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Article

Antibacterial activity by *Dombeya wallichii* plant extracts obtained by ultrasound-assisted extraction

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SUMMARY

The global health community is concerned about the establishment and spread of antibiotic resistance as well as the evolution of new strains of disease-causing bacteria. The creation of alternatives to antibiotics is required for effective treatment. Medicinal plants could be a good source of medication to combat this problem. *Dombeya wallichii*, which is commonly called Pink Ball Tree in the family *Sterculiaceae*, has been documented to have medicinal potential. This species of Dombeya has been studied for a wide range of medicinal properties, including antimicrobial activity. Considering their vast potential, we hypothesized that the extracts of *D. wallichii* would have broad-spectrum antibacterial properties. We also hypothesized that ultrasonic-assisted extraction could also improve the separation and extraction of these medicinal compounds. Here, we screened the antibacterial potential of bark, flower, stem, and leaf extracts against five strains of food spoilage-causing bacteria. Our results indicated that the extraction yield was effectively increased by employing the ultrasonic-assisted approach. We observed the highest antibacterial activity in the stem extracts, followed by leaf and bark extracts. The extracts were more effective against tested Gram-positive bacteria when compared with Gram-negative strains. Hence, these extracts had a narrow spectrum of antibacterial activity and were comparatively less potent than most of the broad-spectrum antibiotics. Further research for potential therapeutic applications should be done in order to better understand the antibacterial activity of *D. wallichii*. This knowledge may help design future antimicrobial compounds.

INTRODUCTION

Antibacterial agents are essential to reducing the global burden of infectious diseases. However, the emergence and spread of multi-drug-resistant bacteria have become a major public health threat because effective antibacterial agents are becoming less and less effective against pathogens, or not effective at all (1). Therefore, finding novel antibacterial medications is critical, especially given the evidence that drug-resistant clinical isolates are rapidly spreading over the world (1). Foodborne disease is another prevalent food safety issue produced by the ingestion of contaminated food products, and it has long been a major public health concern (2, 3). Even in developed countries, food deterioration caused by microbes continues to damage all sorts of food, resulting in food waste and loss. Annual food losses worldwide are estimated to be as high as 40% due to a variety of factors, including spoilage by microbes (4). When these microbes reach food, they use the nutrients to proliferate and make metabolites that cause food to spoil (1). Foodborne bacteria can be transmitted to people by contaminated food and can cause infection. *Salmonella typhi*, *Escherichia coli*, and *Pseudomonas aeruginosa* are Gram-negative bacteria that are the most frequent causes of food poisoning (5). Aside from *Bacillus cereus* and *Staphylococcus aureus*, other Gram-positive bacteria have also been linked to food poisoning and food spoilage (6).

Gram-positive and Gram-negative bacteria are two broad categories of bacteria that are distinguished by differences in their cell wall structure and composition, which can be visualized using a laboratory staining technique called the Gram stain. Gram-positive bacteria have a thick peptidoglycan layer in their cell walls, which retains the crystal violet stain used in the Gram staining process. They also have a relatively simple cell wall structure with no outer membrane (7). Gram-negative bacteria, on the other hand, have a thinner peptidoglycan layer in their cell walls, which does not retain the crystal violet stain. They also have an outer membrane containing lipopolysaccharides, which can contribute to their pathogenicity (7). These bacteria have developed resistance genes against the existing antibiotics as a result of their widespread usage and overuse (8). Because some antibiotics are reported to have negative side effects such as nausea, bone marrow depression, thrombocytopenia, and agranulocytosis, medicinal plants are being investigated as potential new sources of antimicrobial agents with novel mechanisms of action and fewer side effects (9, 10). Also, plant extracts may have unique mechanisms of action that differ from conventional antibiotics and may be effective against bacteria that are resistant to multiple classes of antibiotics (10).

