Chemical compounds, FTIR and *in vitro* antibacterial analyses of the acetone stem bark extract of *Ziziphus mucronata* subsp. *mucronata* (Buffalo Thorn) against potential nosocomial bacterial pathogens

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Abstract

Ziziphus mucronata is a commonly used plant in South Africa for treatment of infections and diseases. The study aimed at determining the antimicrobial activities and the pharmacologically active chemical compounds present in the acetone extract of this plant. The pharmacologically active chemical compounds in this extract were determined using gas chromatography-mass spectrometry (GC-MS). Their antibacterial activity was assayed *in vitro* by agar well diffusion and macrobroth dilution techniques against different bacterial isolates. Fourier transform infrared (FTIR) spectroscopy was used to determine the functional groups of the chemical compounds in the plant extract. The GC-MS result showed 52 chemical compounds with 29 compounds having reported pharmacological activities (while 23 compounds have not been reported), with1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl)cyclohexane, 2,6-β-17-β-Trihydroxy-6-α-pentyl-2,3-seco-5-α-androstan-3-oic acid-γ-lactone, 2,3,4,5-tetrahexyl-dimethyl ester Hexanedioic acid, i-Propyl 9-tetradecenoate, (3-β-22E)-Ergosta-7,22-dien-3-ol, Ergosta-4,22-dien-3-one, 3,4-dimethoxy-methylmonoacetalBenzaldehyde, 6-(acetyloxy)-4-methyl-4-Hexenoic acid and O-α-D-glucopyranosyl-α-D-Glucopyranoside being compounds amounting for ≥1%. The FTIR spectrograph indicated that the identified compounds are amines, amides, alkanes, aldehydes, diketones, nitrosamine, esters, alkyl amine, secondary and primary alcohols and sulfoxide groups which are present in the plant extract. The Minimum Inhibitory Concentrations (MICs) ranged between 0.31 mg/mL and 5.5 mg/mL against the test bacterial isolates. This study shows the biological activities of *Ziziphus mucronata* extract depend greatly on the varied concentrations of the chemical compounds identified in the acetone extract and indicated the potential importance of this plant as a significant source of novel compounds for the treatment of diseases over synthetic antibiotics.

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Introduction

Infectious diseases have become a burden to the global health community due to the alarming public health threat they pose globally.[1] Bacteria and other microorganisms able to cause infections are extraordinarily irrepressible and have developed resistance towards the synthetic and semi-synthetic antimicrobial agents.[2,3] These resistances to antimicrobial chemotherapeutic agents in human pathogenic bacteria have risen to an alarming rate globally, causing a higher morbidity and mortality rate than the susceptible microorganisms. This increase in antimicrobial resistance is due to the disturbing level of the indiscriminate use of antibiotics resulting in a great challenge to human health. Therefore, alternative antimicrobial strategies have been sought for in plants to serve as chemotherapeutic agents against infectious diseases.[3]

Although bacteria have exhibited great levels of resistance to antibiotics and it is estimated that about 75% of useful plant derived pharmaceuticals used globally are discovered by systematic investigation of novel compounds from traditional medicines,[4] an estimated 80% (4.8 billion people) of the world's population depend on medicinal plants for health care because they are generally free or readily available at very affordable cost. Daily dietary intake of flavonoids from fruits, vegetables, wine, tea, chocolate and other cocoa products and similar polyphenols exceeds that of antioxidant vitamins and provitamins.[5] While Macéé and Truelstrup[6] indicated that phytochemicals are responsible for the medicinal activity of plants, Edeoga *et al.*[7] reported that the phytochemicals constitute a reservoir of new natural antimicrobial therapeutic substances to be discovered. These phytochemicals which are primary and secondary metabolites include terpenoids, alkaloids, tannins and phenolic compounds exhibiting diverse important therapeutic potentials and pharmacological activities.

