ANTIMICROBIAL ACTIVITIES OF *Artemisia absinthium* AQUEOUS AND ETHANOLIC EXTRACT ON RESPIRATORY TRACT PATHOGENS (*Escherichia coli* and *Salmonella spp*)

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ABSTRACT
Since modern medicine has been beyond the financial reach of a substantial proportion of the third-world population, using plants as herbal medicine is emerging as a safe and cheap alternative pharmaceutical. The study aimed to determine the antimicrobial activities of *Artemisia absinthium* extract. The respiratory tract pathogens used in this work were obtained from the Microbiology Laboratory Unit of the Department of Science Laboratory Technology Umaru Ali Shinkafi Polytechnic Sokoto. The *Artemisia absinthium* was obtained from the Botanical Garden of the Department of Science Laboratory Technology Umaru Ali Shinkafi Polytechnic Sokoto. It was transferred in a polyethylene bag and then taken for analysis. The isolate was purity tested by sub-culturing the test organisms on fresh agar plates and carrying out biochemical tests such as Gram staining, indole test, catalase and coagulase test to identify the organisms. The incubation temperature was done at 37^{o}C for 24 hours. Agar well diffusion techniques were used. The results above showed that the ethanolic extract of *Artemisia absinthium* was slightly effective against *Salmonella spp and Escherichia coli* at all concentrations. In contrast, the water extracts were not effective on the test organisms. However, the control was sensitive to the test organisms at all concentrations. Therefore, experiments should be conducted at higher concentrations of the aqueous and methanolic extracts to assess their activity on Multidrug-Resistant Organisms.

Keywords: *Artemisia absinthium;* antimicrobial;

1.1 INTRODUCTION
Plants have been widely recognized as an essential source of novel therapeutic compounds since ancient times for treating various diseases and were reported in traditional medicine systems such as the Siddha and Ayurveda (Khanna and Chandra, 2012). Bioactive compounds are known for their antitoxin resistance and as a reliable source of antimicrobial treatments. They are widely used as anti-infective supplements and adjuncts with other compounds (Blaszyle and Holley, 2018). However, the properties of natural-based mixtures need to be carefully investigated to determine their pharmacological effects on biological systems (Ushimaru *et al.,* 2007). Approximately 80% of the world's medicines depend on plant-based bioactive components for curing various diseases (Owolabi *et al.,* 2007).

Respiratory tract infections (RTIs) are among the most common and diverse group of infections that have continually been a major cause of morbidity and morbidity in clinical medicine (Malosh *et al.,* 2017). RTI is any infectious disease of the upper or lower respiratory tract. Upper respiratory tract infections (URTIs) involve the common cold, tonsillitis, laryngitis, pharyngitis, rhinitis, and otitis media. At the same time, lower respiratory tract infections (LRTIs) include acute bronchitis and pneumonia (Wang *et al.,* 2016). Bacteria such as *Pseudomonas aeruginosa, Streptococcus pneumoniae, Moraxella catarrhalis, Haemophilus influenzae* etc, are among the causative agents of RTI (Assane *et al.,* 2018). URTIs are also the cause of most antibiotic use. Approximately 60% of all outpatient antibiotics used are for respiratory infections, particularly acute bacterial sinusitis for adults and acute bacterial otitis for children (Jong *et al.,* 2012).

The most common bacteria that cause respiratory tract infection are *Pseudomonas spp., Streptococcus spp., Proteus spp., Klebsiella spp., Staphylococcus spp., Enterobacter spp., Acinetobacter spp.,* and *Haemophilus influenza* (Sabry 2014) These above-listed bacteria are frequently resistant to commonly used *et al.,* antibiotics like ampicillin, amoxicillin and kanamycin (Raft *et al.,* 2017). Due to this fact, scientists have recently given more attention to extracting active biological compounds from natural species used in herbal medicine (Peters *et al.,* 2011).

