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Ethnomedicinal uses, phytochemical characterization, and antibacterial activity of *Grewia tenax and Albizia anthelmintica* extracts against multidrug-resistant pneumonia-causing bacteria

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The use of *Grewia tenax* and *Albizia anthelmintica* in treating different ailments is attracting significant attention as a primary health care option in Namibia. This study aims to document their ethnobotanical uses, phytochemical composition, antioxidant, and antibacterial activity against multidrug-resistant *Streptococcus pneumonia, Klebsiella pneumonia*, and *Staphylococcus aureus*. The ethnobotanical uses of *G. tenax* and *A. anthelmintica* in treating respiratory conditions were documented. Organic (ethyl acetate) and aqueous extracts were screened for phytochemical composition using the thin-layer chromatography method. The total phenol content was determined using the Folin and Ciocalteu reagent method. *In vitro* antioxidant activity was based on the scavenging activity of the stable 1, 1-diphenyl 2-picrylhyorazyl free radical. Antibacterial activity of extracts (200.0 µg/ml) and antibiotics was determined by the disc diffusion method. *G. tenax* and *A. anthelmintica* are commonly used to treat pneumonic symptoms. Steam inhalation and decoction are the most common methods used in preparing remedies. While alkaloid, flavonoid, and coumarins were detected in all extracts, organic extract of *A. anthelmintica* showed higher total phenol content of 28.5 ± 0. 5 mg GAE/g. *G. tenax* organic extract showed higher *in-vitro* antioxidant activity of 83.3±0.1%. The pathogens showed resistance to 10 µg of penicillin G, and Co-Trimoxazol, however, *A. anthelmintica* organic twig extracts inhibited the growth of the bacteria with average inhibition ranging between 17.5±0.6 - 20.7±0.6, mm and a minimum inhibitory concentration of 50.0 µg/mL. These findings are the first to report on the ethnomedicine of *G. tenax* and *A. anthelmintica* in Namibia and their effectiveness in killing pneumonia-causing bacteria.

Key words: Phytochemical screening, total phenol content, antioxidant activity, antibacterial activity, pneumonia, antibiotic resistance, *G. tenax*, *A. anthelmintica.*

INTRODUCTION

Pneumonia is a common lower respiratory tract infection that affects the alveoli of the lungs. The major causes of

bacterial pneumonia include *Staphylococcus aureus, Klebsiella pneumoniae and Staphylococcus aureu* (Khan et al*.,* 2015). These bacteria use different mechanisms to initiate pneumonia. *S. pneumonia* initiates pneumonia by migrating from mucous to the alveolus of the lungs, where it replicates and initiates host damage responses leading to the definitive progression of lobar pneumonia (Prata and Lacoma*,* 2016). *S. aureus* invades the lung parenchyma where it initiates an infection (Ashurst et al*.,* 2020). *K. pneumonia* has a lipopolysaccharide capsule which is a virulence factor that allows the bacteria to escape opsonophagocytosis by granulocytes and serum complement proteins by the host organism (Reddinger et al*.,* 2018). Regardless of the causing bacteria, the lungs alveoli of pneumonic patients become filled with fluid, causing painful breathing and limiting oxygen intake (Roomaney et al*.,* 2016). Pneumonia can affect people of all ages; however, children aged under 5 years, adults over 65 years, and immune-compromised people tend to be more at risk. An 81% mortality rate due to pneumonia has been reported in children under 2 years old globally (Centers for Disease Control and Prevention, 2012). In Africa, lower respiratory conditions including pneumonia and bronchitis are the leading causes of respiratory deaths. Pneumonia is single-handedly responsible for 16% of global deaths of children under the age of five, with a significantly greater share in Africa (Centers for Disease Control and Prevention, 2017). Lower respiratory ailments including pneumonia were ranked third amongst the top ten causes of death in Namibia in 2012 with a mortality rate of 5%, moving up to being ranked the second highest cause of death in Namibia in 2017 (Henriques-Normark and Tuomanen, 2013; Centers for Disease Control and Prevention, 2017). *S. pneumonia, K. pneumonia,* and *S. aureus* have been reported to be resistant to many antibiotics including vancomycin, chloramphenicol, erythromycin, clindamycin, tetracycline, bacitracin, penicillin G, amoxicillin, oxacillin, methicillin, streptomycin, and gentamicin globally (Ullah et al*.,* 2016; Ahmed et al*.,* 2018).