Although alternative antimicrobial strategies are urgently needed as the efficacy of antibiotics is now significantly limited, it is of utmost importance to qualitatively and quantitatively identify new sources of therapeutically valuable compounds having medicinal values. Thus, various phytochemical analyses have been used for the identification of specific phytochemicals. Of these quantitative phytochemical analyses, Gas Chromatography-Mass Spectrometry (GC-MS) analysis is the ultimate tool to identify biologically active molecules. It separates the components of a mixture and makes use of mass spectrometry to characterize each of the components individually. The predominant compounds identified in such analysis in different crude extracts are biologically active molecules.[8]

Ziziphus mucronata subsp. *mucronata* is a small to medium sized tree with a spreading canopy. It is distributed throughout summer rainfall areas of sub-Saharan Africa extending from South Africa northwards to Ethiopia and Arabia. The members of the taxon are drought tolerant and very resistant to heat.[9] A decoction of the glutinous roots is commonly administered as a painkiller for all sorts of pains as well as dysentery. A concoction of the bark and the leaves is used for respiratory ailments and other septic swellings of the skin. In ethnomedicine, the pastes of the roots and leaves are used to treat boils, swollen glands, wounds and sores,[10] while steam baths from the bark are used to purify and improve skin complexion[11] Its bark and roots are used for the treatment of rheumatism, gastrointestinal complaints and snake bites.[12] In East Africa, the roots are used for treating snakebites, gonorrhoea, diarrhoea, and dysentery.[13] Its bark and leaves are used in folk medicine for the treatment of various deficiencies related to nociception, inflammation, mood and depression.[14] In South Africa, ethnobotanical survey indicated that this plant is used for gastrointestinal disorders including dysentery and diarrhoea.[15] Although *Z. mucronata* subsp. *mucronata* has shown significant therapeutic potentials, this study aimed at investigating the chemical compounds in its acetone stem bark extract, identifying their biological activities and determining its antibacterial activity against potential pathogenic bacteria isolated from the hospital environment.

Materials and Methods

Collection of plant sample

The bark materials of *Z. mucronata* subsp. *mucronata* were collected from the plant growing within the University of Fort Hare campus in Alice, South Africa. The plant was authenticated in the Department of Botany and a voucher specimen (OLAJ/2010/ZM/01) was prepared and deposited in the Griffin Herbarium of the University. The stem bark sample of *Ziziphus mucronata* was broken into small pieces and dried in the hot air oven at 40°C before being pulverized with a mechanical grinding machine. An aliquot (200 g) of the pulverized stem bark sample was soaked in 1 L of acetone for 72 h. The crude extract was filtered using Whatman No. 1 filter paper and the solvent was recovered using a rotary evaporator. The residue was transferred into sterile beakers and dried in a hot air oven at 40°C until completely dried. The extract obtained from the solvent was then stored at room temperature until further experimentation. The extract was re-dissolved in acetone (4 mL) before being diluted with sterile water (16 mL) to the required concentrations for the bioassay analysis. The corresponding concentration was expressed in term of mg of extract per mL of solvent (mg/mL).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the acetone extract

The GC-MS analysis was carried out using GC-MS-QP 2O10 Plus Shimadzu system (Kyoto, Japan) employing the following conditions: Column Elite-1 fused silica capillary column (30 × 0.25 mm ID × µL df, composed of 100% dimethyl Polysiloxane). An electron ionization system with ionization energy of 70 eV was used for the GC-MS detection. Helium gas (99.99%) was used as the carrier gas at constant flow rate of 1 mL/min and an injection volume of 2 µL was employed (Split ratio of 10:1), injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (Isothermal for 2 min) with an increase of 10°C/min to 200°C then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da. Total GC running time was 22 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adapted to handle mass spectra and chromatogram was Turbo-Mass-OCPTVS-Demo SPL software. The detection employed was based on the NIST Ver. 2.0, year 2009 Library.