The World Health Organization (WHO) states that the best place to get a range of medications is from medicinal plants (11). A large variety of medicinal plants have been employed for their antibacterial properties, which are due to the phytochemicals produced by the plants. Numerous phytotherapy sources have described different medicinal plants for treating infectious diseases, including skin infections, respiratory ailments, gastrointestinal problems, and urinary tract infections (12, 13). These medicinal plants are employed in herbal treatments either as a single plant or as a combination of different plant species (14). As a result, plants could serve as an alternative to antibiotics, which might be useful in combating antibiotic resistance. Because of their long history of usage in medicine, medicinal plants are thought

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to be safe as they are generally free from undesirable side effects (15). Additionally, research has been conducted on the antibacterial potential of many natural compounds extracted from medicinal plants against foodborne pathogens (16, 17). Considering the enormous potential of plants as sources of antimicrobial agents, the present study sought to examine the in vitro antibacterial activity of extracts from the unexplored medicinal plant *Dombeya wallichii* against the most prevalent bacteria causing food spoilage.

We selected *D. wallichii*, for which the antibacterial potential has not been previously explored, for use in this study based on indigenous knowledge and its use in traditional medicine (18). *D. wallichii* is traditionally used for medicinal purposes such as nausea, ulcers, stomach pain, and diarrhea (18). However, *D. wallichii* is an invasive plant species in India, so there have been few notable research investigations on it in the past.

Plant species belonging to the *Dombeya* genus have been reported to have moderate antibacterial activities against common food pathogens and other bacteria, but there is very little scientific information on *D. wallichii* being used for modern medicinal purposes (19). The extracts of these plants have shown moderate activity against some diarrhea-causing organisms, but studies did not explore the most common food spoilage bacteria (19). Many of these studies focused on traditional extraction techniques like maceration and solvent extraction and did not explore the most common food spoilage bacteria (19).

Ultrasonication is a branch of acoustics that can be applied to solids, liquids, and gases at frequencies above the normal human range of hearing. The ultrasonic effect leads to the degradation of plants' cell walls and membranes, resulting in the release of soluble ingredients into the surrounding medium (solvent) (20). Ultrasound-assisted extraction (UAE) is the simplest and most economical technique and can be easily scaled up to industrial production (20). UAE has been shown to improve the separation of medicinal compounds, such as saponins and water-soluble polysaccharides (21). So, for the extraction of chemicals from diverse sources and for use in various applications, the use of ultrasound is strongly advised. Since UAE offers excellent extraction yields without compromising the integrity of the extracted bioactive compounds, it is a helpful extraction method for numerous bioactive compounds. Through physical forces generated during sonic cavitation, ultrasound waves break plant tissue, releasing extractable components faster (22). This led us to question if utilizing UAE improves the extraction of medicinal compounds and has better antibacterial potential compared to those using conventional extraction methods. We also wanted to know if these extracts would show a broad spectrum of antibacterial activity against all strains of bacteria tested. As such, the validation of *D. wallichii* to augment its traditional use is required to create public awareness. The bacteria screened in the present study are representative of the most common food pathogens, which include *Salmonella abony, E. coli, P. aeruginosa, B. cereus,* and *S. aureus*, a combination of Gram-positive and negative strains (23). The differences between the five strains of bacteria chosen in our study help determine the spectrum of antibacterial activity. Our results suggest that the aqueous extracts of stem, leaf, and bark obtained by the UAE approach can indeed effectively inhibit the growth of Gram-positive strains of *S. aureus* and *B. subtilis*. The extracts had less of an effect on the Gram-negative bacterial strains, though. As a result, the antibacterial activity spectrum of these extracts was narrow. The flower extracts exhibited no antibacterial activity against

Plant extract	Solvent used for extraction	Treatment	Dilution used	OD at λ_{max}
Bark	Water	Sonicated	1:15	1.245
		Non-sonicated	Without dilution	0.368
	Acetone	Sonicated	1:15	0.968
		Non-sonicated	Without dilution	0.241
Stem	Water	Sonicated	1:15	1.524
		Non-sonicated	Without dilution	0.598
	Acetone	Sonicated	1:15	1.113
		Non-sonicated	Without dilution	0.842
Leaf	Water	Sonicated	1:15	1.874
		Non-sonicated	Without dilution	0.598
	Acetone	Sonicated	1:15	1.447
		Non-sonicated	Without dilution	0.695
Flower	Water	Sonicated	1:15	1.964
		Non-sonicated	Without dilution	0.847
	Acetone	Sonicated	1:15	1.524
		Non-sonicated	Without dilution	0.931