Over 50,000 plant species are used globally in pharmaceutical and cosmeceutical industries. Studies conducted in India, Kenya, South Africa (Yorka et al*.,* 2011), and Namibia (Cheikhyoussef et al*.,* 2011); have demonstrated the status of medicinal plants in treating pneumonia and other respiratory conditions such as fever, cough, asthma, fatigue, and cold. In ethnomedicine, medicinal plants are normally given to eradicate the symptoms that arise with an illness (Shakya*,*2016). Studies conducted on plants have shown medicinal plant extracts and essential oils have anti-pneumonic activity against common pneumococcal infection-causing bacteria such as *Streptococcus pneumonia, Klebsiella pneumonia*, *Haemophilus influenza*, and *Staphylococcus aureus* (2008; Biscevic-Tolic et al*.,* 2013; Fares et al*.,* 2013; Wang et al*.,* 2019; Houdkova et al*.,* 2008). Extracts and essential oils from *Alpinia brevilabris* C. Presl, *Alpinia cumingii* K. Schum., *Alpinia elegans* (C. Presl) K. Schum., *Callicarpa micrantha* Vidal, *Cinnamomum mercadoi* S. Vidal, and *Piper quinqueangulatum* Miq. *Salvadora persica* fruits have been reported to possess anti-pneumococcal activity (Almaghrabi, 2018; Houdkova et al*.,* 2018). The ethnomedicinal uses of *G. tenax* and *A. anthelmintica* and the validation findings from different parts of the world (Basri et al*.,* 2014; Nawinda, 2016)**.** An increase in microbial resistance to the available medicine used in managing pneumonia and other lower respiratory ailments has raised concern and there is an urgent need for new antimicrobial agents to be used in bacterial anti-pneumococcal therapy and in fighting other respiratory diseases caused by *S. pneumonia, K. pneumonia* and *S. aureus* (Sharma and Patni, 2012; Felmingham et al., 2015)). This study was conducted to document the ethnomedicinal uses in Namibia and determine the phytochemical composition, antioxidant profile, and the *in vitro* antibacterial activity of *G. tenax* and *A. anthelmintica* against multidrug-resistant *S. pneumonia, K. pneumonia*, and *S. aureus* (Methicillin-Resistant Strain).

MATERIALS AND METHODS

Ethnomedicinal survey, plant collection

An ethnobotanical survey on the uses of *G. tenax* (Voucher no. BRL 33) and *A. anthelmintica* (Voucher no. BRL 34) in managing respiratory conditions was conducted in the Omusati region in Namibia in April 2018. A total of 7 community members from Iikokola village were approached in this study. A research permit was obtained from the National Commission on Research Science and Technology and a collection form from the Ministry of Environmental and Tourism of Namibia. The survey focused on the uses of *G. tenax* and *A. anthelmintica* in treating pneumonia and complications that arise with it, parts used, dosages, and methods of preparing herbal remedies from *G. tenax* and *A. anthelmintica*. Twigs and roots of *G. tenax* and *A. anthelmintica* were collected for laboratory analysis. Voucher specimens were collected and sent to the National Botanical Research Institute for botanical identification.

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Extracts preparation

Twigs and roots of *G. tenax* and *A. anthelmintica* were rinsed with distilled water, cut into smaller pieces, and shade dried for 4 weeks. The dry twigs and roots were blended into a fine powder using an industrial bladder. Twenty grams of the plant materials were added to ethyl acetate and distilled water to prepare organic and extracts respectively. Mixers of plant materials and solvents were macerated on a shaker for 48 h at room temperature. After filtration through Whatman no 1 filter papers, the filtrates were concentrated by rotary evaporation and then freeze-dried to form a powder. The powdered extracts were stored at -20ºC. The percentage yield was calculated using the formula:

$$\% \text{ Yield} = (\text{Mass of plant extract}/\text{Mass of plant material}) \times 100.$$

Qualitative phytochemical screening

Ethyl acetate and aqueous twig and root extracts of *G. tenax* and *A. anthelmintica* were screened to detect the presence of alkaloids, flavonoids, coumarins, tannins, anthraquinones, saponins, triterpenoids, and steroids by thin-layer chromatography (Nawinda, 2016). Organic and aqueous roots and twig extracts were spotted onto Thin Layer Chromatography (TLC) plates at a single spot with capillary tubes. The spotted TLC plates were placed in ethyl acetate: toluene: formic acid (4:4:1) as a solvent system. The TLC plates were viewed under the UV chamber at 366 nm and Rf values were calculated (Nawinda*,* 2016).

Determination of total phenol content

The total phenol content of the aqueous and ethyl acetate extracts was determined using the Folin and Ciocalteu reagent, with slight modifications (Chandra et al., 2014). Sample and standard readings were measured using a UV-Vis Spectrophotometer at 550.0 nm against the reagent blank. The extracted sample (4 mg in 2 ml of the extraction solvents) was mixed with 0.6 mL of water and 0.2 ml of Folin-Ciocalteu's phenol reagent (1: 1). After 6 min, 1 ml of saturated sodium carbonate solution (8% w/v in water) was added to the mixture and the volume was made up to 3.0 ml with distilled water. The reaction was kept in the dark for 90 min and after centrifuging the absorbance of blue color from different samples was measured at 550 nm. The phenolic content was calculated as gallic acid equivalents GAE/g of dry plant material based on a standard curve of Gallic acid (0.0–0.1 mg/L). All determinations were carried out in triplicate.