Fourier Transform Infrared (FTIR) Spectroscopy analysis

Fourier Transform Infrared (FTIR) Spectroscopy of the powdered materials was carried out by Potassium Bromide (KBr) technique adopted from a standard source.[16] The spectra were recorded with PerkinElmer FTIR Spectrum RX1 (PerkinElmer Inc., Chorley, UK) at spectral range of 4000–400 cm^{-1} at room temperature (25±2ºC). Interpretations of peaks of the spectra were done by referring to standard FTIR tables to determine the functional groups of the chemical compounds.[17]

Test organisms

The bacteria used in this study were isolated from hospital bedside tables in our University Teaching Hospital. The samples were carefully collected by swabbing bedside tables with swabs saturated with sterile peptone water. The swabs were tightly sealed and immediately transported to the laboratory and cultured in nutrient broth. The swab sticks were streaked on Eosin Methylene Blue agar, Cetrimide agar (OXOID CM0085 – Oxoid Ltd., Basingstoke, Hants, UK and *Salmonella-Shigella* agar (Lab M - Neogen Corporation, 620 Lesher Pl, Lansing, MI 48912, USA) which were

incubated overnight at 37°C.[18] The bacterial isolates were characterized phenotypically by being subjected to Gram staining, microscopic appearance, colony pigmentation, colony morphology and biochemical tests including glucose fermentation, oxidase production, Indole, Methyl red; Voges-Proskauer and Citrate tests specifically for Gram-negative isolates, nitrate reduction tests, and urease tests according to standard protocols.[19] According to their main morphological and physiological characteristics, the potential nosocomial bacterial pathogens isolated were identified as *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli* as described by O'Hara.[19] The control organisms included *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 13311 and *Pseudomonas aeruginosa* ATCC 19582. Each isolate was maintained on sterile nutrient agar slants and was recovered for testing by culturing in sterile nutrient broth for 24 h. Before usage, each bacterial culture was subcultured into 10 mL of fresh sterile nutrient broth.

Antibiotic sensitivity test

The standard disc diffusion test was performed according to the recommendations of the Clinical Laboratory Standards Institute.[20] Four separated colonies of each isolate grown onto nutrient agar plates overnight were suspended with inoculating loop in 2 mL sterile normal saline solution and vortexed to get uniform bacterial suspensions. Each strain's suspension was adjusted to 0.5 McFarland Standards turbidity to give a resultant concentration of 1.5×10^6 Colony Forming Unit/mL (CFU/mL). The susceptibility of the isolates was determined according to the modified Kirby–Bauer disk diffusion technique.[21] Mueller-Hinton agar (MHA) (OXOID CM0085 – Oxoid Ltd., Basingstoke, Hants, UK) plates were swabbed with the resultant adjusted culture of each of the test isolates. Multidiscs containing eight different antibiotics including Ceftazidime (CAZ) (30 µg) (Cephalosporin), Gentamycin (GEN) (10 µg) (Aminoglycoside), Cefuroxime (CRX) (30 µg) (Cephalosporin), Ciprofloxacin (CPR) (5 µg) (Fluoroquinolone), Ofloxacin (OFL) (5 µg) (Fluoroquinolone), Amoxicillin-Clavulanate (Augmentin - AUG) (30 µg) (Penicillin), Nitrofurantoin (NIT) (300 µg) (Nitrofuran) and Ampicillin (AMP) (10 µg) (Penicillin) were aseptically placed on the inoculated agar with sterile forceps, and incubated at 37°C for 24 h. After 24 h of incubation, the plates were examined for inhibition zones. The diameter of the inhibition zones produced by each antibiotic disk were measured to the nearest millimeter, recorded and interpreted using the Clinical and Laboratory Standard Institute Zone Diameter Interpretative Standards.[20]

Determination of minimum inhibitory concentration (MIC)