Table 1: UV-visible spectrophotometric analysis (optical density) of water or acetone extracts of all plant parts at their λ_{max}. The spectra showed that, in comparison to samples extracted using the ultrasound approach, all samples obtained using the conventional method had a low extraction yield. Additionally, when compared to acetone extracts, water extracts demonstrated a higher extraction yield.

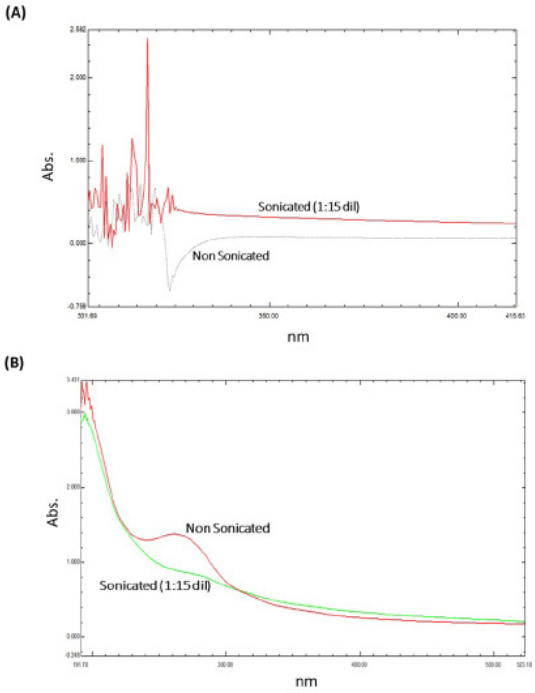

Figure 1: Spectrophotometric analysis of the flower extracts. UV-VIS spectrum of **(A)** water and **(B)** acetone flower extracts of D. wallichii. In ultrasonic assisted extraction, the yield of water and acetone extracts for all tested plant parts was much higher than extracts obtained by conventional method, so extractions were diluted 1:15.

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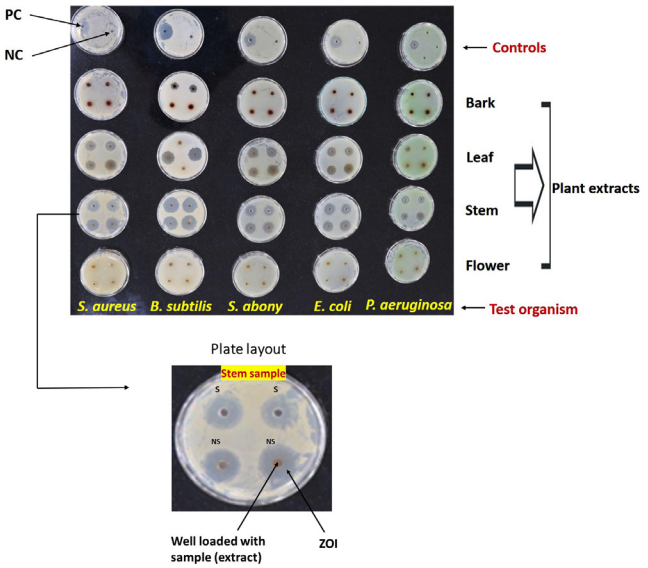

Figure 2: Zones of inhibition (ZOI) plates for S. aureus, B. subtilis, S. abony, P. aeruginosa and E. coli against water extracts. Stem and leaf extracts showed bigger ZOI representing their potent antibacterial property but the bark extract was less effective and the flower extracts represented the no ZOI. Positive control: Gentamicin, Negative control: 10% v/v DMSO.
S: Sonicated, NS: Non-sonicated, PC: Positive control, NC: Negative control.

all the tested bacterial strains.

