are used by people of diverse ethnicities for different medications.

Nigeria, like many sub-Saharan tropical countries in Africa, is endowed with an almost unlimited number of useful plants with medicinal properties. In the various cultural communities of the country, several plant leaves, stem bark, roots, etc are employed in medications according to tradition and with surprisingly positive results. One such useful

plant is the *Kanachoeppinnata* family called *Bryophyllum pinnatum*. It is a succulent herbaceous plant in the family *Crassulaceae* and has its native origin in Madagascar (Southern African Indian Ocean Island). Presently, the plant is found adapted to all tropical and subtropical zones of different continents

The use of *Bryophyllum pinnatum* in ethnomedicines for treating diseases including colds, abscesses, insect bites, skin disorders, asthma, and hypertension has been reported (Nagaratna & Prakash, 2015). In Igboland of South East Nigeria, the plant is used for the treatment of cough and in the post-partition care of the umbilical cord in newborns. Given the testimonial reports of the plant, the possibility of its extracts being effective against a wide range of microorganisms associated with food contamination, spoilage, and intoxication appears to have a positive odd.

A wide variety of health issues can be caused by food-borne bacteria pathogens ranging from short-lived ailments like fevers to severe cases like invasion of the bloodstream with life-threatening infections and organ damage. Presently, foodborne diseases have become an increasing public health concern globally, and many bacteria species are involved (Santos, *et al.*, 2023). It has also been reported that foodborne diseases constitute a significant major cause of hospitalization and death worldwide. There are reports of increasing emergence and spread of antibiotic-resistant strains of the causative microorganisms. This sustains the challenge of overcoming the development of antibiotic-resistant strains of bacteria against

existing drugs hitherto used for their control (Awala *et al.*, 2017).

The emergence of these resistant strains has led to the search for alternative antibacterial agents. Researchers have always agreed that plant extracts are useful as antimicrobials (Ildiz *et al.*, 2018; Al *et al.*, 2022). Medicinal plants are reported to contain a wide variety of phytochemicals with therapeutic properties that can be exploited in treating and managing many human diseases (Tyagi *et al.*, 2017; Wong *et al.*, 2022; Kumar *et al.*, 2023). Again, research findings agree that phytochemicals like alkaloids, tannins, saponins, flavonoids, phenols, essential oils, etc act as medicinal agents in plants and thus, confer on them (the plants) antimicrobial and other activities against disease-causing microorganisms (Ivan *et al.*, 2018; Aggarwal *et al.*, 2021). In most spheres, plants are considered an unlimited source of bioactive components and their use as antimicrobials is being strongly exploited.

According to Anyanwu and Okoye (2017), resistance of antibiotics by foodborne pathogens has been on the increase and has become a global dilemma as one of the significant threats facing humanity. This is believed to be due to the continued proliferation of multi-drug resistant strains of pathogens partly occasioned by abuse and misuse of available antimicrobial drugs some of which have become ineffective while others are associated with dangerous side effects to some individuals. The need for alternative antimicrobial agents especially those of biological sources, is not in doubt. Plant-based antimicrobials have become more needful as their use represents the development of a safe

alternative to synthetic drugs and an effective way to handle the problem of antimicrobial resistance of pathogens. It is because of the foregoing that this research was designed to study the antibacterial properties of this common readily available herbaceous plant, *Bryophyllum pinnatum*.

Materials and Methods

Collection and Identification of Plant Specimen

The plant *Bryophyllum pinnatum* (Miracle leaf) was randomly picked from the farm of Michael Okpara University of Agriculture, Umudike, South East Nigeria. The plant specimen was identified in the Forestry Department of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Preparation of Plant Materials

The plant specimen was air-dried at ambient temperature for three days and pulverized into a fine powder in an electric blender (SONIK R, Japan).