The antibacterial activity of the acetone extract of the stem bark of *Ziziphus mucronata* was assayed *in vitro* by the macrobroth dilution assay method to determine the Minimum Inhibitory Concentrations (MIC) of the extract. The MIC of the acetone extract (0.02 and 20 mg/mL) and Ciprofloxacin (0 – 5 µg/mL) for antibacterial activity was determined using the macrodilution bioassay according to Olajuyigbe and Afolayan.[22] The inocula of the test bacteria were prepared using the colony suspension method.[23] Colonies picked from overnight cultures grown on nutrient agar were used to make suspensions of the test organisms in saline solution to give an optical density of approximately 0.1 at 600 nm. The suspension was then diluted 1:100 before being used by diluting with sterile nutrient broth. One milliliter (1 mL) of the extract was serially diluted in sterile double strength Mueller Hinton broth in test tubes to get concentrations ranging between 0.01 mg/mL and 5.5 mg/mL. Each tube was inoculated with 100 µL of each bacterial suspension before incubating at 37°C for 24 h. Blank Mueller-Hinton broth was used as negative control. Bacterial growth was indicated by turbidity of the test tube shown while the MIC value was recorded as the lowest concentration of the extract showing no visible growth.

Results

In the study, the therapeutic potential of the acetone stem bark extract of *Ziziphus mucronata* was determined by GC-MS analysis to identify its chemical compounds. The chromatogram of the identified chemical compounds in the extract is shown in Figure 1, while the name, molecular formula, molecular weight, chemical structure and area percentages of the identified chemical compounds along with their biological activities are shown in *Supplementary Materials Table 1*. The GC-MS result showed that there are 52 chemical compounds in the acetone stem bark extract of *Z. mucronata*. Of these identified compounds, 4-hydroxy-4-methyl-2-Pentanone (24.86%), 1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl)cyclohexane (9.92%), Resorcinol (8.41%), Octacosanol (7.37%), 2,6-β-17-β-Trihydroxy-6-α-pentyl-2,3-seco-5-α-androstan-3-oic acid-γ-lactone (3.32%), n-Hexadecanoic acid (3.16%), Hexanedioic acid, 2,3,4,5-tetrahexyl-, dimethyl ester (2.41%), 1,3-Benzenediol, 4-propyl- (2.31%), Bis(2-ethylhexyl) phthalate (2.13%) and 3-O-Methyl-d-glucose (1.99%) were the most prominent chemical compounds. Of the 52 compounds, 29 have been reported to have significant pharmacological activities of therapeutic importance while 23 compounds have not been reported to have any pharmacological activity in literature.

The FTIR spectrum of the compounds in the extract is presented in Figure 2. The data on the peak values and the probable functional groups in the extract are presented in Table 1. The spectrograph indicated the presence of amines, amides, alkanes, aldehydes, diketones, nitrosamine, esters, alkyl amine, primary & secondary alcohols and sulfoxide group of compounds.

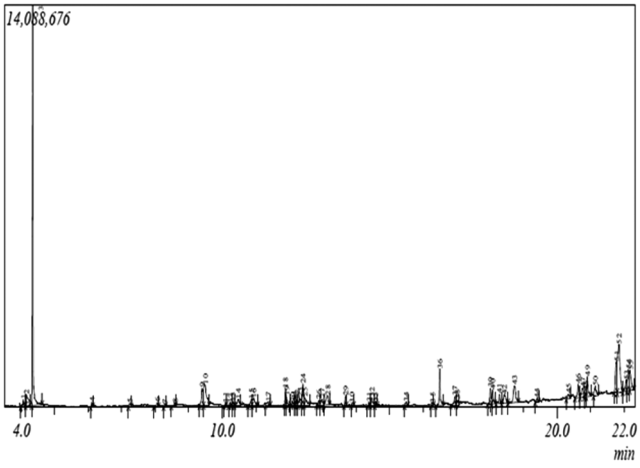

Figure 1. Chromatogram of the chemical compounds in the acetone stem bark extract of *Ziziphus mucronata*.