RESULTS

Ultrasound-assisted extraction (UAE)

The concentration of bioactive molecules in plant extract may vary due to the target material, technique applied, solvent system used, and agro-climate changes (24). As per our knowledge, there is no report available about the extraction yield for *D. wallichii* plant extraction by UAE. For the purpose of conventional extraction, we dissolved the powdered samples of leaf, bark, stem, and flower in water or acetone under continuous shaking for 3 h at room temperature. For the purposes of the UAE, we dissolved the powdered samples in

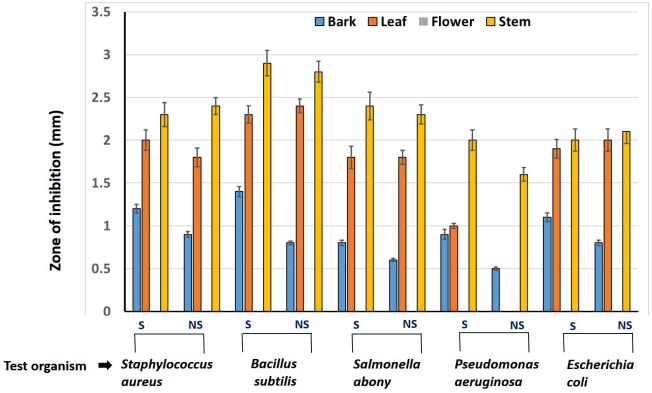

Figure 3: Zone of inhibition (ZOI) measurements for the water extracts (n = 3). The bars represent the average ZOI measurements for the tested bacterial plates. Stem and leaf extracts demonstrated a larger ZOI, indicating their antibacterial potential and bark extract was less efficient. Since the ZOI measurements for the flower plates are zero there are no representative bars. There was only a marginal difference between sonicated (S) and non-sonicated (NS) extracts.

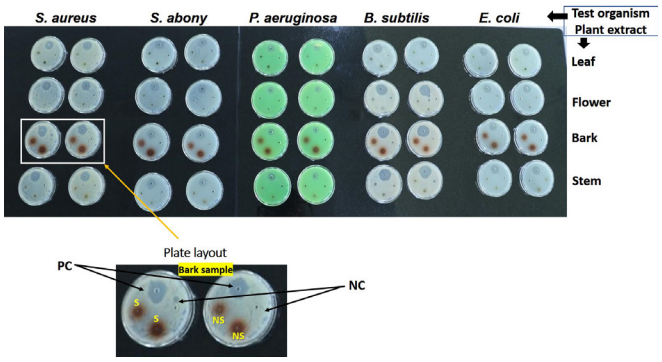

Figure 4: Zones of inhibition (ZOI) plates for S. aureus, B. subtilis, S. abony, P. aeruginosa and E. coli against acetone extracts. The extracts had a smaller ZOI than most of the corresponding water extracts suggests ineffective acetone extraction. Positive control (PC): Gentamicin, Negative control (NC): 10% v/v DMSO.

water or acetone and then exposed them to ultrasonic waves for 30 min at a frequency of 30 kHz. To regulate the heat generated during the ultrasonic treatment throughout the process, we measured the temperature of the samples with an infrared thermometer every 2 min and maintained at a lower temperature using ice around the sample container.

The water and acetone extracts of *D. wallichii* parts which came from different areas of the plant (leaf, bark, stem, and flower) obtained by sonication and conventional methods showed great differences. Notably, the concentration of the ultrasonic-assisted extracts was 15 times higher than the normal extracts **(Table 1)**. The spectra revealed the extraction yield (concentration) of each tested plant extract, prepared by using conventional and ultrasound methods using water or acetone **(Figure 1)**. We have observed a low extraction percentage yield in all the samples obtained by the conventional extraction method compared to those using the ultrasound method **(Table 1)**.