The phytochemicals such as alkaloids, saponins, steroids, tannins, flavonoids, amino acids, and trigonillin were responsible for biological activity. The leaves and seeds have been widely used to prepare concentrates and powders for restorative uses (Swati *et al.,* 2014). The therapeutic properties of medicinal plants come from their phytochemical components. The general phytochemicals that significantly cause effective results in human health care are alkaloids, flavonoids, tannins and glycosides. The phytochemical screening of *A. nilagirica* revealed the presence of various phytochemicals such as tannins, alkaloids, flavonoids, terpenoids and glycosides (Arokiyaraj e*t al.,* 2012). In another study, the presence of phytochemicals such as alkaloids, phenol, tannins, flavonoids, amino acids, quinines and terpenoids were reported from this plant. *A. absinthium* has been reported to have efficiency against various neurological disorders, antimicrobial, dermal infection, antifungal, larvicidal, and anti-inflammatory activities (Ahameethunisa and Hopper, 2010). Also, various secondary metabolites such as terpenoids, flavonoids, polysaccharides and saponins were characterized using Gas Chromatography–Mass Spectrophotometer (GC–MS), Performance Liquid Chromatography (HPLC) and Nuclear magnetic resonance spectroscopy (NMR) (Xie *et al.,* 2008, Avula *et al.,* 2009). The antimicrobial compounds from *Artemisia* sp. were used as alternate medicine in the food industry (Ng, 2004).

1.2 Statement of the Problems

Bacterial species such as *Salmonella, Pseudomonas* and *Staphylococcus* cause various diseases. *Pseudomonas aeruginosa* causes respiratory tract infections or sepsis in patients with cystic fibrosis or suppression of the immune system (Esen *et al.,* 2001). *Salmonella enterica* serovar Typhi causes typhoid fever (Dougan and Baker, 2014). The genus *Proteus* includes facultative anaerobic, Gram-negative, proteolytic, and heterotrophic rods, which are human opportunistic pathogens (Drzewiecka, 2016). In recent years, the rates of antibiotic resistance in *Pseudomonas aeruginosa* are increasing worldwide. The multidrug-resistant phenotype in *P. aeruginosa* could generally be mediated by various mechanisms, including enzyme production, multidrug efflux systems, loss and target mutations and outer membrane protein (Hirsch and Tam, 2010). Multiple drug resistance is a significant health problem in the treatment of staphylococcal infections, mainly infections of methicillin-resistant *Staphylococcus aureus*, which occurs mainly due to the extensive use of antimicrobial substances, coupled with the transmission of pathogenic organisms by person-to-person contacts (Okeke and Lamikanra, 2003). Hence, effective control of antibiotic applications and prevention of the transmission of these pathogenic strains are essential to eradicate this highly infectious organism. Among the world's medicinal plants, the biological activity of the genus Artemisia has been comparatively less explored against various pathogenic bacteria and anti-inflammatory activity. Hence, the anti-respiratory and antibacterial properties were carried out in this study.

1.3 Justification of the Study

In recent decades, antimicrobial herbal products have been included in the special interests of researchers because of a rapid increase in antibiotic resistance in microorganisms (Essawi and Srour, 2000). Many members of the Genus *Artemisia* are important medicinal plants. Previously, the antibacterial effects of the *Artemisia* species have been reported (Guangrong *et al.,* 2008). *Artemisia absinthium*, a species of wormwood, grows in temperate regions of Eurasia and Northern Africa. Plant extracts have been shown

to exhibit vigorous antimicrobial activity, especially against Gram-positive pathogenic bacteria (Fiamegos *et al.*, 2011). According to WHO (2008), there is a lack of scientific evidence to evaluate the safety and efficacy of traditional medicine. Therefore, there is a need to screen medicinal plants for better understanding of their properties, safety and efficacy, validate their traditional uses, and identify the active compounds. This study aims to determine the antibacterial activities of *Artemisia absinthium* ethanolic extract on some Respiratory tract pathogens.