Preparation of Aqueous Extracts

Aqueous extraction was carried out using the modified maceration technique (Remington, 2000). The powdered plant specimen (50 g) was macerated using 150 mL of distilled water for 3 h with intermittent stirring. This was followed by vacuum filtration with Whatman (No. 1) filter paper placed in a Buchner funnel. The extraction process was conducted twice on the residue using 100 mL followed by 50 mL of water at the second and third extraction, respectively. The portions of aqueous extracts were combined in

small conical flasks, sealed with parafilm and stored frozen at -4 °C before drying. The portions were dried to completion in a hot air oven (SELLECTA CE 0505, USA) at 45°C under constant monitoring. The extract was tightly sealed with two layers of parafilm and placed in a desiccator to avoid the extracts picking up moisture. The extract was stored at 4°C until used for experiments.

Preparation of Ethanol Extracts

The ethanol extract was prepared using the same process (Remington, 2000). The powdered plant specimen (50 g) was extracted using 150 mL of the solvent in a foil-sealed flask for 3 h with intermittent shaking. This was followed by vacuum filtration with Whatman (No. 1) filter paper placed in a Buchner funnel. The extraction process was conducted twice on the residue using 100 mL followed by 50 mL of the solvent at the second and third extraction, respectively. The filtrates were concentrated in an oven maintained at 50°C, then transferred to a glass petri dish and dried in a vacuum oven at 60°C for 24 h. The extract was kept in sealed vials at 4°C.

Phytochemical Analysis of the Plant Extract

Screening for qualitative preparation of phytochemicals in the test plant extract was done with conventional methods (Osugwu *et al.*, 2014; Ekwenye & Okorie, 2010).

Test for tannin

The tannin chloride test was used (Ekwenye & Okorie, 2010). In a test tube, about 2 mL of each extract was measured out and mixed with an

equal volume of distilled water. Drops of acidified ferric chloride were added to the mixture and mixed well. The development of dark (Greenish-black) precipitates gave a positive result. A dilute tannic acid solution was used as a positive standard for the test.

Test for Alkaloid

Alkaloids in the plant extract were tested with Meyers extract and Hagler's reagent. In each case, the extract of about 2 mL was mixed with drops of the test reagent in a test tube, and monitored for the formation of an orange-brown precipitate as positive results (Ekwenye & Okorie, 2010).

Test for Steroids and Triterpenes

This was carried out by Dragendorf's test (Osuagwu *et al.*, 2014). A sample of each extract, about 2ml was dispensed into a clean, dry test tube, and mixed with 1mL acetic anhydride. The tube was kept in ice for several minutes before 1 mL of concentrated sulphuric acid was carefully added to the tube by the side to form a layer below the extract. Extra caution was exercised as the reaction was very violent. The positive result was obtained when a steroidal ring, brownish colour was formed at the interphase of the acid and the extract in the test tube.

Test for Saponin

The froth formation test was used. Two (2) mL of the extract was added to 5 mL of distilled water in a test tube. The mixture was shaken vigorously and monitored for the formation of a stable froth (foam). The development of a stable

froth (foam) indicates a positive result while the non-formation of froth gives a negative result (Osuagwu *et al.*, 2014).

Test for Flavonoid

The alkaline acid test was used (Ekwenye & Okorie, 2010). About 2 mL of each extract was dispensed into a test tube, followed by the addition of drops of bench ammonia solution. The development of yellow colouration which cleared with the addition of bench hydrochloric acid solution gave a positive result.

Test for Cyanogenic Glycoside (HCN)

The method involving alkaline picrate (Egbuna *et al.*, 2018) was used. About 20 mL of distilled water was added to a test tube and a strip of alkaline picrate paper was hung over the mixture in the test tube. Caution was exercised so as to avoid contact between the paper strip and the extract solution. The arrangement was allowed to stand for 24 h while the strip was held in place with the stopper used to cover the test tube. The development of orange colour or brick red indicated the presence of cyanogenic glycoside.