Antibacterial activity of plant extracts using Agarose well diffusion method

We determined the antibacterial activity of the water and acetone plant extracts of leaf, bark, stem, and flower against two Gram-positive *(B. subtilis* and *S. aureus*) and three

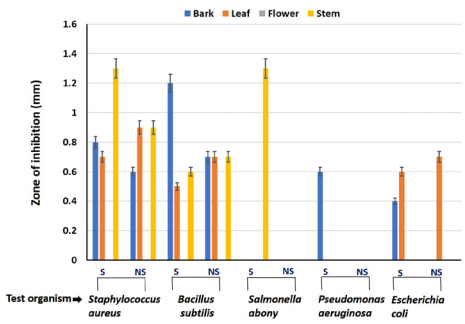

Figure 5: Zone of inhibition (ZOI) measurements for the acetone extracts (n = 3). The bars represent the average ZOI measurements for the tested bacterial plates. The extracts had only a negligible effect on Gram-positive bacteria and almost no effect at on Gram-negative bacteria. Since the ZOI measurements for the flower plates are zero there are no representative bars. The ZOI was often smaller in the acetone extracts than it was in the corresponding water extracts. S: Sonicated, NS: Non-sonicated.

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Gram-negative strains (*E. coli, S. abony,* and *P. aeruginosa*) by measuring their zones of inhibition (ZOI, mm) around the loaded wells on Mueller Hinton Agar (MHA) plates. We inoculated these plates by spreading the bacterial inoculum over the entire agar surface.

Our findings showed that all plant extracts, except for the flower, had varying degrees of efficacy in inhibiting the growth of the tested bacteria. At a dosage of 50 mg/ml, leaf and stem extracts were the most effective at inhibiting the growth of all bacteria tested, but the bark extract was only effective against *B. subtilis* and *S. aureus* **(Figure 2–5)**. The flower extracts showed no ZOI against all tested bacteria **(Figure 2–5)**. The maximum growth inhibition zone for the water plant extracts of stem, leaf, and bark was found to be 2.9, 2.4, and 1.4 mm, respectively, against B. subtilis **(Figures 2 and 3)**. Similarly, the maximum growth inhibition zone for the acetone plant extracts of stem, leaf, and bark was found to be 1.3, 0.9, and 0.8 mm against S. aureus **(Figures 4 and 5)**. The solvent used for the preparation of compound solutions (10% dimethyl sulfoxide (DMSO)) did not show any inhibition against the tested organisms (as a negative control). The positive control gentamicin showed a large ZOI for all the tested bacterial strains.

The water extracts exhibit significant activity against *B. cereus* and *S. aureus* and moderate activity against *E. coli, S. abony,* and *P. aeruginosa*. However, the acetone extracts had a smaller ZOI than most of the corresponding water extracts. So, for additional investigation to study the MIC and MBC, just the aqueous extracts showing good ZOI were selected.

Antimicrobial assays

We determined the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the aqueous plant extracts against all susceptible bacterial strains to evaluate their bacteriostatic and bactericidal properties **(Table 2)**. We performed MIC and MBC only for those organisms that showed a ZOI and were sensitive to the plant extracts in the previous antimicrobial assay by agar well diffusion method. The MIC was considered as the lowest concentration which inhibited the growth of the respective bacterial strains. The MIC was measured by employing the broth microdilution technique where the tested bacteria are inoculated into Mueller Hinton broth (MHB) medium in the presence of different concentrations of plant extracts. Growth was assessed after incubation for 48 h at 37°C, and the MIC value was defined as the lowest concentration of the extract at which there is no visible growth as demonstrated by the lack of turbidity. The MBC, which is the lowest concentration of the plant extract required to kill the tested bacteria, was confirmed by the absence of bacterial growth when streaked onto MHA plates from the MHB tubes corresponding to their lowest MIC's.