***In vitro* evaluation of antioxidant activity (DPPH radical method)**

Antioxidant activity of the ethyl acetate and aqueous extracts was measured based on the scavenging activity of the stable 1, 1-diphenyl 2-picrylhyorazyl (DPPH) free radical with a few modifications (Sahu et al*.,* 2013). One ml of 0.1 mM DPPH solution in ethyl acetate was mixed with 1.0 ml of plant extract solution of varying concentrations (12.5, 25.0, and 50.0 µg/ml) for 60 min. Corresponding blanks were prepared and L-ascorbic acid in distilled water and ethyl acetate (12.5, 25.0, and 50.0 µg/ml) were used as a reference standard. Ascorbic acid was dissolved in different solvents to evaluate whether the solvents used affects the % inhibition. A mixture of 1.0 ml ethyl acetate and 1 ml DPPH solution was used as a control. The experiment was done in triplicate. The absorbance of samples was measured using a UV-Vis Spectrophotometer at 517 nm. A decrease in absorbance indicated the antioxidant activity. Radical scavenging activity was expressed as percentage inhibition of DPPH and was calculated using the formula:

$$\text{Inhibition } \% = [(A_c - A_s)/A_c] \times 100$$

Where A_c is the absorbance of the control and A_s is the absorbance of the sample. This experiment was repeated three times.

Antibacterial assays

Microorganisms

Organic and aqueous extracts were screened for antibacterial activity. Antibacterial activity for extracts was determined against *S. pneumonia* ATCC 27336*, K. pneumonia* ATCC *13882,* and *S. aureus* ATCC 33591 (MRS). The bacteria strains were revived in Muller Hinton broth and incubated at 37°C for 24 h. After incubation, the turbidity of the bacteria cultures was adjusted to match 0.5 McFarland standard using Muller Hinton broth.

Preparation of plant extracts for antibacterial testing

Antibacterial activity of the *G. tenax* and *A. anthelmintica* twig and root extracts were tested at 200.0 µg/ml. For each extract, a stock solution of 200.0 µg/ml was prepared by dissolving 400.0 µg of the plant extract into 2 ml of ethyl acetate. Antibacterial activity of *G. tenax* and *A. anthelmintica* extracts was determined by the Kirby-Bauer disc diffusion method on Mueller Hinton agar plates (Krishnaveni et al*.,* 2016; Ali and Khan*,* 2018). The results were measured and expressed in terms of zone of inhibition of the bacterial growth around each disc in millimeters whereby: 1-7mm: no activity, 8.0-13.0 mm moderate activity, and ≥14.0 mm: higher activity (Ali and Khan*,* 2018). Antibiotic discs of Gentamycin (10 µg), Erythromycin (15 µg), Co- Trimoxazol (25 µg) and Penicillin (10 µg) were used as positive controls against *S. pneumonia, S. aureus* and *K. pneumonia.* Sterile Whatman no 1 paper discs drenched with ethyl acetate and distilled water were used as a negative control. The experiment was done in triplicate. The minimum inhibitory concentration was determined by the broth dilution method at 37 ºC for 24 hours in Muller Hinton broth. This was done using serially diluted plant extracts of the concentrations between 3.1 and 200.0 µg/ml using the equation: $C_1V_2=C_2V_2$. The Minimum Bactericidal Concentrations assay was determined to confirm the MIC for each extract by culturing the MIC dilution tube on sterile Nutrient agar at 37 ºC for 24 h.

Statistical analysis

All experiments were done in triplicates and statistical analysis was performed employing Graph Pad Prisms software version 7.0. Comparison between groups was done using Two-way ANOVA, followed by Bonferroni posttests' test. All data were presented as mean ± Standard deviation. Results for total phenol quantification, antioxidant activity, and antibacterial activity were considered to be statistically significant $P<0.005$.

RESULTS

Ethnomedicinal uses of *A. anthelmintica* and *G. tenax* in Northern Namibia

A total of 7 community members from Iikokola village were approached in this study. Of all the 7 traditional health practitioners approached in Iikokola village, Omusati region in Namibia, only 6 knew the ethnobotanical uses of *A. anthelmintica* and *G. tenax* in treating pneumonia and conditions associated with it. *A. anthelmintica* and *G. tenax* are mostly used as traditional remedies to treat respiratory pneumonia as well as fever, cough, breathing difficulties, fatigue, and flu that are associated with pneumonia and other respiratory conditions. In Northern Namibia, remedies for pneumonia are prepared in form of decoction and steam inhalation using either root or twigs of *A. anthelmintica* and *G. tenax* as shown in Table 1. Infusions, vapor bathing are also used to manage other respiratory conditions associated with pneumonia as depicted in Table 1.