Culture Preparation

Typed bacterial cultures were obtained from the Diagnostic Centre of National Veterinary Research Institute, Vom, Plateau State, Nigeria. The strains were *Staphylococcus aureus* ATCC 12340, *Bacillus subtilis* ATCC 12430, *Escherichia coli* ATCC 12530 and *Pseudomonas aeruginosa* ATCC 10250. Glycerol stock cultures of each organism were prepared and kept at -4 °C prior to use. The strains were reactivated on sterile Tryptone Soy

Agar (Oxoid, UK) and incubated at 37°C for 18 h. Selective media were employed to verify their identity. After incubation, the organisms were transferred into sterile Tryptone Soy Broth (TSB) and incubated overnight at 37°C. In order, to standardize the overnight culture to a concentration of 1.0×10^6 CFU/mL, TSB was used to dilute the overnight cultures to obtain an absorbance (OD_{590 nm}) of 0.02 for all bacteria (Sandasi *et al.*, 2008).

Antimicrobial Activity Test

The sensitivity of the test organism to the ethanolic extract of the leaves of *Bryophyllum pinnatum* was carried out using the diffusion method of Daoud *et al.* (2019). Twenty (20) mL of molten nutrient agar was seeded with 0.2 mL of broth culture of standardized test organism in a sterile Petri dish. The petri dish was rotated slowly to ensure a uniform distribution of the organisms. They were left to solidify and wells of 8.0 mm diameter were made in the agar with a sterile cork borer. Then, 0.1 ml of the extracts of different concentrations was used to fill each well. The petri dish was allowed to stand for approximately 30 min at $28 \pm 2^\circ\text{C}$ to enable the proper diffusion of the extracts. The plates were then incubated at 37°C for 24 h. The zone of inhibition in millimeters was measured and recorded.

Determination of Minimum Inhibitory Concentration and the Minimum Bactericidal Concentration

The lowest inhibitory concentration of each extract was assessed using the tetrazolium microplate assay described by Ohaegbu *et al.*

(2022) with slight modifications. The assay was conducted using round-bottomed polystyrene 96-well clear microtitre plates with a standard plate layout. Briefly, each extract was reconstituted in 0.5% dimethyl sulfoxide (DMSO), and different concentrations of 25 – 250 mg/mL were made. Following the serial dilutions, 100 µL of the standard culture (1.0×10^6 CFU/ mL) was then added to all the wells. The plates were sterile-sealed and incubated for 24 h at 37°C. The MIC was detected following the addition of 50 µL of 0.2 mg/mL of INT (2-4-iodophenyl-3-4-nitrophenyl-5-phenyl-2H-tetrazolium chloride) in all the wells and incubating for a further 30 min at 37°C. Bacterial growth was assessed by examining the colour change of INT in the microplate wells. Biologically active bacterial cells reduce the colourless tetrazolium salt which acts as an electron acceptor to a red-coloured formazan product (Berridge *et al.*, 2005). When the solution in the well became clear after incubation with INT, the inhibition of bacterial growth was observed. The MIC is defined as the lowest extract concentration that completely suppresses the growth of microorganisms and it is indicated by the first clear well in the column. For the measurement of minimum bactericidal concentration (MBC), 20 µL of culture medium was taken from the microtitre plate wells that displayed no changes in colour were re-inoculated on Mueller Hinton (MH) agar plates. After incubating for 24 h at 37°C, the MBC was identified as the lowest concentration that displayed no bacterial growth on MH agar plates. The MIC and MBC determinations were carried out in duplicate. The positive and negative

controls are Gentamycin (V.S. International Pvt Ltd. India) and TSB (Oxoid, UK) respectively.

Statistical Analysis

Single-factor analysis of variance (ANOVA) test was used to evaluate significant differences between the mean absorbance values obtained without the extracts (controls) and those

obtained with the different extracts used. Differences were considered statistically significant at $P < 0.05$. The means were separated using Duncan's Multiple Range Test. The statistical variables were evaluated using the International Business Machines (IBM) Statistical Software for Social Sciences 16.0 (IBM, USA) for Windows software.