Our findings showed that there was variation in the MIC among plant extracts; the lowest MIC values (160 and 320 µg/ml) against S. aureus and B. subtilis, respectively, were displayed by sonicated extracts of the stem and leaf **(Table 2)**. Among all plant extracts tested, sonicated stem and leaf water extracts showed strong antibacterial activity against Gram-positive strains. The plant extracts were not effective against all tested Gram-negative bacteria with MIC values of more than 5000 mg/ml **(Table 2)**. The results of MIC and MBC tests on the potent plant extracts indicated that *D. wallichii* leaf and stem extracts may be utilized to regulate and inhibit the growth of some food-borne pathogens and illnesses caused by food poisoning (25). MIC and MBC tests have proven effective tools for comparing the ability of various antimicrobial agents to kill bacteria and are helpful in antibacterial product development and quality control (26). In general, antimicrobial agents are thought to be bactericidal if the MBC is no more than four times the MIC (23). Sometimes an antimicrobial agent's MBC is extremely near to its MIC (27).

Bacteria	Sample	MIC (µg/ml)	MBC (µg/ml)
Staphylococcus aureus	Bark (Sonicated)	≥ 20480	≥ 20480
	Leaf (Sonicated)	320	1280
	Leaf (Non-Sonicated)	640	5120
	Stem (Sonicated)	160	640
	Stem (Non-Sonicated)	640	2560
Bacillus subtilis	Bark (Sonicated)	20480	≥ 20480
	Bark (Non-Sonicated)	≥ 20480	≥ 20480
	Leaf (Sonicated)	320	1280
	Leaf (Non-Sonicated)	≥ 20480	≥ 20480
	Stem (Sonicated)	160	160
	Stem (Non-Sonicated)	320	640
Salmonella abony	Leaf (Sonicated)	10240	20480
	Leaf (Non-Sonicated)	≥ 20480	≥ 20480
	Stem (Sonicated)	20480	20480
	Stem (Non-Sonicated)	20480	≥ 20480
Escherichia coli	Leaf (Sonicated)	10240	20480
	Leaf (Non-Sonicated)	≥ 20480	≥ 20480
	Stem (Sonicated)	10240	≥ 20480
	Stem (Non-Sonicated)	10240	10240
Pseudomonas aeruginosa	Leaf (Sonicated)	≥ 20480	≥ 20480
	Stem (Sonicated)	5120	10240
	Stem (Non-Sonicated)	5120	20480

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values (µg/ml) of plant water extracts against the tested bacteria. The lowest MIC and MBC values against S. aureus and B. subtilis, respectively, were displayed by sonicated extracts of the stem and leaf. The samples against S. abony, E. coli, and P. aeruginosa showed higher MIC and MBC values, which indicated a lower level of antibacterial activity against Gram-negative strains.

Finally, the inhibitory effects of stem and leaf water extracts demonstrated the greatest antimicrobial effects and significant activity against *S. aureus* and *B. cereus*. We concluded that the tested microorganism has evolved resistance to the tested antimicrobial agent if the MBC of the tested agent against the tested microorganism is greater than 32 times the MIC.

DISCUSSION

When compared to those produced using the ultrasound approach, the extraction yield of each examined plant part acquired using the conventional method was lower **(Table 1, Figure 1)**. According to earlier reports, when plant cell walls were broken by ultrasonic waves, chemicals and molecules

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were released into the solvent (28). The lack of thermal treatment used in this procedure helps to protect the bioactive compounds and increases the amount of sample materials that may be collected (29).