Qualitative phytochemical screening and total phenol quantification

This study confirmed the higher presence of phytochemical compound classes such as tannins, coumarins, and anthraquinones in *A. anthelmintica* ethyl acetate extracts*;* and flavonoids, coumarins, tannins, and saponins in ethyl acetate extracts of *G. tenax* as shown in Table 2. Phytochemical compounds such as saponins, steroids, and triterpenoids were not detected in aqueous extracts although they were detected in organic extracts as depicted in Table 3. Meanwhile, organic extract of *A. anthelmintica* showed higher total phenol content of 28.5 ± 0.6 mg GAE/gram of dry weight of plant extract as depicted in Figure 1. There was a significant difference ($p<0.0001$) in the total phenol content of *A. anthelmintica* and *G. tenax* aqueous and organic extracts.

Antioxidant activity

Since higher phytochemical composition was observed in twig extract in comparison to root extracts, only twig extracts were evaluated for their antioxidant activity. DPPH radical scavenging activities induced by various tested concentrations of aqueous and ethyl acetate twig extracts derived from *G. tenax* and *A. anthelmintica* as depicted in Table 4. Ethyl acetate and aqueous twig extracts showed *in vitro* antioxidant activity with a concentration-dependent effect in all plants. The percentage inhibition values of ethyl acetate extracts raged between 74.8 ± 0.0 and 83.3 ±0.1%, while for aqueous extracts ranged between 60± 0.0 and 69± 0.0%; with higher antioxidant activity observed in organic extracts in comparison to aqueous extracts. For organic extracts, there was a significant (P-value < 0.0001) dose-dependent increase in the percentage of antioxidant activities for most tested concentrations of *G. tenax* and *A. anthelmintica* when compared with ascorbic acid; except for ascorbic acid and *G. tenax* (P-value= 0.4) at 100.0 µg/ml; as well as ascorbic acid and *A. anthelmintica* (P-value = 0.4) at 200.0 µg/ml. For aqueous extracts, there was a significant (P-value < 0.0001) dose-dependent increase in the percentage of antioxidant activities for most tested concentrations of *G. tenax* and *A. anthelmintica* when compared with ascorbic acid, except for *G. tenax* verses ascorbic acid (P-value= 0.1) at 50.0 µg/ml. Based on the effect of solvent on the antioxidant activity, ethyl acetate showed higher antioxidant activity compared to the aqueous-ascorbic acid standard.

Antibacterial activity of standard antibiotics and plant extracts

Table 5 summarizes the antibacterial activities of (4) antibiotics and the ethyl acetate twigs and roots extracts of *G. tenax* and *A. anthelmintica* against *S. pneumonia, S. aureus,* and *K. pneumonia*. Since none of the aqueous extracts showed inhibitory activity, their results are not showed in Table 5 however the lack of inhibition zones is shown in Figure 2. Only Gentamycin (10 µg) and Erythromycin (15 µg) were able to inhibit the growth of the pneumonia-causing pathogens. While resistance to Penicillin G (10 µg) was recorded against *S. pneumonia, S. aureus,* and *K. pneumonia*; only *S. aureus* showed sensitivity to Co- Trimoxazol (25 µg). Medicinal plant extracts evaluated in this study showed higher phytochemical composition in the organic extract in comparison to aqueous extracts hence, only ethyl acetate extracts were evaluated for their antibacterial activity. *G. tenax* showed a narrow spectrum with efficacy only observed *S. aureus*. However, *A. anthelmintica* showed broad-spectrum moderate to higher antibacterial activity against multi-drug resistant strains of *S. pneumonia, K. pneumonia*, and *S. aureus.* Moreover, it is interesting to notice that *A. anthelmintica* twig extracts showed strong growth inhibitory patterns against *S. pneumonia, K. pneumonia,* and *S. aureus* ranging between 17.5±0.6 - 22.5±0.6 mm which is in the same range as gentamycin and erythromycin 15 µg standard antibiotics (Figure 2).

Table 1. Ethnomedicinal uses of *A. anthelmintica* and *G. tenax* and in treating conditions associated with the respiratory system in Northern Namibia.