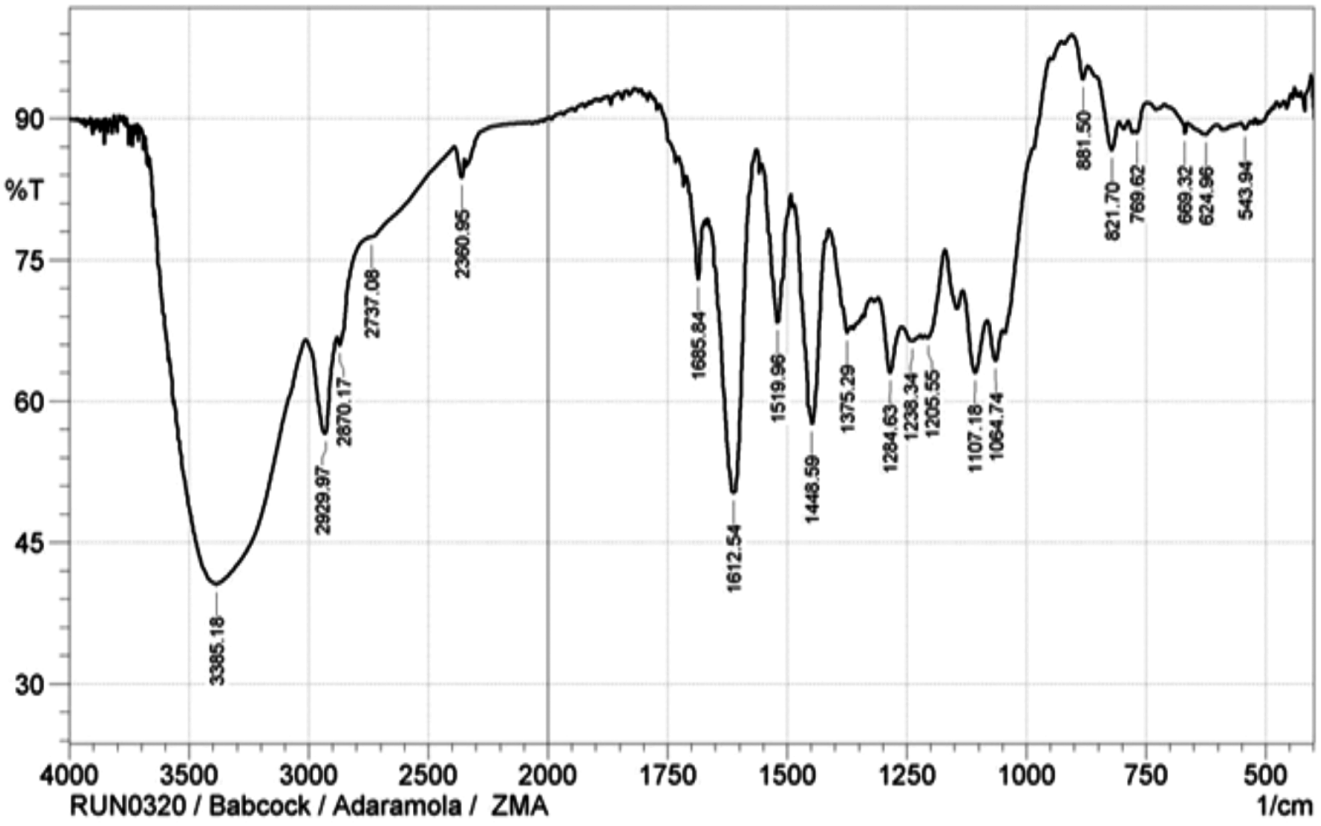

Figure 2. Fourier Transform Infra-Red (FTIR) spectrograph of the acetone stem bark extract of *Ziziphus mucronata*.

Table 1. Peak of Fourier Transform Infra-Red (FTIR) absorption and functional groups present in the acetone stem bark extract of *Ziziphus mucronata*.