Results

Detected Phytochemicals in the Test Plant *Bryophyllum pinnatum*

The result shows variations in the level of detected phytochemicals in the different extracts, water, and ethanol. Alkaloids and flavonoids were detected at high levels (+++) in the ethanol but were moderate (++) in the aqueous solution. Saponin and Cyanogenic glycoside (HCN) were

both moderate (++) in the aqueous extract but low (+) in the ethanolic extract of the plant.

On the other hand, Tannin was detected at a high level (+++) in the aqueous solution but was moderate (++) in the ethanol extract but the level of detection of steroids was moderate in both extracts (++) . In all, the plant contains many different phytochemicals at varying levels of detection.

Table 1: Qualitative Screening of Phytochemicals in *Bryophyllum pinnatum* Extracts

| Nature of extract | Alkaloid | Saponin | Flavonoid | Tannin | Steroids and triterpenes | Glycoside HCN |
|-------------------|----------|---------|-----------|--------|--------------------------|---------------|
| Water extract | ++ | ++ | ++ | +++ | ++ | ++ |
| Ethanol extract | +++ | + | +++ | ++ | ++ | + |

Key : + = Detected at low level, ++ = Detected at moderate level, +++ = Detected at high level

The mean inhibition diameters of extracts from *Bryophyllum pinnatum* and standard antibiotics is shown in Table 2 and 3. The results show varying levels of antibacterial

properties of the extracts against the different food-borne bacteria pathogens. Generally, the results indicate that the ethanolic extracts of the plant are more potent as an antimicrobial agent than the water extract while both extracts were

less potent than the standard antibiotic. However, there were variations in their respective activity against the different pathogens.

The aqueous solution at its highest concentration caused inhibitions with diameters ranging from 10.33 mm to 11.67 mm. *Staphylococcus aureus* ATCC 12340, and *Bacillus subtilis* ATCC 12430, were inhibited with diameters of 10.33mm and 10.63mm respectively by the water extract while *E. coli* ATCC 12530, and *Pseudomonas aeruginosa* ATCC 10250 were inhibited with diameters of 11.54mm, and 11.67mm, respectively (Table 2).

The antimicrobial properties of the ethanolic extract of *Bryophyllum pinnatum* ranges from 15.33mm (*Staphylococcus aureus*) to 20.33mm (*Pseudomonas aeruginosa*). The mean diameter of the inhibition of the highest concentration of ethanol extract against the food-borne pathogens was in the order of *Staphylococcus aureus* (15.33mm) < *Bacillus subtilis* (17.00mm) < *E. coli* (19.67mm) < *Pseudomonas aeruginosa* (20.33mm) whereas the performance of the two plant extracts was lower compared to that of the standard drug.

Table 2: Antimicrobial Activity of *Bryophyllum pinnatum* Aqueous Extracts against Food-borne Pathogens

| Test organisms | Conc.(mg/mL) | | of zone 150 | of inhibition 100 | (mm) | |
|---------------------------------|--------------|------|----------------|----------------------|------|---------|
| | 250 | 200 | | | 50 | Control |
| <i>S. aureus</i> ATCC 12340 | 10.33 | 8.33 | 4.93 | NA | NA | 23.67 |
| | ± | ± | ± | | | ± |
| | 1.16 | 1.12 | 1.16 | | | 2.52 |
| <i>B. subtilis</i> ATCC 12430 | 10.63 | 7.13 | 3.83 | NA | NA | 22.67 |
| | ± | ± | ± | | | ± |
| | 0.58 | 0.16 | 1.16 | | | 1.16 |
| <i>E. coli</i> ATCC 12530 | 11.54 | 7.98 | 4.55 | NA | NA | 25.33 |
| | ± | ± | ± | | | ± |
| | 1.53 | 1.16 | 0.16 | | | 1.16 |
| <i>P. aeruginosa</i> ATCC 10250 | 11.67 | 7.82 | 10.33 | NA | NA | 25.33 |
| | ± | ± | ± | | | ± |
| | 0.58 | 0.16 | 1.22 | | | 1.16 |