Extract ID	Oshiwambo name	Ethnobotanical uses in Namibia	Preparation Method in Namibia	Method description, partly used and dosage in Namibia
G. tenax	*Oshishegele*	Chest pain	Steam inhalation and vapor bath	Roots, bark, leaves, and twigs are crushed, and boiled, the mixture is poured into the basin. The patient is sited near the basin and covered with a blanket and inhale the steam for 10 min. After steam inhalation, the patient is soaked in the basin with the tepid plant water mixture later bathed and massaged with this medicine using a cloth to massage the body.
		Fatigue	Steam inhalation and vapor bath	Roots and bark are crushed, and boiled, the mixture is poured into the basin. The patient is sited near the basin and covered with a blanket and inhale the steam for 10 min. After steam inhalation, the patient is soaked in the basin with the tepid plant water mixture later bathed and massaged with this medicine using a cloth to massage the body.
		Cough	Decoction and steam inhalation	Roots are ground, mixed with boiling water, and The mixture is allowed to boil for about 10 min. The filtrate is collected and taken orally while lukewarm three times daily. After taking decoction roots, bark, leaves, and twigs are crushed, and boiled, the mixture is poured into the basin. The patient is sited near the basin and covered with a blanket and inhale the steam for 10 min. This is done once per day.
		Fever	Steam inhalation and infusion	Roots, leaves, and bark are crushed, and boiled, the mixture is poured into the basin. The patient is sited near the basin and covered with a blanket and inhale the steam for 10 min. After steam inhalation, an infusion prepared by grinding roots, mixing the paste or powder with boiling water, and covering for about 5 min is taken orally three times per day while lukewarm.
		General health respiratory	Decoction and steam inhalation	Roots are ground, mixed with boiling water, and The mixture is allowed to boil for about 10 min. The filtrate of the decoction is collected and taken orally while lukewarm three times daily. After taking decoction roots, bark, leaves, and twigs are crushed, and boiled, the mixture is poured into the basin. The patient is sited near the basin and covered with a blanket and inhale the steam for 10 min. This is done once per day.
		Pneumonia	Decoction	Roots or twigs are ground, mixed with boiling water, and the mixture is allowed to boil for about 10 min. The filtrate of the decoction is collected and taken orally while lukewarm three times daily.
A. anthelmintica	*Omupopo*	Cough	Decoction and infusion	Roots are ground, mixed with boiling water, and The mixture is allowed to boil for about 10 min. The filtrate of the decoction is collected and taken orally while lukewarm 1 time daily. An infusion prepared by grinding roots, mixing the paste or powder with boiling water, and covering for about 5 min is taken orally two times per day while lukewarm.
		Breathing difficulties	Infusion and steam inhalation	An infusion prepared by grinding roots, mixing the paste or powder with boiling water, and covering for about 5 min is taken orally three times per day while lukewarm three times a day. Steaming is done one time per day using crushed roots and twigs and The paste or powder is added to water and boiled, the mixture is poured into the basin. The patient is sited near the basin and covered with a blanket and allowed to inhale the steam for 10 min.
		Pneumonia,	Decoction and steam inhalation	A patient with pneumonia can be given a decoction and steam inhalation treatment. For a decoction, Twigs are ground, mixed with boiling water, and The mixture is allowed to boil for about 20 min. The filtrate is collected and taken orally while lukewarm three times daily. After taking a decoction leaves and twigs are crushed, and boiled, the mixture is poured into the basin. The patient is sited near the basin and covered with a blanket and inhale the steam for about 10 min. This is done once per day.

Table 1. Contd.

	Runny nose	Infusion and steam inhalation	An infusion prepared by grinding roots or twigs, mixing the paste or powder with boiling water, and covering the remedy for about 5 min is taken orally three times per day while lukewarm three times a day. Steaming is done one time per day using crushed roots, bark, and twigs. The paste or powder is added to water and boiled, the mixture is poured into the basin. The patient is sited near the basin and covered with a blanket and allowed to inhale the steam for 10 min.
	Chest pain	Steam inhalation, decoction, and vapor bath	After taking a decoction the patient is soaked in the basin with the tepid plant water mixture later bathed and massaged with this medicine using a cloth to massage the body. This is done once per day

Table 2. Phytochemical screening of ethyl acetate extracts.

Extract ID	Alkaloid	Flavonoid	Coumarin	Tannin	Anthraquinone	Saponin	Triterpenoid	Steroid
G. tenax*: *twigs	+	+++	+++	+++	++	+++	+	-
Roots	++	++	-	++	+	+++	+	-
A.anthelmintica: twigs	+	++	++	+++	+++	+++	-	-
Roots	-	++	++	+	+	-	-	+

Low: + Moderate: ++ High: +++ Absent: -

Table 3. Phytochemical screening of aqueous extracts.

Extract ID	Alkaloid	Flavonoid	Coumarin	Tannin	Anthraquinone	Saponin	Triterpenoid	Steroid
G. tenax*: *twigs	+	+	++	++	-	-	-	-
roots	+	-	++	++	+	-	-	-
A. anthelmintica: twigs	+	++	+	+	++	-	-	-
: roots	-	+	+	+	+	-	-	-

Low: + Moderate: ++ High: +++ Absent: -

For *A. anthelmintica* ethyl acetate extract, the minimum inhibitory concentration (MIC) ranges from 100.0 to 50.0 µg/ml, while for *G. tenax* ethyl acetate extract the MIC value was 200.0 µg/ml (Table 6).

