Antibacterial and phytochemical evaluations of *Alternanthera repens* (L.) and honey on *Pseudomonas aeruginosa* of clinical origin

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Received: 14 January 2022
Revised: 27 February 2022
Accepted: 5 March 2022
Published: 25 July 2022

Egyptian Pharmaceutical Journal 2022, 21:273–281

Context
Medicinal plants have long been used as a source of therapeutic agents worldwide, and herbal medicines have increasingly been employed in the treatment of diseases. *Alternanthera repens* is one of the underexploited plant species for its antimicrobial potentials.
Aims
This study aimed at investigating the antibacterial efficacy of the leaf and stem ethanolic extracts of *A. repens* and honey against *Pseudomonas aeruginosa*.
Materials and methods
The phytochemical analyses were performed on ethanolic plant extracts using the universal laboratory techniques for qualitative and quantitative determination. The agar-well diffusion method was used for the in-vitro antibacterial bioassay. The antibacterial activities of the honeys, ethanolic leaf and stem extracts, and antibiotics were compared. The minimum inhibitory concentrations and minimum bactericidal concentrations of the honeys and extracts were determined.
Statistical analysis used
The students' *t*-test was employed to determine the significant differences between the phytochemical constituents in the extracts and also the antibacterial activities of the ethanolic leaf and stem extracts against *P. aeruginosa*.
Results
Phytochemical screening showed the presence of total phenols, saponins, tannins, total flavonoids, alkaloids, cyanogenic glycosides, phytate, and terpenoids in the plant extracts. The extracts and honeys were able to inhibit the growth of the *P. aeruginosa* at varying concentrations (25, 50, 75, and 100%). The combinations of the honeys and ethanolic extracts of the plant parts exerted significantly higher antibacterial effects on *P. aeruginosa*.
Conclusion
The ethanolic extracts of *A. repens* possessed antibacterial properties against *P. aeruginosa*, which was more pronounced in combination with honey. The presence of various phytochemicals in the plant indicated its high potential for possible drug production.

Keywords:
antimicrobial resistance, *Apis mellifera* honey, ethanolic extract, medicinal plant, phytochemicals

Egypt Pharmaceut J 21:273–281

1687-4315

Introduction

Alternanthera repens (L.), which is one of the underexploited medicinal plants, is a member of the family Amaranthaceae. It is commonly called Khakiweed or creeping chaffweed. It is a burgundy foliage plant that spreads on the ground and works well for edging, annual groundcover, or in a formal knot garden [1]. It performs well in high heat, where its color becomes deeper and richer. It is a dense mat-forming plant with annual tops, a fleshy, perennial rootstock, reddish, hairy stems, and prickly burrs. The plant has been used as medication for gastrointestinal diseases [2] and as a traditional Mexican medicine for the treatment of diarrhea and dysentery [3]. African honey bees (*Apis mellifera*) are important pollinators of many fruits, nuts, vegetables, and field crops. Honey bees assist in maintaining the ecosystem by pollinating different wild flowering plants. The medicinal properties of honey had been earlier documented [4]. Pure honey possesses the ability of inhibiting a wide array of pathogenic organisms, including oral bacteria and food spoilage bacteria. Honey has been employed in the treatment and management of diseases

over 2000 years ago, and its use has been incorporated into modern medicine [4].

Resistance to antibiotics is a major public health threat that has extended to the 21st century [5]. Infectious diseases account for approximately one-half of all deaths in tropical countries and are the number one cause of death. Moreover, an increase in the rate of morbidity and mortality because of microbial infections has been regarded as a major problem. However, the emergence and dissemination of multidrug-resistant strains of pathogenic bacteria has become a significant public health threat as there are fewer, or even sometimes no effective antimicrobial agents available for the infection caused by such pathogenic bacteria. Therefore, based on the proofs of the rapid development and spread of resistant pathogens, the need to explore novel antimicrobial agents becomes imperative. This is important to tackle the global burden of infectious diseases [6].