S/N	Peak values	Functional group
1	3385.18	Bonded N-H/C-H/O-H stretching of amines and amides
2	2929.97	C-H stretch Alkanes
3	2870.17	C-H stretch Alkanes
4	2737.08	C-H Aldehyde
5	1685.84	Quinone or conjugated ketone/C=O Conjugated aldehyde
6	1612.54	Diketones /C=C (α,β-unsaturated ketone)
7	1519.96	N-O Nitro compound
8	1448.59	Nitrosamine
9	1375.29	C-H alkane/Isopropyl group
10	1284.63	Alkyl ketone/C-O Aromatic ester
11	1238.34	Ester carbonyl/Alkyl aryl ester
12	1205.55	P-O-C stretch (Aromatic phosphate)/Alkyl amine
13	1107.18	Si-C-C (Organic siloxane or silicone) /C-N Alkyl amine/C-O Secondary alcohol
14	1064.74	Si-O-Si (Organic siloxane or silicone) /C-O Primary alcohol Sulfoxide
15	881.50	1,2,4-trisubstituted
16	821.70	Aromatic compound/Alkene
17	769.62	C-H Mono-substituted benzene derivative
18	666.32	Halogen compounds (Bromo-compounds (C-Br))/C=C (Alkene)
19	624..96	Halogen compound (C-Br)
20	543.94	Halogen compound (C-I)

The antibacterial activities of the antibiotics against the different test bacterial strains are presented in Table 2. The bacterial isolates showed varied degree of susceptibility to the used antibiotic. They were all resistant to Amoxicillin-Clavulanate (Augmentin - AUG), Ampicillin (AMP), Cefuroxime (CRX) and Ceftazidime (CAZ) but more susceptible to Gentamicin (GEN), Ciprofloxacin (CPR), Ofloxacin (OFL) and Nitrofurantoin (NIT). Although bacterial isolates were multidrug resistant, being resistant to more than 3 classes of antibiotics, fluoroquinolones were considered the most effective against the bacterial isolates. The zones of inhibition ranged from 17 ± 1.00 mm to 38 ± 1.00 mm.

The antibacterial activity of the extract was assayed *in vitro* by the macrobroth dilution assay method against the different microorganisms used in the study. The susceptibility of the bacterial isolates to the extract, as shown in Table 3, indicated that the MICs of the extract ranged between 0.31 mg/mL and 5.5 mg/mL. While four of the isolates were inhibited at MICs equal to 0.34 mg/mL and ten isolates had MICs equal to 5.5 mg/mL, two isolates each had MICs equal to 2.75 mg/mL and 1.38 mg/mL, respectively. The extract inhibited *E. coli* strains at MICs ranging between 0.31 and 5.5 mg/mL and *S. typhi* at MICs ranging between 0.31 and 1.38 mg/mL. While *P. aeruginosa* (C38) was inhibited at MIC equal to 0.31 mg/mL, other strains of the species were inhibited at MICs equal to 5.5 mg/mL. *S. typhi* ATCC 13311, *P. aeruginosa* ATCC 19582 and *E. coli* ATCC 25922, used as control, were inhibited at MICs equal to 0.31, 0.31 and 0.63 mg/mL, respectively. On the other hand, the isolates were susceptible at MICs ranging between 0.02 and 1.25 µg/mL of Ciprofloxacin used as control.