There was a significant difference (P<0.0001) in

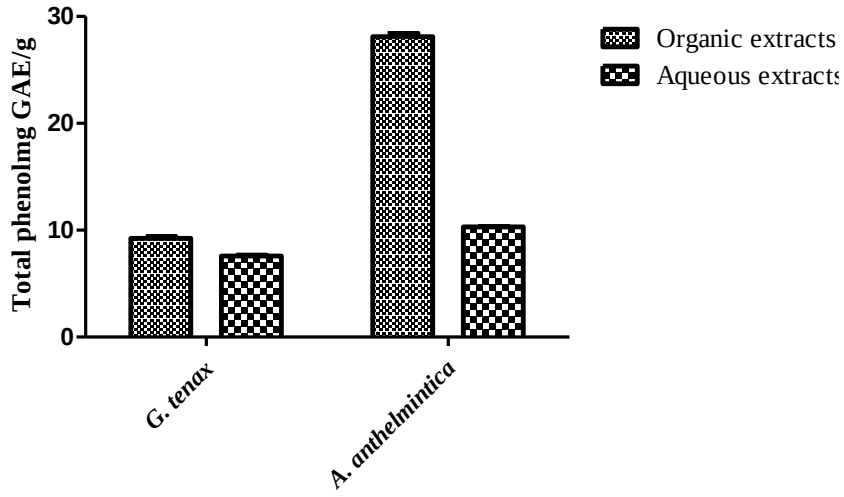

Figure 1. A comparison of total phenolic content (mg GAE/g dry weight of plant extract.) in organic and aqueous twig extracts of *G. tenax* and *A. anthelmintica*; data represent mean ± SD, n =3, p<0.0001.

Table 4. The *In vitro,* antioxidant activity of *G. tenax* and *A. anthelmintica* twig extracts at 12.5, 25, and 50 µg/ml

Sample name	Concentration (µg/ml)	% Inhibition at 517 nm Mean ± SD	
		Ethyl acetate twig extracts	Aqueous twig extracts
Ascorbic acid	12.5	N/A	60± 0.041*
	25	76.80±0.005*	62± 0.042*
	50	82.98± 0.012*	65± 0.043
G. tenax	12.5	75.16±0.460	61± 0.042*
	25	77.54±0.065*	63± 0.060*
	50	83.26±0.01*	65± 0.0442
A. anthelmintica	12.5	77.80±0.662*	65± 0.014*
	25	79.53±0.116*	67± 0.043*
	50	83.08±0.481	69± 0.044*

SEM: standard error of the mean; *: P < 0.0001 (tested concentrations vs. Ascorbic acid), n=3, N/A: Not Analyzed

Table 5. Antibacterial activity of organic twig and roots extracts compared to 4 standard antibiotics against *S. pneumonia, S. aureus,* and *K. pneumonia* at 200 µg/ml (mm ±SD).

Sample name	Part used	Inhibition zones in mm		
		Organic extracts		
		S. pneumonia	*K. pneumonia*	*S. aureus*
G. tenax	Twigs	0	0	10.7±0.58*
	Roots	0	0	0

Table 5. Contd.

A.anthelmintica	Twigs	20.7±0.58*	17.5±0.58*	18.3±1.00*
	Roots	15.5±0.58*	12.5±0.58*	10.3±0.58*
Gentamycin		22.5±0.58*	20.5±0.58*	21.0±0.00*
Penicillin G		0	0	0
Erythromycin		16±0.58	19±0.58	20±0.58
Co-Trimoxazol		0	0	20±0.58
Methanol		0	0	0