Pseudomonas aeruginosa is a gram-negative, aerobic, rod-shaped bacterium, belonging to the *Pseudomonaceae* family. It is commonly found in soil, water, as well as in and/or on animals, humans, and plants. *P. aeruginosa* is an opportunistic pathogen and a highly resistant microorganism commonly associated with infections resulting in long hospital stays. It exploits the weaknesses in host defense to mount an infection. The organism does not normally infect uncompromised tissues but can invade tissues beleaguered by immunodeficiency. It causes infection in the urinary tract, respiratory system, dermis, soft tissue, gastrointestinal tract, blood, bone, and joint. *P. aeruginosa* causes chronic infections in the lungs of patients with cystic fibrosis and chronic obstructive lung disease, chronic urinary tract infections in patients with permanent bladder catheter, and ventilator-associated pneumonia in intubated patients. *P. aeruginosa* is responsible for burns and wound infections; it is also an important cause of wound infections in diabetic individuals and infected wounds following surgeries [7]. Infections caused by *P. aeruginosa* strains are usually life-threatening and are re-emerging worldwide as a public health threat. This could not be dissociated from the range of adaptation, survival, and resistance mechanisms possessed by the organism. *P. aeruginosa* has also been identified to cause chronic infections as a result of its ability to produce biofilms, leading to intrinsic antibiotic tolerance and development of mutational antibiotic resistance and making it difficult or impossible to get eradicated by conventional antibiotic treatment [8]. The aim of this research was to evaluate the phytochemical and antibacterial potential of selected honeys and the leaves and stem of *A. repens* for the presence of secondary metabolites and to evaluate the antibacterial effects of its ethanol extract on selected clinical isolates.

Materials and methods

Sources of organism and honey samples

P. aeruginosa associated with wound infection was obtained from Don Bosco Catholic Medical Centre, Araromi Street, Ondo State, Nigeria. *A. repens* used for this research was obtained from a farmland in Akure, Ondo State. The plant was identified at the laboratory of the Plant Biotechnology Unit of the Department of Biological Sciences, University of Medical Sciences, Ondo City, Ondo State. Two honey samples produced by *A. mellifera* used in this research were obtained from accredited beekeepers in Akure, Ondo State, and Ondo City, Ondo State, Nigeria. The research was carried out in the laboratory of the Department of Biological Sciences, University of Medical Sciences, Ondo City, Ondo State.

Confirmation of organism

The confirmation of *P. aeruginosa* was carried out by Gram's stain and biochemical reactions as described by Osuntokun *et al.* [9] Gram's staining, coagulase, urease, indole, methyl-red, Voges-Proskauer, hydrogen sulfide production, catalase, oxidase, nitrate reduction, Simmons citrate, arginine dihydrolase, starch hydrolysis, gelatin hydrolysis, maltose, sucrose, and mannitol tests were carried out.

Preparation of extract

The fresh plant parts (leaf and stem) were harvested and washed thoroughly under running tap water to remove every form of earth on it. The plant parts were spread, and one portion was room dried at 25°C for 14 days without contact with the sun, whereas the other portion was oven dried at 60°C for 24 h. The plant parts were ground into fine powder using a heavy-duty silver crest blender (4500 W, Model-SC-5003, China). Exactly 80 g of the pulverized plant leaves and stems was separately macerated in ethanol to saturation for 72 h. The mixtures were agitated after the addition of the solvent and sieved with muslin cloth into a clean beaker and then filtered using No. 1 Whatman filter paper. The filtrates were dried using a rotary evaporator. These made the preparations of the ethanolic extracts of room-dried and oven-dried leaves and room-dried and oven-dried stems of *A. repens*. The different concentrations (25, 50, 75, and 100%) of ethanol were used as solvent in the extraction

process [4], whereas sterile distilled water was used to dilute the pure honey (100%) to lower concentrations investigated.

Antimicrobial analyses

The antimicrobial effects of the different concentrations of the preparations of *A. repens* including honeys against *P. aeruginosa* were separately determined using the agar-well diffusion method. A young actively growing culture of the test organism, cultured on nutrient agar plates and incubated for 18 h at 37°C, was used. The bacterial cell was prepared to make a suspension equivalent to McFarland standard to obtain a final inoculum size of 0.5×10^8. The bacterial suspension was inoculated on solidified Mueller Hinton agar (MHA) plates using the spread plate method. Six-millimeter-diameter wells were bored on the MHA plates into which 0.5 ml of the different concentrations of ethanolic extracts of room-dried and oven-dried leaves and room-dried and oven-dried stems of *A. repens* and 0.5 g of honeys 1 and 2 were separately introduced. These plates were allowed to stand for 30 min and incubated at 37°C for 24 h. All experiments were performed in triplicates under aseptic conditions. The antimicrobial activities of the preparations were indicated by the appearance of clear zones of inhibition (mm) around the wells, and the average values were calculated and recorded [10]. Using the 50% concentrations, the synergistic antimicrobial (ratio 1 : 1) activities of honey 1 and room-dried leaf, honey 1 and room-dried stem, honey 2 and room-dried leaf, honey 1 and oven-dried leaf, honey 2 and room-dried stem, honey 1 and oven-dried stem, honey 2 and oven-dried leaf, and honey 2 and oven-dried stem against *P. aeruginosa* were determined using spread plate and agar-well diffusion methods according to the aforementioned protocols.