Discussion

Antimicrobial screening of traditional medicinal plants has become a source of innumerable therapeutic agents. While many complementary and alternative medicines have enjoyed increasing popularity recently, efforts to validate their use have seen their assumed effective therapeutic properties being increasingly scrutinized *in vitro* and *in vivo*. This is because a large number of antimicrobial agents derived from traditional medicinal plants are available for treating various diseases caused by microorganisms.[24] There are evidences affirming that the therapeutic potential of plant-based antimicrobial compounds are without any side effects often associated with synthetic antimicrobials. An innumerable amount of chemotherapeutic agents have been isolated from natural sources over the years proving nature to be of importance in pharmaceutical microbiology. As seen in many cultures in Nigeria, traditional medicine is indispensable in treating infections and diseases. This makes plants a great source of chemotherapeutic agents.[25] Explorations into the possible antimicrobial properties in the medicinal plants have led to investigating phytoconstituents in alcohol extracts. Consequently, many reports have indicated the antimicrobial effects of medicinal plants against bacteria and fungi of medical importance. Though several antibiotics and antimicrobials are available, increasing capability of microbes to develop multidrug resistance has further encouraged search for new, safe and effective chemical agents of plant origin while there is dearth of knowledge on chemical compounds in medicinal plants with novel drug potentials.

Table 2. Determination of the multidrug resistance profile of different microorganisms by the disc diffusion assay using different antibiotics discs.

	GEN	CPR	OFL	AUG	NIT	AMP	CPX	CAZ
Bacterial isolates	**Diameter of inhibition zones (± 1.00 mm)**							
E. coli (E4)	21	32	30	6	27	6	6	6
E. coli (E6)	22	25	17	6	24	6	6	6
E. coli (E30)	21	29	24	6	22	6	6	6
E. coli (E23)	21	23	22	6	24	6	6	6
E. coli (S7)	28	29	24	6	27	6	6	6
E. coli (E19)	21	23	17	6	21	6	6	6
E. coli (E3)	16	25	20	6	25	6	6	6
S. typhi (S11)	21	23	17	6	26	6	6	6
S. typhi (S16)	22	23	23	6	26	6	6	6
S. typhi (S4)	6	27	16	6	6	6	6	6
P. aeruginosa (C38)	29	30	25	6	28	6	6	6
P. aeruginosa (C30)	20	20	20	6	24	6	6	6
P. aeruginosa (C13)	20	23	23	6	24	6	6	6
P. aeruginosa (C27)	19	32	26	6	22	6	6	6
P. aeruginosa (C7)	22	30	26	6	31	6	6	6
P. aeruginosa (C14)	20	18	18	6	22	6	6	6
P. aeruginosa (C19)	20	40	30	6	25	6	6	6
P. aeruginosa (C35)	23	25	24	6	25	6	6	6
E. coli ATCC 25922	27	29	26	6	25	6	6	6
S. typhi ATCC 13311	20	24	23	6	27	6	6	6
P. aeruginosa ATCC 19582	23	33	28	6	26	6	6	6

Key: Gen, Gentamicin (10 µg) (Aminoglycoside); CPR, Ciprofloxacin (5 µg) (Fluoroquinolone); OFL, Ofloxacin (5 µg) (Fluoroquinolone); AUG, Amoxicillin-Clavulanate (Augmentin) (30 µg) (Penicillin); NIT, Nitrofurantoin (300 µg) (Nitrofuran); AMP, Ampicillin (10 µg) (Penicillin); CRX, Cefuroxime (30 µg) (Cephalosporin); CAZ, Ceftazidime (Cephalosporin)

Table 3. Minimum Inhibitory Concentrations of acetone extracts of *Ziziphus mucronata* against the different microorganisms used in this study.

Organisms	Minimum Inhibitory Concentration	
	Extract (mg/mL)	Ciprofloxacin (µg/mL)
E. coli (E4)	0.31	0.08
E. coli (E6)	5.5	1.25
E. coli (E30)	5.5	1.25
E. coli (E23)	0.31	0.04
E. coli (S7)	1.38	0.08
E. coli (E19)	5.5	0.63
E. coli (E3)	2.75	0.31
S. typhi (S11)	2.75	0.04
S. typhi (S16)	0.31	0.02
S. typhi (S4)	1.38	0.02
P. aeruginosa (C38)	0.31	0.02
P. aeruginosa (C30)	5.5	0.02
P. aeruginosa (C13)	5.5	0.02
P. aeruginosa (C27)	5.5	0.02
P. aeruginosa (C7)	5.5	0.02
P. aeruginosa (C14)	5.5	0.02
P. aeruginosa (C19)	5.5	0.02
P. aeruginosa (C35)	5.5	0.02
E. coli ATCC 25922	0.63	0.08
S. typhi ATCC 13311	0.31	0.08
P. aeruginosa ATCC 19582	0.31	0.02