Sonicated and non-sonicated extracts had distinct UV-visible spectra **(Figure 1)**. This might be because sonication causes cavitation and implosion, which rupture cell walls and increase the number of disrupted cells (29). When the cell is damaged, the solvent enters the cell and incorporates the intracellular plant material. As a result, the molecules in the sonicated and non-sonicated extracts can be different (29). The differences in how water and acetone extracts dissolve various phytochemicals may be the likely causes of the variances in the spectra of the two extracts **(Table 1)** (30). For instance, ethanol can extract more carotenoids, and water can extract more polyphenols (30). Phytochemicals such as flavonoids, keratin, phenolic acids, tanshinone, tocols, terpenoids, xanthones, a-mangostin, carrageenans, isoflavones, genistin, apigenin, and many more are extracted using a variety of procedures. The process of extraction of these compounds is based on number of variables such as the raw material, the organic solvent, and the applied technique (31). We assessed the antimicrobial properties of acetone and water extracts of *D. wallichii* at a 50 mg/ml concentration against *E. coli, S. abony, P. aeruginosa, B. subtilis* and *S. aureus* **(Figure 2–5)**. Our results of the antibacterial properties of acetone and water extracts revealed that bark, leaf, and stem extracts efficiently suppressed the growth of food pathogens and spoilage microorganisms with variable potency, whereas flower extracts showed no activity **(Table 2)**. In general, the acetone extracts had a smaller ZOI than most of the corresponding water extracts, so for additional investigation (MIC and MBC), just the aqueous extracts were selected. According to earlier reports, water extracts from various plants typically provide much larger yields than alcoholic extracts from the same plants (32). The increased polarity of water may be the cause of this (33).

Our study validates earlier findings in the literature that show an increase in extract concentration is directly correlated with an increase in antibacterial activity (34). The sonicated extract of the stem yielded the lowest MIC values against *S. aureus,* and the sonicated extract of the leaf yielded the lowest MIC values against *B. subtilis* **(Table 2)**. According to a study conducted by Mostafa et al., the variety in chemical contents and the volatile nature of plant extracts' components is a cause of the differences in MIC (35). Most of the corresponding non-sonicated extracts had higher MIC values than the sonicated aqueous extracts, on average **(Table 2)**. The aqueous extracts from the sonicated stem and leaves exhibited the highest effect on the Gram-positive bacterial strains *S. aureus* and *B. subtilis*; however, the extracts had less impact on Gram-negative bacterial strains. Gram-negative bacteria are more resistant than Gram-positive bacteria because of their unique structure, which also contributes to their widespread global burden of sickness and mortality (36). The main cause of Gram-negative bacteria's resistance to a variety of antibiotics is their outer membrane (36). To reach their targets, most antibiotics need to penetrate the outer membrane. Gram-negative bacteria can modify the outer membrane in any way, including by mutating porins or changing its hydrophobic characteristics (36). This can result in resistance. This crucial layer is absent from Gram-positive bacteria, giving Gram-negative bacteria greater antibiotic resistance than Gram-positive bacteria (37).

The results indicate that the extraction yield can be effectively increased by employing the UAE approach. The aqueous extracts had significant antibacterial activity against tested Gram-positive bacteria and showed moderate effects against Gram-negative bacteria. Species belonging to this genus such as *Dombeya rotundifolia, Dombeya torrida,* and *Dombeya tsaratananensis* have shown moderate antibacterial activities against common food pathogens and other bacteria (38, 39). These results suggest that the plant extracts examined in this study may be utilized as natural preservatives in food to prevent or reduce the growth of harmful microbes and spoiling.

MATERIALS AND METHODS

Sample collection

The plant materials were collected from the Prayoga campus (Ravugodlu, Bengaluru 560082, Karnataka, India) along with the details of their botanical name, family, and parts used. The plant parts were collected for the extraction were leaf, bark, stem, and flower. These parts were collected in a sterile container by the student researchers.

Preparation of plant extracts

The collected samples were first washed under running tap water and then dried using a hot air oven, at 40°C for 5 days. Using a mortar and pestle and then a grinder (IKA MultidriveB, India) the plant parts were coarsely powdered. The weight of the ground powder was taken, and 10 g of this fine powder from each plant was dissolved in 100 ml of acetone and shaken for 3 h at room temperature. The supernatant was filtered through Whatman filter paper while the residues were used for a second and third extraction. The dissolved components were always filtered and kept in a glass bottle. After the third extraction, the filtrates were evaporated using a rotary evaporator (Buchi R-205, Switzerland) at decreased pressure and 50°C to produce the concentrated crude extract. The crude extracts were collected in glass vials and then lyophilized to obtain a dried form of the extract. For lyophilization, the extracts were placed in Petri dishes, then frozen at -80°C and finally freeze-dried at -50°C under vacuum (FreeZone 2.5 Liter -50C Benchtop Freeze Dryer, Labconco, USA) for 20 h. The same protocol was followed for the preparation of extracts using water instead of acetone.