Determination of minimum inhibitory concentrations and minimum bactericidal concentrations

The determination of the minimum inhibitory concentrations (MICs) was carried out using the broth dilution method. Test tubes containing an 8-ml portion of nutrient broth were prepared. One 1 ml of the standard inoculum from the bacterial suspension was inoculated in each of the broths. Four different concentrations (25, 50, 75, and 100%) of the extracts and honeys were investigated. These tubes were incubated at 37°C for 24 h and observed for bacterial growth. The growth in the medium is indicated by turbidity of the medium. Subsequently, a loopful of broth from each of the test tubes not showing bacterial growth was inoculated into nutrient agar plates for the determination of minimum bactericidal concentrations (MBCs). The plates were incubated at 37°C for 24 h and observed for growth. The MBC was considered as the lowest concentration of antimicrobial agent that arrested the viability of the initial bacterial inoculum by, at least, 99.9% [11,12].

Antibiotic sensitivity of *Pseudomonas aeruginosa*

The disc diffusion technique was employed for the determination of antibiotic susceptibility pattern of the test organism following standard procedures. A 0.5 ml of the standard inoculum from the bacterial suspension was introduced onto the surface of solidified MHA plates using the spread plate technique. The plates were allowed to stand for 15 min before the placement of gram-negative antibiotic discs and which were then incubated at 37°C for 24 h. The antibiotics investigated in this study included ofloxacin (10 μg), reflacine (10 μg), ciprofloxacin (10 μg), augmentin (30 μg), gentamycin (10 μg), streptomycin (30 μg), ceporex (10 μg), nalidixic acid (30 μg), septrin (30 μg), and ampicillin (30 μg). The sizes of the inhibition zones developed around the paper discs were measured and recorded in millimeters [13].

Phytochemical analyses

The phytochemical analyses were performed using the universal laboratory techniques for qualitative and quantitative determination. The plant extracts were screened for alkaloids, saponins, phenols, flavonoid, tannin, phytate, terpenoids, and cyanogenic glycoside as described by Trease and Evans [14].

Qualitative phytochemical analysis

Phenol analysis

A 2 g proportion of the extract was emerged in 20 ml of methanol and then extracted by filtration using filter paper. Then, 1 ml of the filtrate was tested by adding 1 ml of Folin-Concalteon with 1 ml of 20% $NaCO_3$. Dark blue color formation indicated the presence of phenol.

Alkaloids

The extract was mixed with 2 ml of Wagner's reagent, and the formation of reddish-brown colored precipitate indicated the presence of alkaloids.

Cyanogenic glycosides

Fehling's test: ~5 ml of mixture of equal parts of Fehling's solutions I and II was added to about 3 ml of the extract and boiled for 5 min. A denser brick red precipitate indicated the presence of glycoside.

Saponins

Foam test was performed to test the presence of saponins. To 2 ml of the extract, 6 ml of water was

added in a test tube and shaken vigorously. Then, the mixture was observed for the formation of persistent foam. This confirmed the presence of saponins.

Flavonoids

The test for the presence of flavonoids was determined by the alkaline reagent test. The extracts were mixed with 2 ml of 2% solution of NaOH. An intense yellow color was formed, which turned colorless on addition of few drops of diluted acid. This confirmed the presence of flavonoids.

Terpenoids

The presence of terpenoid was determined through the Salkowski test. One milliliter of chloroform was mixed thoroughly with the sample extract solution. Then, a 3-ml portion of concentrated sulfuric acid was gently introduced to form a layer. A reddish-brown coloration on the interface indicated the presence of terpenoids.

Tannins

Approximately 5 g of the extracts was boiled with 40 ml of water. This was filtered and used for the ferric chloride test. For ferric chloride test, 3 ml of the filtrate was added to few drops of ferric chloride solution. A greenish black precipitate indicated the presence of tannin.

Phytate

One gram of the extract was soaked in 2% hydrochloric acid for 3 h and filtered through a No. 1 Whatman filter paper. Then, 25 ml of the filtrate was transferred into a conical flask and 5 ml of ammonium thiocyanate solution was added as an indicator. After which, distilled water was added to give it the proper acidity, and this was titrated against standard iron (III) chloride solution until a brownish yellow color persisted for 5 min.

Quantitative phytochemical analysis

Total phenols

This was determined using the Folin-Ciocalteu method. Distilled water and Folin-Ciocalteu's reagent were added to 125 µl of the plant extract. The mixture was allowed to stand for 6 min before the addition of 7% sodium carbonate solution. The mixture was allowed to stand for 90 min. Absorbance was read at 760 nm on a SpectrumLab70 spectrophotometer, and the result was expressed as gallic acid equivalents.