Values are mean ± standard error of three replicates. NA: no activity, Control= Gentamycin

Table 3: Antimicrobial Activity of *Bryophyllum pinnatum* Ethanol Extracts against Food-borne Pathogens

| Test organisms | Conc. (mg/mL) | | Of zone 150 | Of inhibition 100 | (mm) 50 | Control |
|---------------------------------|---------------|-------|----------------|----------------------|------------|---------|
| | 250 | 200 | | | | |
| <i>S. aureus</i> ATCC 12340 | 15.33 | 12.12 | 9.25 | 5.36 | NA | 23.67 |
| | ± | ± | ± | ± | | ± |
| | 1.16 | 1.12 | 1.16 | 0.16 | | 2.52 |
| <i>B. subtilis</i> ATCC 12430 | 17.00 | 14.25 | 10.20 | 6.02 | NA | 22.67 |
| | ± | ± | ± | ± | | ± |
| | 1.00 | 0.14 | 1.00 | 1.16 | | 1.16 |
| <i>E. coli</i> ATCC 12530 | 19.67 | 14.00 | 10.50 | 7.14 | NA | 25.33 |
| | ± | ± | ± | ± | | ± |
| | 1.16 | 1.22 | 1.00 | 1.44 | | 1.16 |
| <i>P. aeruginosa</i> ATCC 10250 | 20.33 | 15.40 | 11.35 | 6.16 | NA | 25.33 |
| | ± | ± | ± | ± | | ± |
| | 1.16 | 1.00 | 1.42 | 1.20 | | 1.16 |

Values are mean ± standard error of three replicates. NA: no activity, Control= Gentamycin

Minimum inhibitory concentration of *Bryophyllum pinnatum* extracts against the food-borne pathogens

The ethanol extract was the most active against the target organisms with MIC values of 50 and 100 mg/mL. The control (gentamycin) showed inhibitory activity with MIC values of 20 and 25 mg/mL against the gram negative and gram positive organisms respectively. With the aqueous extract, the minimum concentration of 100mg/mL caused inhibition with *E. coli* ATCC 12530 and *Pseudomonas aeruginosa* ATCC 10250 while the least concentration of 150mg/mL initiated inhibition in *Staphylococcus aureus* ATCC 12340 and *Bacillus subtilis* ATCC 12430. Generally, the gram negative organisms recorded a high susceptibility to the extracts than the gram positive organisms as evidenced by its lower MBC values.

Table 4: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the Plant Extract against the Food Borne Pathogens

| Solvent | MIC (mg/mL) | | | | MBC (mg/mL) | | | |
|-----------------|------------------|----------------|----------------------|--------------------|------------------|----------------|----------------------|--------------------|
| | <i>S. aureus</i> | <i>E. coli</i> | <i>P. aeruginosa</i> | <i>B. subtilis</i> | <i>S. aureus</i> | <i>E. coli</i> | <i>P. aeruginosa</i> | <i>B. subtilis</i> |
| Water extract | 150 | 100 | 100 | 150 | 250 | 250 | 250 | 250 |
| Ethanol extract | 100 | 50 | 50 | 100 | 200 | 150 | 150 | 150 |
| Control | 25 | 20 | 20 | 25 | 30 | 25 | 25 | 30 |

Values are means of triplicate experiments. Control = Gentamycin

Discussion

Our study on the phytochemical content and *in vitro* antibacterial activities of aqueous and ethanol *Bryophyllum pinnatum* extracts on selected food-borne pathogens showed that the plant extract is rich in phytochemicals and has the potential to impact health benefits including antimicrobial activity (Daniel *et al.*, 2020; Amala *et al.*, 2021). The observed variations in phytochemical composition between aqueous and ethanolic extracts of *Bryophyllum pinnatum* align with findings from other studies that have highlighted the influence of the extraction solvents on phytochemical profiles (Truong *et al.*, 2019). Due to their differing polarities, ethanol and water selectively extract distinct groups of bioactive compounds, resulting in variations in the phytochemical content.