Ultra-sonic assisted extraction

For ultrasound techniques, 10 g of powder of each tested plant material was mixed with 180 ml of acetone in a beaker. The samples were sonicated at cold temperature using a tip ultra-sonicator (Trans-O-Sonic 250 W) for 30 min at 30 kHz **(Figure 6)**. Afterwards, the beakers were transferred to the shaking incubator and kept shaking for 3 h at room temperature. The supernatant was filtered, while the residues were used for a second and third extraction. Each time the dissolved parts were filtered and stored in a glass bottle **(Figure 6)**. After the third extraction, the filtrates were evaporated under reduced pressure at 50°C using a rotary evaporator to yield the crude extract **(Figure 6)**. The crude extracts were then collected in vials for further use. Dried extracts were dissolved in 10% DMSO. The crude extracts were then stored at -20°C for further study **(Figure 6)**. For

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the UV-visible spectrophotometric analysis, the sonicated samples were diluted up to 15 times as the absorbance was beyond the spectral limit in the undiluted extracts **(Figure 1)**. The non-sonicated samples showed absorbance in the measurable range, so the samples were not diluted **(Figure 1)**.

Microbial culture

Five food spoilage microbial strains were used in the study: three strains of Gram-negative (E. coli, S. abony and P. aeruginosa) and two strains of Gram-positive (*B. subtilis* and *S. aureus*) bacteria. The cultures were purchased from American Type Culture Collection (ATCC). The bacteria were pre-cultured in MHB medium (Make: Microexpress) overnight in a rotary shaker at 37°C. After incubation, each strain was adjusted using the 0.5 McFarland standard to a concentration of 108 cells/ml. At a final concentration of 108 cells/ml, a spectrophotometer (λ = A595 nm) was used to regulate the bacterial density.

Antimicrobial assay of plant extracts

Extracts from four plant parts (leaf, bark, stem, and flower) were examined to determine their antibacterial activity against all five bacterial strains using the agar well diffusion method in MHA plates as displayed previously described (40). To bring the turbidity to 0.5 optical density (OD), the test organisms were inoculated in nutrient broth and cultured overnight at 37°C. With standardized microbial culture broth, the MHA plate was lawn cultivated. Plant extracts of 50 mg/ml concentration were prepared in DMSO. Six wells of 6 mm diameter were bored in the inoculated media with the help of a sterile cork-borer (6 mm). Each well was filled with 50 µl extracts from different plants, a positive control (Gentamicin 10 µg/ml), or a negative/solvent control (10% DMSO), respectively. The plates were incubated for 18–24 h at 37°C after being allowed to diffuse for about 30 min at room temperature. After incubation, the test compounds' antimicrobial activity was determined by looking at the plates for the development of a clear zone around the well. The observed ZOI was measured and reported in mm.

Determination of MIC and MBC of the plant extracts

The MIC value of the extract was determined as the lowest concentration that completely inhibited bacterial growth after 48 h of incubation at 37°C (41). The broth microdilution method was used to determine the MIC (26). The dilutions of extracts were prepared in test tubes containing MHB to obtain various concentrations. The bacterial inoculum was added to give a final concentration of 5 × 105 colony-forming units (CFU)/ml in each tube. The positive control containing Gentamicin (10 µg/ml) was used as a standard drug. For the determination of MBC, a portion of liquid (5 µl) from each tube that exhibited no growth was taken and then incubated at 37°C for 24 h. The MBC was confirmed by the absence of bacterial growth of the tested strains streaked from the test tubes corresponding to their lowest MICs. The lowest concentration that revealed no visible bacterial growth after sub-culturing was taken as MBC.

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