Saponins

The saponin content was determined using the spectrophotometric method. Two grams of the sample was weighed into a beaker, and isobutyl alcohol (but-2-ol) was added. The mixture was filtered using No. 1 Whatman filter paper into a beaker containing 40% magnesium carbonate ($MgCO_3$) solution. Approximately 1 ml of the solution was transferred into a volumetric flask. Then, 2 ml of iron (III) chloride ($FeCl_3$) solution was added and made up to mark with distilled water. This stood for 30 min, and development of color and absorbance were recorded on a SpectrumLab70 spectrophotometer at 380 nm.

Tannins

The total tannin content was assessed by the standard protocol of Keerthana *et al.* [15]. This was done by diluting 0.5 ml of the sample extract with 80% ethanol. A 0.1 ml portion of the diluted sample was mixed with 2 ml of Folin-Ciocalteu reagent and allowed to stay for 7 min. Then, 7.5 ml of 7% sodium carbonate solution was introduced, and this stood for 2 h. The absorbance measurement was taken at 760 nm, and the tannin content was estimated employing tannic acid curve as the standard.

Flavonoids

Approximately 0.25 g of the plant extract was dissolved in 1 ml of distilled water; 5% $NaNO_2$ solution, 0.150 ml of freshly prepared aluminum chloride ($AlCl_3$), and 1 M NaOH solutions were added. The mixture stood for 5 min; absorbance measurement was recorded at 510 nm on a spectrophotometer (SpectrumLab70), and the result expressed as equivalents to quercetin.

Alkaloids

Overall, 5 g of the sample was weighed into a beaker, and 200 ml of 10% acetic acid. Ethanol was added, and this stood for 4 min; it was filtered, and the extract was concentrated in a water bath to one quarter of the original volume. Ammonium hydroxide solution was added drop-wise to the extract until the precipitation was completed. The obtained precipitate was washed with dilute ammonium hydroxide and filtered, and the residue was dried and weighed. The residue dried and weighed is the alkaloid.

Cyanogenic glycosides

Approximately 2 ml of the sample dissolved in water was transferred into a conical flask containing chloroform, and the mixture was filtered into a conical flask. Then, 2 ml of pyridine and 29% sodium nitroprusside was added to the mixture and thoroughly shaken for 10 min. Thereafter, 20% NaOH was added. Then, color development and absorbance

were read at 510 nm on a SpectrumLab70 spectrophotometer.

Terpenoids

From the extract, 100 mg was taken and soaked in 9 ml of ethanol for 24 h [16]. The extract was filtered. After filtration, it was extracted with 10 ml of petroleum ether using a separating funnel. The ether extract was separated in preweighed glass vials and dried completely. Ether was evaporated, and the yield percentage of total terpenoid contents was measured.

Phytate

From each of the extracts, 1 g was weighed into conical flasks, and a 100 ml portion of 2% HCl was added to digest the samples for 3 h. The filtration of digested samples was done with a filter paper, and 25 ml of the filtrates was measured into 250-ml conical flasks. A 5-ml volume of 0.3% NH_4SCN solution was introduced. The resulted mixtures were titrated against 0.1 M ferrous chloride ($FeCl_3$) until a brownish-yellow color end point that persisted for 5 min was obtained. The percentage phytate content was calculated as follows: titer value ×0.00195. Each of the determination process was carried out in triplicate, and the average titer value was obtained.

Statistical analysis

The data obtained were statistically analyzed using the Statistical Package for the Social Sciences (SPSS), IBM 25. The Student *t* test was used to determine the differences in the phytochemical constituents in the plant extracts and also the antibacterial activities of the ethanolic leaf and stem extracts against *P. aeruginosa*. Values were considered significant at *P* value less than or equal to 0.05.

Results

P. aeruginosa strain was characterized as being negative to Gram's reaction, coagulase, urease, indole, methyl-red, Voges-Proskauer, hydrogen sulfide production, maltose, and sucrose tests. It is positive to catalase, oxidase, nitrate reduction, Simmons citrate, arginine dihydrolase, starch hydrolysis, gelatin hydrolysis, and mannitol tests (Table 1).

As not unexpected, the activity of honeys and extracts increased with concentrations, and at the highest concentration, pure honey 1 elicited the highest anti-pseudomonal activity (21.2 mm), followed by the room-dried leaf extract (20.2 mm), whereas the least activity was elicited by oven-dried leaf extract (11.0 mm) (Table 2). The antibacterial effects exerted by the ethanolic extracts of oven-dried leaf and room-dried leaf showed no statistical difference [t (3) =−3.069, 0.055 (−9.6759 and 1.759)]. The ethanolic extracts of the stem of the plant using two treatments of drying also showed no significant difference [t (3) =−2.970, 0.059 (−10.3569 and 0.3569)]. The antibacterial effect exerted by honey 1 was stronger than that of honey 2, although the difference was not statistically significant [t (3)=−1.769, 0.175 (−4.5930 and 16.0930)].