The high levels (+++) of alkaloids and flavonoids in the ethanolic extract of *B. pinnatum* are consistent with findings by Olaniyi *et al.* (2021), who reported that ethanol, being a polar organic solvent, is effective at extracting alkaloids and flavonoids due to their moderate polarity. These phytochemicals are recognized

for their strong antimicrobial, antioxidant, and anti-inflammatory properties. Chibli *et al.* (2014) similarly observed higher flavonoid content in ethanol extracts in comparison to aqueous extracts, attributing it to ethanol's capacity to penetrate plant cell walls and dissolve lipophilic and hydrophilic compounds. The moderate levels (++) of saponins and cyanogenic glycosides in the aqueous solution and their lower levels (+) in the ethanol extract align with findings by Tanwar *et al.* (2018). These researchers observed that water is more effective at extracting saponins and glycosides due to their hydrophilic nature. Cyanogenic glycosides, although less emphasized in therapeutic applications due to potential toxicity, are still noted for their antimicrobial and anticancer potentials when present in controlled amounts (Tahir *et al.*, 2024). Arunkumar *et al.* (2020) support the high tannin content (+++) in the aqueous solution and its moderate levels (++) in the ethanol extract. They noted that highly polar tannins are more readily extracted by water. Tannins are recognized for their astringent, antioxidant, and antimicrobial

properties, contributing to the therapeutic potential of plant-based preparations.

The moderate levels (++) of steroids in both extracts corroborate findings by Ogungboye *et al.* (2022), who reported that steroids, being amphipathic compounds, can be extracted in comparable amounts by both water and ethanol. Steroids in plants are often associated with anti-inflammatory and hormone-regulating activities, making them valuable in medicinal applications.

The overall phytochemical richness of *B. pinnatum* observed in this study reinforces its therapeutic potential, as documented in previous studies. Sharma *et al.* (2024) highlighted that the diversity and abundance of bioactive compounds in *B. pinnatum* make it a valuable source for developing natural remedies for various ailments. The variation in the levels of phytochemicals across extracts suggests that the choice of the solvent can be tailored to specific therapeutic needs. Several studies have linked the phytochemicals detected in *B. pinnatum* to significant health benefits. Alkaloids and flavonoids are associated with antimicrobial, anti-inflammatory, and anticancer properties (Sudevan *et al.*, 2019). Saponins are recognized for their cholesterol-lowering and immune-boosting effects, while tannins have been reported to exhibit strong antioxidant activity (Soldado *et al.*, 2021). The variations in phytochemical composition across aqueous and ethanolic extracts underscore the importance of solvent selection in maximizing the therapeutic potential of plant extracts. The findings are consistent with existing literature and highlight

the potential of *B. pinnatum* as a rich source of bioactive compounds.

Our findings on the antibacterial properties of *B. pinnatum* align with existing research on the antibacterial characteristics of *Bryophyllum pinnatum* extracts, highlighting the plant's potential as an antimicrobial agent. In this study, the ethanolic extract of *B. pinnatum* showed superior antibacterial activity against the experimental organisms compared to the aqueous extracts. In agreement with our findings, Akinyele *et al.* (2021) demonstrated that ethanolic extracts of *B. pinnatum* exhibited larger inhibitory zones against Gram-negative bacteria like *E. coli* and *Pseudomonas aeruginosa*, as observed in the current study (19.67mm and 20.33mm, respectively). The high efficacy of ethanol extracts is often attributed to their ability to extract more bioactive compounds, such as alkaloids, flavonoids, and saponins, which are potent antimicrobial agents.

The lower antibacterial activity of the water extract at the highest concentration (10.33mm to 12.33mm) against pathogens like *Staphylococcus aureus* ATCC 12340 and *Bacillus subtilis* ATCC 12430 is consistent with findings by Olaniyi *et al.* (2020). Their study noted that the limited spectrum of antimicrobial activity of aqueous extracts may be due to the reduced solubility of certain bioactive phytochemicals in water.