1.4 Aim of the study

The aim is to study the antibacterial activities of *Artemisia absinthium* ethanolic extract on some selected respiratory tract pathogens.

1.5 Objective of the study

The specific objectives are:

i. To determine the antibacterial potential of *Artemisia absinthium* ethanolic extract
ii. To determine the minimum inhibitory concentration of *Artemisia absinthium* ethanolic extract on some selected respiratory tract pathogens.

MATERIALS AND METHODS

Source of Sample

The respiratory tract pathogens used in this work were obtained from the Microbiology Laboratory Unit of the Department of Science Laboratory Technology Umaru Ali Shinkafi Polytechnic Sokoto.

Source of Extracts

The *Artemisia absinthium* was obtained from the Botanical Garden of the Department of Science Laboratory Technology Umaru Ali Shinkafi Polytechnic Sokoto. It was transferred in a polyethylene bag and then taken for analysis.

Identification of test organisms

The test organism was collected from the Microbiology Laboratory in the Department of Science Laboratory Technology Umaru Ali Shinkafi Polytechnic Sokoto. The isolate was purity tested by subculturing the test organisms on fresh agar plates and carrying out biochemical tests such as Gram staining, indole test, catalase and coagulase test to identify the organisms.

Gram staining

This reaction is done to identify organisms that are Gram-positive (+ve) and Gram-negative (-ve)

Procedure – A smear of the isolate was made on a clean, grease-free slide, air-dried, and heat-fixed. The slide was flooded with 0.5% solution of crystal violet and allowed for 30 seconds. The stain was washed off with water, flooded again with lodine solution (mordent), and allowed for 10 seconds, after which it was washed off. The slide was counter-stained with safranin for 30 seconds, rinsed with water, and air-dried. The stained slide was viewed under the microscope using immersion oil under an x100 objective lens (Cheese Bough, 2002).

Biochemical Tests

Indole Test

Procedure – The test organism (isolate) was inoculated in a tube containing 3 ml of sterile tryptone water. Incubation was done at 37^0C for 24 hours. 0.5ml of Kovac's reagent was added and shaken gently. The examination for a red ring-like color on the surface of the layer within 10 minutes was done. Ochei et al. (2001).

Catalase Test

Procedure – This was performed by dropping a loopful of hydrogen peroxide on a clean, grease-free slide and mixing the loopful of isolate with the hydrogen peroxide on the slide. The production of gas bubbles from the mixture, which occurred almost immediately, is a positive reaction

$2H_20_2 \rightarrow 2H_2 + O_2$

Coagulase test

This test is used to differentiate *staphylococcus aureus* from *streptococcus sp*. It is known to cross-link the β chain of fibrinogen in plasma to form a fibrin clot that deposits on the cell wall. A loopful of the test isolate is smeared on a slide, mixed with normal saline and treated with a drop of serum, which is then mixed. Agglutination or clumping occurs within 5- 10 seconds, which shows positive.

Antibacterial Sensitivity Test

The antibacterial activity of *Artemisia absinthium* against the three pathogens was tested in vitro using the suitable diffusion method (Kirby Bauer's method). The test materials were prepared by diluting each honey in sterilized double distilled water at different dilutions (concentrations) of 20%, 40%, 60%, and 80% net honey (100%). Nutrient agar plates were prepared, and each plate was properly inoculated with each test organism using the streaking method with the help of a sterile wire loop. Wells were made using a sterile cork borer, and each well was filled with different concentrations of honey. The plates were incubated at 37^0C for 24 hours and observed for the zone of inhibitions. This in-vitro experiment was compared using a sensitivity disc (Augmentin) as a control.

RESULTS

Artemisia absinthium's ethanolic and water extract was slightly effective against Salmonella spp and Escherichia coli at all concentrations. However, the controls were sensitive to the test organisms at all concentrations.