The MICs and MBCs of honey, room-dried, and oven-dried ethanolic leaf and stem extracts of *A. repens* against *P. aeruginosa* ranged from 25 to 75% concentrations (Fig. 1). The antibiotic sensitivity profile of *P. aeruginosa* ranged from 0 to 25 mm, with the highest activity exhibited by gentamycin. The synergistic antibacterial effects of honey and ethanolic extracts of oven-dried and room-dried leaf and stem of *A. repens* on *P. aeruginosa* (Fig. 2) show that

Table 1 Morphological and biochemical characteristics of *Pseudomonas aeruginosa*

Tests	Reaction
Arginine dihydrolase	+
Catalase	+
Coagulase	−
Gelatin hydrolysis	+
Gram's reaction	−
Hydrogen sulfide production	−
Indole	−
Maltose	−
Mannitol tests	+
Methyl-red	−
Nitrate reduction	+
Oxidase	+
Simmons citrate	+
Starch hydrolysis	+
Sucrose	−
Urease	−
Voges-Proskauer	−

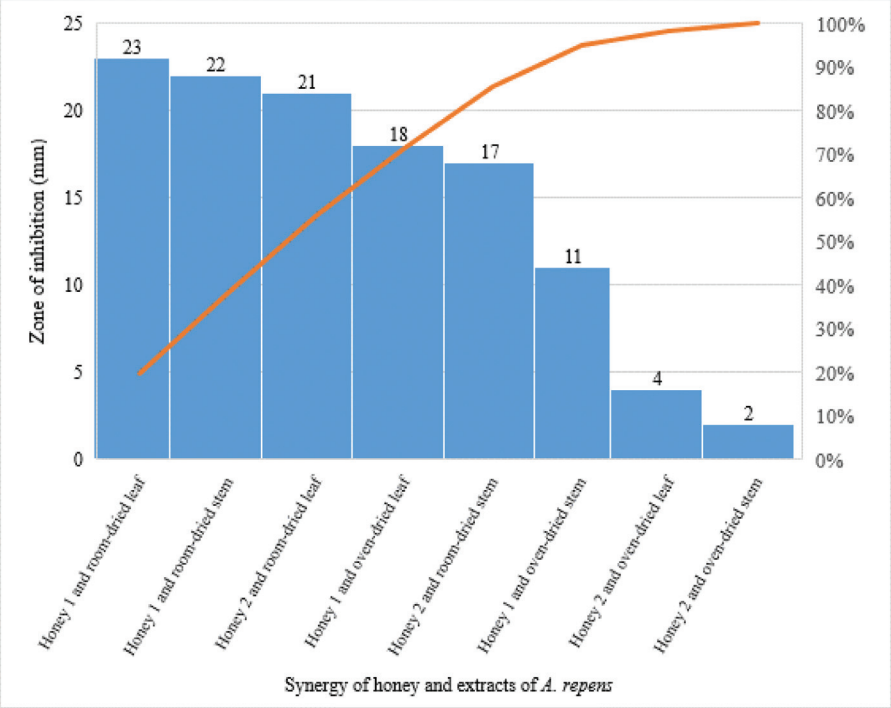
−, negative;+, positive.

Table 2 Anti-pseudomonal activities of honey and ethanolic leaf and stem extracts of *Alternanthera repens*

	Zone of inhibition (mm)			
Antimicrobials	25%	50%	75%	100%
Honey 1	0.0 ± 0.0^{a}	15.2 ± 1.7^{c}	18.0 ± 1.9^{b}	21.2 ± 2.2^{a}
Honey 2	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	13.2 ± 1.2^{a}	18.4 ± 1.9^{a}
Oven-dried leaf	0.0 ± 0.0^{a}	7.3 ± 0.5^{b}	9.1 ± 0.7^{a}	11.0 ± 0.10^{b}
Room-dried leaf	5.3 ± 0.2^{a}	9.0 ± 0.9^{b}	12.2 ± 1.1^{a}	20.2 ± 1.5^{c}
Oven-dried stem	0.0 ± 0.0^{a}	5.3 ± 0.3^{a}	7.2 ± 0.6^{a}	9.3 ± 1.1^{b}
Room-dried stem	4.4 ± 0.6^{a}	8.1 ± 0.6^{ab}	10.0 ± 0.9^{a}	19.3 ± 1.3^{a}

Values show the mean of triplicate experiments±SD.
Data with same alphabetical superscripts along same column show no statistical difference at $P < 0.05$.