The current study also reveals the varying susceptibility of pathogens to the plant extracts. *Pseudomonas aeruginosa* exhibited the highest

susceptibility to the ethanol extract (20.33mm at the highest concentration), corroborating reports by Chibli *et al.* (2019), which emphasized the vulnerability of Gram-negative bacteria with less rigid peptidoglycan structures to plant-derived compounds. Conversely, the relatively lower activity of the extracts against *Staphylococcus aureus* (15.33mm with ethanol extract and 10.33mm with water extract at the highest concentration) is supported by Adegboye *et al.* (2022), who suggested that the dense peptidoglycan layer in Gram-positive bacteria can inhibit the diffusion of bioactive compounds.

In comparison to the standard antibiotic, the plant extracts displayed significant but lower antimicrobial activity (inhibition diameters of 22.67mm to 25.33mm). This aligns with findings by Swetha *et al.* (2022), who reported that while plant extracts demonstrate notable antimicrobial potential, their efficacy often lags behind synthetic antibiotics, likely due to lower concentrations of active compounds and possible synergistic effects in the latter.

The order of inhibition by the ethanol extract (*Staphylococcus aureus* < *Bacillus subtilis* < *E. coli* < *Pseudomonas aeruginosa*) suggests pathogen-specific differences in susceptibility, a pattern that has been observed in other studies. For instance, the work by Arunkumar and Rajasekaran (2020) highlighted similar orders of susceptibility among food-borne pathogens, indicating consistent behavior across studies. These results reinforce the antimicrobial potential of *B. pinnatum*, especially when ethanol is used as the extraction solvent.

The inhibitory concentrations of *Bryophyllum pinnatum* extract against food-borne pathogens observed in this study align with previous research that emphasizes the plant's antimicrobial potential. The current findings, which highlight the ethanol extract's superior activity at lower minimum inhibitory concentrations (MIC) compared to the water extract, are consistent with results from other studies. Itaman *et al.* (2022) reported similar MIC values, where ethanol extracts of *B. pinnatum* were more effective in inhibiting microbial growth than aqueous extracts. Their study attributed this difference to the ethanol extract's ability to solubilize a broader range of phytochemicals, including flavonoids, phenols, and alkaloids, which are well-known antimicrobial agents. These compounds are often poorly soluble in water, limiting the efficacy of aqueous extracts.

The MIC values observed for the ethanol extract (50mg/ml for *E. coli* and *Pseudomonas*) corroborate findings by Chandra *et al.* (2017), who noted that Gram-negative bacteria are often more susceptible to plant-based antimicrobial agents. The relatively low MIC for these bacteria may be due to ethanol's effectiveness in extracting compounds that disrupt the lipid-rich outer membrane characteristic of Gram-negative pathogens.

Conversely, the higher MIC of the water extract (150mg/ml) for most pathogens reflects findings from Oulahal, and Degraeve, (2022), who demonstrated that water extracts generally require higher concentrations to achieve

comparable antimicrobial effects. This reduced potency could stem from the lower concentration of active compounds in water-based preparations and the limited bioavailability of these compounds.

Conclusion

Natural products serve as potential alternatives to chemical disinfectants, providing consumers with safer options and a wider selection. The results of this study support the traditional use of *Bryophyllum pinnatum* leaves in treating bacterial infections. Therefore, we conclude that *B. pinnatum* exhibits antibacterial properties and may serve as a potential source of active preservatives for food and pharmaceutical applications.

Recommendations

1. Further investigations focusing on the isolation and identification of active phytochemicals and their synergistic interactions could pave the way for the development of plant-based antimicrobial agents.
2. Future studies could also optimize extraction methods to enhance their efficacy for pharmaceutical applications.

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Conflicts of Interest

The authors declare no conflict of interest.

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Author Contributions

All authors conceptualized the study. CGO and CAN were responsible for the methodology, analysis, data curation, and manuscript preparation. Review and editing of the manuscript were done by CGO and CVA but all authors agreed on the content before submission.

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