Table 1: Antibacterial activity of *Artemisia absinthium*

Sample	**Isolate**	**Extract**		**Control**	
		Conc (mg/mg)	**Zone inhibition (mm)**	**Antibiotics (mg)**	**Zone of inhibition (mm)**
Ethanolic extract	*Salmonella species*	10	2.6	Cefraxone	5.9
		20	2.6	Ciprofloxacine	6.0
		30	3.0		
		40	3.4		
Water extract	*Salmonella species*	10	3.1	Cefraxone	5.9
		20	0.0	Ciprofloxacine	6.0
		30	2.4		
		40	0.0		
Ethanolic extract	*Escherichia coli*	10	0.0	Cefraxone	5.0
		20	0.0	Ciprofloxacine	5.3
		30	2.4		
		40	2.1		
Water extract	*Escherichia coli*	10	2.0	Cefraxone	5.0
		20	0.0	Ciprofloxacine	5.3
		30	0.0		
		40	0.0		

The results above showed that the ethanolic extract of *Artemisia absinthium* was slightly effective against *Salmonella spp and Escherichia coli* at all concentrations. In contrast, the water extracts were not effective on the test organisms. However, the control was sensitive to the test organisms at all concentrations.

DISCUSSION

The present study showed that *Salmonella spp and Escherichia coli* were resistant against *Artemisia absinthium* while the control Ciprofloxacine and cefrazine were sensitive to *Streptococcus spp*. Thus, the test organisms' resistance in this study may be related to geographical location, which may have affected the plant due to weather and environmental conditions. This finding contrasts with the work of Paramestwari et al. (2019), who found that *Artemisia absinthium* possesses antibacterial and antifungal activities. The results above showed that the ethanolic extract of *Artemisia absinthium* was slightly effective against *Salmonella spp and Escherichia coli* at all concentrations. In contrast, the water extracts were not effective on the test organisms. However, the control was sensitive to the test organisms at all concentrations.

Artemisia is a large, diverse genus of plants with about 500 species belonging to the daisy family Asteraceae (Saban *et al.*, 2013). Some species of Artemisia are *Artemisia abrotanum* L., A. afra, *A. annua L., A. arborescens, A. arenicola, A. maritima, A. capillaris, A. dracunculus, A. stricta, A. laciniata, A. wallichiana, A. Japonica* and *A. siversiana*. *Artemisia absinthium* Linn. is a vital member of this genus and recognized as the source of a Unani drug "Afsanteen." A. absinthium is commonly called wormwood and is locally known as 'Tethwen' in the Kashmir Valley, India. It is used in indigenous medicine as a vermifuge, an insecticide, an antispasmodic, an antiseptic, and in the treatment of chronic fevers and inflammation of the liver (Ahamad *et al.*, 2012).

It is noted that the antibacterial activity against *Staphylococcus aureus* (sensitive and resistant strains), *Salmonella typhi, E. coli, Proteus vulgaris, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* and antifungal activity against *Candida albicans, C. utilis* and *Aspergillus niger* by the serial dilution method. The essential oil at 1:1000 dilutions was active against both sensitive and resistant strains of *S. aureus, K. pneumoniae* and *P. aeruginosa*. According to an investigation, ethanol extracts of A. absinthium branches inhibit *Staphylococcus aureus* with inhibition zones 10-15 mm in diameter. However, it has not shown antibacterial potential against *Candida albicans, E. coli, Streptococcus faecalis* and *Bacillus subtilis* (Juteau *et al.*, 2013).

CONCLUSION

In conclusion, Artemisia absinthium's ethanolic and water extract was slightly effective against *Salmonella spp and Escherichia coli*.

RECOMMENDATIONS

From the results obtained in this study, it is recommended that:

i. More experiments should be carried out at higher concentrations on methanolic extracts to assess their activity on *Streptococcus spp*
ii. The Ministry of Health should ensure that all herbal products are subjected to scientific verifications before being sold as a remedy.

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