Figure 1



Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of honey and room-dried and oven-dried ethanolic leaf and stem extracts of *Alternanthera repens* against *Pseudomonas aeruginosa*.

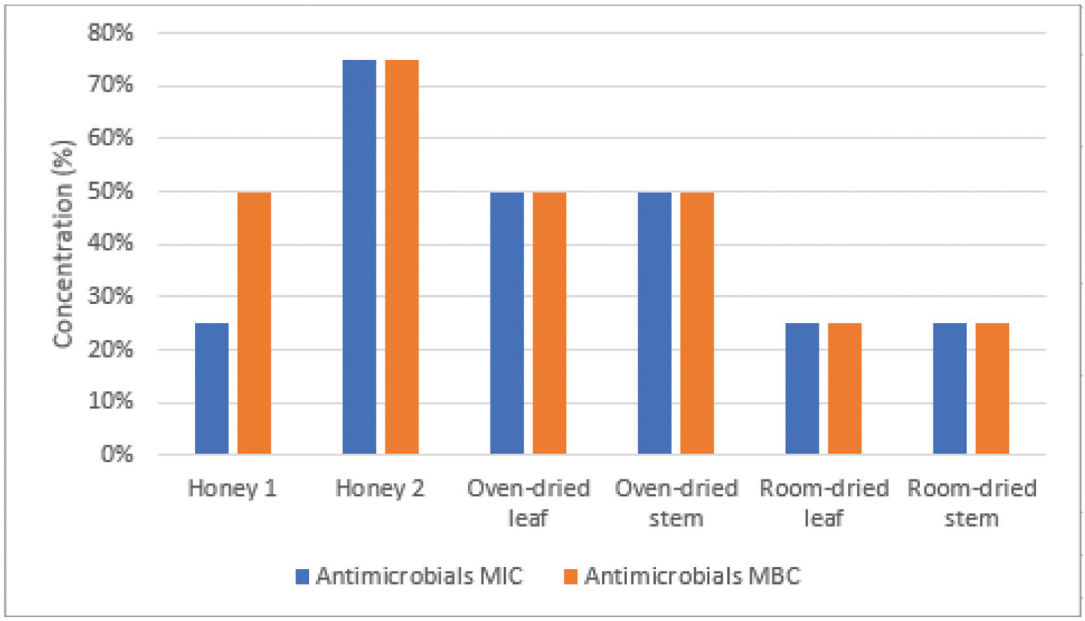
the highest antibacterial activity (23 mm) was exerted by synergy of honey 1 and room-dried leaf, followed by synergies of honey 1 and room-dried stem (22 mm), honey 2 and room-dried leaf (21 mm), honey 1 and oven-dried leaf (18 mm), honey 2 and room-dried stem (17 mm), honey 1 and oven-dried stem (11 mm), and honey 2 and oven-dried leaf (4 mm), whereas the lowest was shown to honey 2 and oven-dried stem (2 mm). The organism exhibited total resistance to augmentin, streptomycin, nalidixic acid, septrin, and ampicillin (Fig. 3).

Qualitative phytochemical screening showed that terpenoids, phytate, cyanogenic glycosides, alkaloids, total flavonoid, tannins, saponin, and total phenols were present in the extracts (Table 3). The quantitative phytochemical constituents of ethanolic extracts of room-dried and oven-dried leaf and stem of *A. repen* showed that total phenol was highest in the room-dried leaf (279.28 mgGAE/g) and lowest in the oven-dried stem (129.55 mgGAE/g) (Table 4). The room-dried stem had the highest quantity of saponin (23.51 mg/g), whereas the lowest was present in oven-dried leaf. The room-dried stem had the highest quantity of tannins (90.59 mg/g), whereas the oven-dried leaf had the lowest (70.5 mg/g). Total flavonoid was highest in room-dried leaf (73.82 mgGAE/g) and lowest in oven-dried stem (30.6 mgGAE/g). Furthermore, the highest quantity of alkaloids was found in room-dried leaf (235.75 mg/g) and lowest in oven-dried stem (105.74 mg/g). The quantities of cyanogenic glycosides, phytate, and terpenoids in the extracts ranged from 0.01 to 1.82 mg/g, 1.29 to 3.01 mg/g, and 2.72 and 3.87 mg/g, respectively. There was a significant difference in the phytochemical constituents of ethanolic extracts of room-dried and oven-dried leaf and stem of *A. repens* (L.) [t (7)=2.633, 0.034 (−0.44925 and 8.37825)].