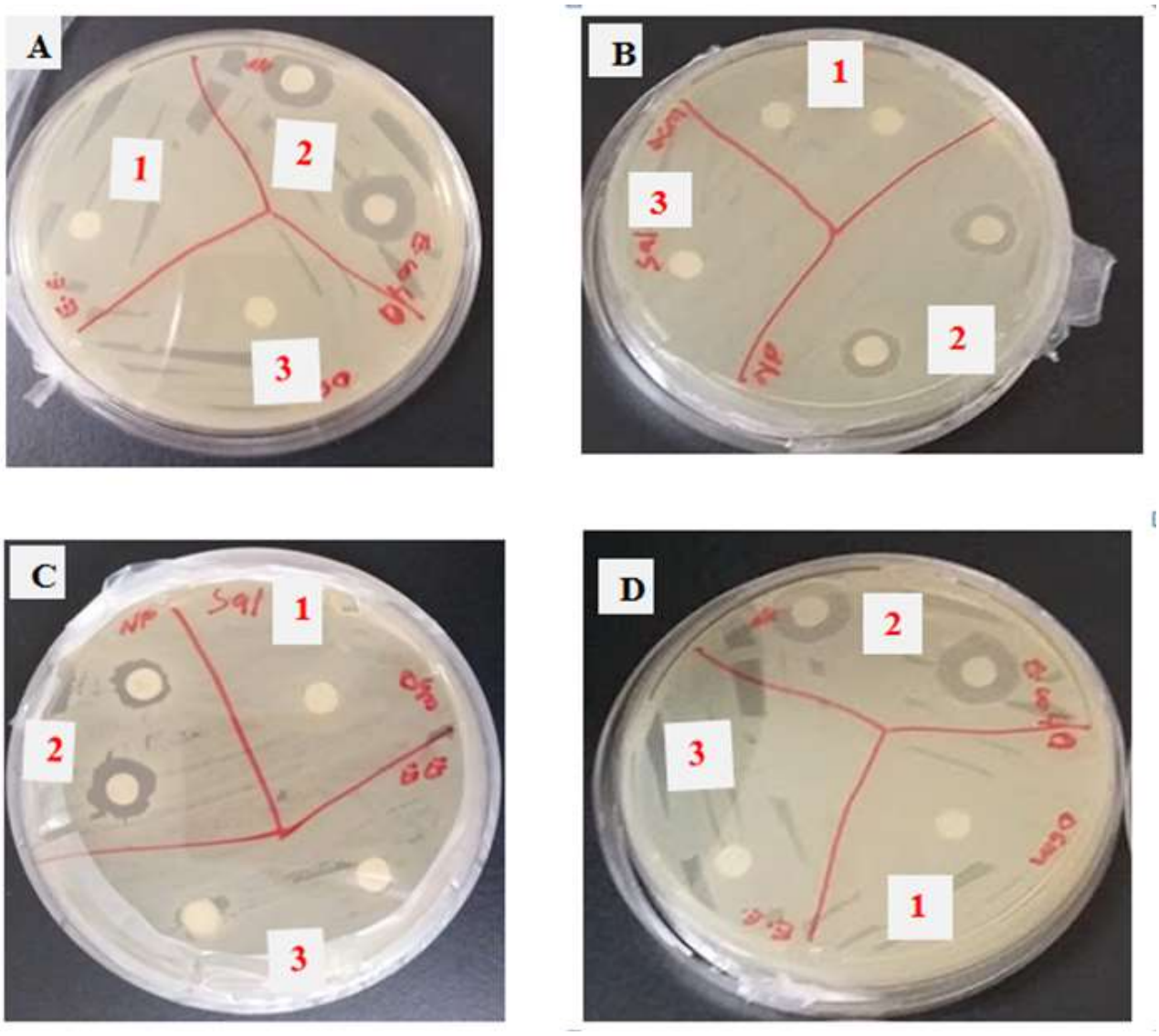

Figure 2. Growth inhibition of Aqueous (1), Organic (2) extracts, and ethyl acetate solvent (3) obtained from *A. anthelmintica* against (A) *S. pneumonia*, (B) *S. aureus*, and (D) K. *pneumonia*, as well as Aqueous (1) and Organic (2) extracts obtained from *G. tenax* against (C) *S. aureu.*

Table 6. Minimum inhibitory concentration *G. tenax* and *A. anthelmintica* organic extracts.

Plant name	Part used	MIC of plant extract		
		Organic extracts		
		S. pneumonia	***K. pneumonia***	***S. aureus***
G. tenax	Twigs	0	0	200±0.00
	Roots	0	0	0
A. anthelmintica	Twigs	50±0.00	50±0.00	100±0.00
	Roots	100±0.00	100±0.00	100±0.00

MIC values are expressed in µg/ml; SEM: standard error of the mean; n=3; 0: No activity.

the antibacterial activity of *A. anthelmintica* and *G. tenax* ethyl acetate extracts when compared to gentamycin. Moreover, *A. anthelmintica* twig and root extracts showed antibacterial activity against *S. pneumonia, K. pneumonia*, and *S. aureus* even though these bacteria showed resistance to penicillin 10 µg and Co-Trimoxazol 25 µg as depicted in Table 4. The ethyl acetate and distilled water used as negative control did not show any growth inhibitory properties against the test organisms. This shows that the observed antibacterial activity was due to the plant and not the solvents used in extraction. To compare the different parts used, twig extracts of *A. anthelmintica* showed higher antibacterial activity than the root extracts.