In this study, the acetone stem bark extracts of *Ziziphus mucronata* with varied percentages of the chemical compounds may act as a source of novel drug compounds with significant therapeutic applications. The various functional groups observed in the extract through the FTIR results most likely indicated the presence of fatty acids, alcohols, aldehydes, ketones, hydrocarbons, amino acids and amides which have been reported in different plants.[26] Among the functional groups observed in the extract, the C-H functional group was the most prominent. Although hydroxyl bundles in flavonoids scavenge free radicals and chelate metal particles[27] the presence of the -OH group in the extract suggested the reason for the degree of inhibitory activity against the microorganisms. The -OH group has the ability of forming hydrogen bonding capacity and could indicate the higher potential of the acetone extract towards inhibitory activity against the microorganisms as indicated by Ashokkumar and Ramaswamy.[28]

The extract showed antibacterial activity against the test bacterial isolates but the varied values for the MICs could be due to the differences in the genetic makeup of the different bacteria species. In agreement with Rossolini and Mantengoli,[29] Olajuyigbe *et al.*,[30] Venier *et al.*,[31] and Olajuyigbe and Adeoye,[32] the extract inhibited the selected bacteria which are usually implicated as major culprits for human urinary tract infections, intestinal infections, food poisoning and nosocomial infections. The ability of this extract to inhibit *P. aeruginosa*, which is usually resistant to the commonly used antimicrobial agents, is significant as this organism is a major cause of morbidity and mortality in patients with chronic pulmonary infection.[33] The presences of several secondary metabolites such as phenolics, alkaloids, flavonoids and tannins[34] have been implicated in antimicrobial activities of many medicinal plants. The antibacterial activity of the extract could be due to the fact that the compounds penetrated the cellular membrane of test bacteria, enhanced the fluidity and permeability of the membrane, altered the topology of membrane proteins and caused disruption in the respiratory chain.[35] While the phenolic compounds in the extract could disrupt the cell membrane leading to the inhibition of the cell metabolism to cause the leakage of cellular content, the disturbed permeability of the cytoplasmic membrane may lead to cell death.[36] Olajuyigbe *et al.*,[37] however, reported that the mechanism of the antibacterial activity of medicinal plants against bacteria could be due to metabolic processes inhibition, cell wall and cell membrane disruption and interference with the stability of cell membranes leading to efflux of lipid and protein in test bacteria. Although Rojas *et al.*[38] indicated that extracts with known antimicrobial properties are of immense significance in therapeutic treatments and their active principles have been considered as a cheap but effective herbal treatment against common microbial infections, based on the structural diversity of the identified chemical compounds and their activity against the selected bacterial isolates, this family of compounds constitutes an interesting group for the development of new antibacterial drugs.

Conclusions

In conclusion, this study identified chemical compounds of therapeutic importance in *Ziziphus mucronata* which are with and without biological activities through GC-MS analysis. It showed the multipurpose therapeutic potentials of this plant scientifically and further justifies its ethno-medicinal use in the treatment of

infectious diseases. The ability of the extract to inhibit Gram-negative bacteria showed that *Ziziphus mucronata* contains interesting biopharmaceutical substances with ability to attract significant scientific attention. Further research on the isolation and characterization of these chemical compounds of economic, pharmaceutical and therapeutic efficacy from *Ziziphus mucronata* are ongoing in our laboratory in order to provide alternative measures for treating diseases caused by these potential pathogens.

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