Discussion

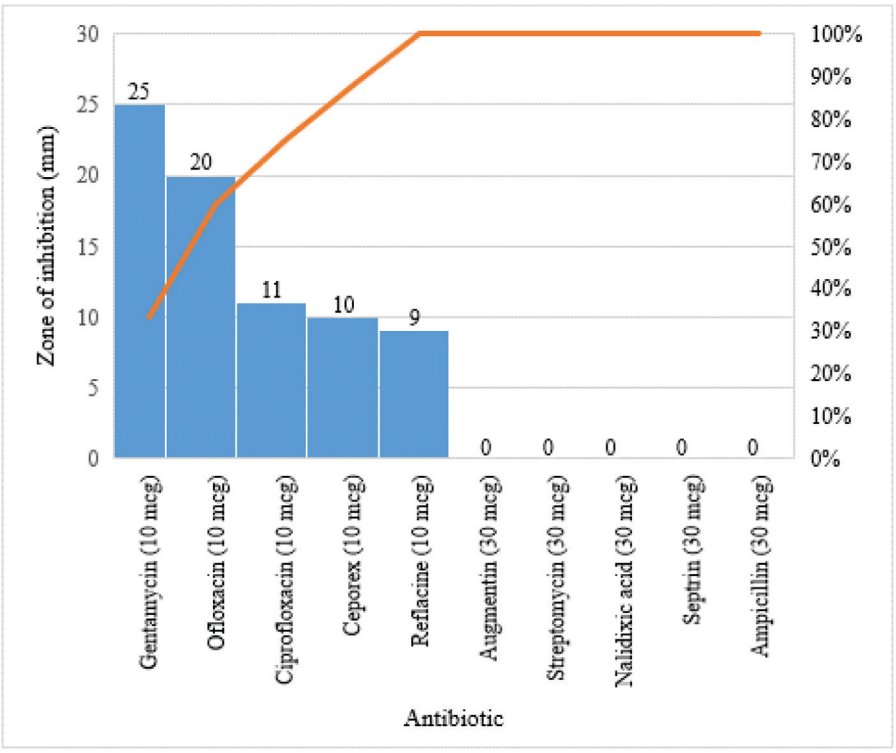
Plants of the genus *Alternanthera* are believed to possess antimicrobial and antiviral properties, although scanty reports were available on the antimicrobial efficacy of *A. repens* at the time of this investigation. Sunil *et al.* [17] reported that the wound healing property of *A*lternanthera *sessilis* might be owing to the inhibitory effect of the plant extract. Adela *et al.* [2] had earlier reported the antidiarrheic potentials of the aqueous and ethanolic extracts of *A. repens* and *Bidens odorata* in Mexican traditional medicine. The morphological and biochemical characteristics of *P. aeruginosa* are given in Table 1. The ethanolic extracts of room-dried and oven-dried leaf and stem of *A. repens* elicited antibacterial

Figure 2



Synergistic antibacterial effects of honey and ethanolic extracts of oven-dried and room-dried leaf and stem of *Alternanthera repens* on *Pseudomonas aeruginosa*.

Figure 3



Antibiotic sensitivity profile of *Pseudomonas aeruginosa*.

activities against *P. aeruginosa* at varying concentrations. This is an indication that *A. repens* can be possibly used in the treatment of diseases and wound infections associated with *P. aeruginosa*. The highest antimicrobial activities of the extracts and honeys were observed at 100% concentration, whereas 25% concentration had the lowest activity (Table 2). This was actually not unexpected because the higher the concentration of an antimicrobial agent, the higher the efficacy.

An earlier study by Oni *et al*. [10] buttressed the ability of the honey to inhibit the growth of *P. aeruginosa* strain at 50, 75, and 100% concentrations. However, at 50% concentration, honey sample 2 did not inhibit the growth of the organism. Honey sample 1 was found to be more effective than honey sample 2, which affirmed that antimicrobial constituents of honey could vary from one source to the other. The difference in antimicrobial potency of different honeys is dependent on their geographical, seasonal, and botanical source as well as harvesting, processing, and storage conditions [18]. It was also observed that room-dried leaf and stem extracts showed higher efficacy than the oven-dried leaf and stem extract (Table 2). The MICs and MBCs of honey and room-dried and oven-dried ethanolic leaf and stem extracts of *A. repens* against *P. aeruginosa* ranged from 25 to 75% concentrations (Fig. 1).

The combined anti-pseudomonal effects of the antimicrobials at reduced concentrations (50%) were significantly higher than the activities elicited unilaterally by the antimicrobials at all concentrations. The combination of honey 1 and room-dried leaf at the reduced concentration exerted the highest antimicrobial activity, whereas the oven-dried stem elicited the lowest efficacy (Fig. 2). The effect of the combination of *A. repens* extracts with honey could lead to increased activity through the use of compounds with synergistic or additive activity. These combinations possess the potentials to thwart drug resistance, decrease required doses of drugs, and thus reduce both cost and adverse effects of drugs, and increase the spectrum of activity [19].