DISCUSSION

According to the World Health Organization, 80% world population use Phyto-therapy as a primary health care option to manage different diseases. Plants such as *G. tenax* and *A. anthelmintica* have been used for centuries to manage different ailments. While *A. anthelmintica* is reportedly used to treat complications associated with the respiratory system by people in the Oshikoto region in Northern Namibia (Ouarhacha et al*.,* 2020), there is no published data on the ethnobotanical uses of *G. tenax* in Namibia and this makes the findings of this study first to report its ethnobotanical uses in treating respiratory complications in Namibia. This knowledge had guided the validation assays conducted in this study. The traditional methods such as steam inhalation, vapor bath, infusions, and decoctions reported in this study are recommended when caring for patients with respiratory problems as they provide relief to patients with respiratory complications by thinning mucus, relieves congestion, and coughing (Nawinda, 2016). With drug-resistance being a never-ending problem, it is important to look for alternative treatment options, such as the use of natural products and the application of traditional healing methods specially to manage respiratory diseases. There is limited data on the global ethnomedicinal methods for managing pneumonia and symptoms that manifest with it, hence the finding of this study is the first to document the detailed ethnomedicinal methods as depicted in Table 1.

The effectiveness of medicinal plants in healing diseases is due to the presence of different phytochemical compounds and antioxidant activity (Alamgeer and Asif, 2018). Nawinda (2016)'s report on the phytochemical screening and total phenol and flavonoid quantification of *A. anthelmintica* roots, bark, and leaves agrees with the findings of this study on phytochemical profiling. However, coumarins, triterpenoids, and steroids reported in this study were not reported by other studies (Basri et al*.,* 2014; Berbadeta et al*.,* 2020). While antioxidants are crucial for normal lung function, it is important to understand the right amount required since both increased oxidants or decreased antioxidants may reverse the physiologic oxidant-antioxidant balance in favor of oxidant leading to lung damage (Kurutas, 2016).

Multi-drug resistant pathogens such as *S. pneumonia, K. pneumonia*, and *S. aureus* were reported to cause 138 million pneumonia cases globally in children in 2015. In 2017, a total global mortality rate due to pneumonia was 2.56 million of which 800,000 were children under 5, with most cases caused by *S. pneumonia, K. pneumonia*, and *S. aureus* (Nawinda*,* 2016). The global burden of antibiotic resistance among respiratory pathogens calls for alternative effective medicine to combat pneumonic infections, especially in children. The higher antibiotic resistance pattern reported daily toward pneumonia-causing pathogens calls for an agent need for alternative treatments for pneumonia that are affordable and safe for use especially for pediatrics. To determine a new source of natural antibiotics, this study focused on the ethyl acetate extracts of *G. tenax* and *A. anthelmintica* and

their antibacterial activity against resistant bacteria strains. A study by Alamgeer and Asif (2018) listed other plants commonly used to treat respiratory diseases and their pharmacological properties.

These extracts can serve as potential alternative sources of life-threatening pneumonia-causing pathogens. In this study, the chemical composition and efficacy of two commonly used plants in Namibia were evaluated against common respiratory pathogens. Natural antioxidants exhibit a wide range of biological effects (Basri et al*.,* 2014). However, despite the higher antioxidant activity observed in *G. tenax,* it showed narrow-spectrum antibacterial activity. The different phytochemical compounds present in *G. tenax* could be responsible for the antibacterial activity detected in this study against *S. aureus*. The lowest minimum inhibitory activity of *A. anthelmintica* crude extract observed against *S. pneumonia* and *K. pneumonia* at 50.0 µg/ml makes these extracts valuable for further *in vivo* investigation against pneumonia-causing pathogens as shown in Table 6.

This study indicates the competence of the plants obtained from the ethnobotanical knowledge holders in Iikokola village. The results from this study form a basis for further investigation into the potency of these plants, to isolate the compounds responsible for the anti-pneumococcal activity, and suggest that the plants tested may be a source of new antibiotic compounds against these antibiotic-resistant bacteria. Different secondary metabolites present in the plant extracts could be responsible for the antibacterial activity reported in this study. Phytochemical compounds and antioxidants in plants make plants effective in eliminating multidrug-resistant pathogens by utilizing different mechanisms to destroy pathogens (Biscevic-Tokic et al*.,* 2013).

Conclusion

This study is the first report on the ethnomedicinal uses of *A*. *anthelmintica and G. tenax* in treating respiratory conditions in Namibia. The ethyl acetate extracts derived from the roots and twigs of *A. anthelmintica and G. tenax* possess antioxidant, and antimicrobial properties and have a variety of phytochemical compounds. In particular, *A. anthelmintica* ethyl acetate extracts are a potent source of bioactive molecules that can be further utilized as a prominent alternative bio-resource in drug discovery efforts in attempting to fight pneumonia-causing bacteria caused by multidrug-resistant *S. pneumonia, S. aureus,* and *K. pneumonia*. The observed efficacy collates with the ethnomedicinal uses of these plants especially in fighting bacterial pneumonia. These findings add value to the ethnomedicinal uses of the plants and form a significant basis for further studies aiming for drug development for pneumonic infections. Moreover, the ethnomedicinal methods used to manage pneumonia reported in this study could be standardized and incorporated into the western health care system to manage pneumonia and other respiratory system complications.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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