Among the antibiotics, gentamycin elicited the highest activity against *P. aeruginosa*. The organism was not sensitive to augmentin, streptomycin, nalidixic acid, septrin, and ampicillin (Fig. 3). Gentamicin is an aminoglycoside. Its high efficacy is attributable to the mechanism of action of this class of antibiotics which enables it to bind irreversibly to the 16 S rRNA subunit of the 30 S ribosome, resulting in inhibition of bacterial protein synthesis [5,20,21].

The phytochemical analysis of *A. repens* leaf and stem revealed the presence of terpenoids, phytate, cyanogenic glycosides, alkaloids, total flavonoid, tannins, saponin, and total phenols in the extracts, and in different quantities (Tables 3 and 4). Therefore, efficacy of the plant against *P. aeruginosa* could be attributed to the presence of these active ingredients. For instance, Umar *et al*. [22] reported that tannins and alkaloids possessed great antibacterial activity. Tannins had been earlier reported to exert antibacterial activity by binding to and precipitating proteins, whereas alkaloids act by affecting a wide range of cellular molecular targets, such as biological membranes and nucleic acids [4]. The antibacterial effects exerted by the ethanolic extracts of oven-dried leaf and room-dried leaf showed no statistical difference (P=0.055). Honey 1 exerted stronger efficacy than honey, although the difference was not statistically significant (P=0.175). This could be buttressed by the submission of Osho and Bello [4], who reported no statistical difference between the two honey samples used in their study. The difference in the activities of the honeys could be as a result of their

Table 3 Qualitative screening of ethanolic leaf and stem extracts of *Alternanthera repens*

Phytochemicals	Room-dried leaf	Oven-dried leaf	Room-dried stem	Oven-dried stem
Phenol	+	+	+	+
Saponin	+	+	+	+
Tannin	+	+	–	–
Flavonoid	+	+	+	+
Alkaloids	+	+	+	+
Cyanogenic glycosides	+	+	+	+
Phytate	+	+	+	+
Terpenoids	+	+	+	+

+, present.

Table 4 Quantitative phytochemical constituents of ethanolic extracts of room-dried and oven-dried leaf and stem of *Alternanthera repens*

Phytochemicals	Room-dried stem	Oven-dried stem	Room-dried leaf	Oven-dried leaf
Total phenols (mgGAE/g)	131.29±4.30^a	129.55±3.39^a	279.28±4.85^a	269.20±3.52^c
Saponin (mg/g)	23.51±0.99^a	23.06±1.62^a	20.01±2.36^a	13.06±1.11^b
Tannins (mg/g)	90.59±2.27^a	85.63±2.21^b	78.75±1.99^c	70.50±1.48^d
Total flavonoid (mgQE/g)	31.32±2.33^a	30.60±2.69^a	73.82±3.65^b	72.00±3.97^b
Alkaloids (mg/g)	107.31±2.87^a	105.74±2.33^a	235.75±4.31^b	226.23±4.22^c
Cyanogenic glycosides (mg/g)	0.15±0.00^a	1.82±0.04^b	0.01±0.00^a	0.01±0.00^a
Phytate (mg/g)	1.29±0.10^a	1.32±0.09^a	2.85±0.27^b	3.01±0.33^b
Terpenoids (mg/g)	3.53±0.29^a	3.10±0.21^b	2.72±0.19^c	3.87±2.23^a

Data show the mean values of triplicate experiments±SDs.
Data with different alphabetical superscripts along same row showed statistical differences at $P < 0.05$.

different chemical compositions and geographical locations from where they were obtained [4]. There were significant statistical differences between the antibacterial activities exerted by the room-dried leaf and room-dried stem (P=0.015) and honey and the antibiotics (P=0.023).

Conclusions

This study revealed that the ethanolic extracts of *A. repens* possessed antibacterial activity against *P. aeruginosa*, which was more pronounced in combination with honey. The presence of various phytochemicals in the plant indicated that it has a high potential for possible drug production. The results from this study also asserted that room drying is a more reliable method of drying medicinal plants than the oven-drying method. In Nigeria, continuous research needs to be carried out on drugs and pharmaceuticals. This should encompass further exploration of medicinal plants, especially those that are underexploited like *A. repens*. Further studies are needed to fully explore and comprehend bioactive and antibacterial profiles of *A. repens* and its synergy with honey to foster their efficacies.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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