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Phytochemical Screening, *In-vitro* Antimicrobial Activity and Antioxidant Characteristics of *Tetrapleura tetraptera* Extracts

Okwute Simon Koma[1*], Olajide Olutayo Olawumi[1,2], Etuk-Udo Godwin[3] and Orishadipe Abayomi Theophilus[2]

[1]Department of Chemistry, University of Abuja, Abuja, Nigeria.
[2]Chemistry Advanced Research Centre, Sheda Science and Technology Complex (SHESTCO), Abuja, Nigeria.
[3]Biotechnology Advanced Research Centre, Sheda Science and Technology Complex (SHESTCO), Abuja, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. The bench work was done by author OOO as a Ph. D student under author OSK, who designed and supervised the project. Authors OOO, EUG and OAT also drafted and read the report while author OSK did the final moderation of the manuscript for publication. Authors OOO and OSK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OOO and EUG managed the analyses of the study and the literature searches. Authors OOO and OSK read and approved the final manuscript.

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ABSTRACT

Aims: In order to provide scientific evidence for the ethno-medical claims, this study was conducted to determine the phyto-constituents, antimicrobial activity and the antioxidant activity of the crude leaf and stem bark extracts of *Tetrapleura tetraptera* (Schum. & Thonn.) Taub.
Study Design: The plant was authenticated by a taxonomist at Forest Research Institute of Nigeria (FRIN) and a voucher specimen was kept for future reference. The leaf and stem bark were

**Corresponding author: E-mail: profokwute@yahoo.com;*

extracted using 95% ethanol and the crude extracts were screened for phytochemicals, antimicrobial and antioxidant potentials and then fractionated.
Place and Duration of Study: The study was undertaken between October 2015 and June 2016, at Nigeria Institute of Leather and Science Technology (Microbiology Laboratory) Zaria, Kaduna State and the Chemistry Advanced Research Centre, Sheda Science and Technology Complex, Sheda, Abuja, Nigeria.
Methodology: Air-dried leaves and stem bark of *T. tetraptera* were pulverized to powder with a mortar and a pestle. The powdered leaves and stem bark were then kept in airtight containers until required for further work. Each of the ground plant samples (1 Kg) was exhaustively extracted with 95% ethanol using a Soxhlet extractor. The extracts were concentrated at 40℃ to dryness using a rotary evaporator to obtain the crude extracts. The crude extracts were screened for phytochemicals and antimicrobial activity against some selected pathogens such as *Staphylococcus aureus*, *Streptococcus pneumonia*, *Streptococcus pyogenes, Corynebacteruim ulcerans, Candida albicans, Candida tropicalis, Klebsiclla pneumonia, Candida krusei* and antioxidant activity using standard procedures.
Results: Alkaloids, phlobatanins, flavonoids, volatile oils and tanins were present in the crude extract of each plant part. However, steroids and saponins were detected only in the stem bark while phenols, resin and terpenoids were detected only in the leaf extract. The antioxidant activity of the extracts was determined by the DPPH inhibition method. The crude stem bark extract exhibited a stronger free radical scavenging activity than the leaf (69.81: 63.46% at 5 mg/ml and 71.54: 63.85% at 4 mg/ml, respectively). The results of antimicrobial screening also showed that the crude stem bark extract was more active with inhibitory activity between 27-32 mm compared to that of the crude leaf extract which was between 24-29 mm, against the test organisms, *Staphylococcus aureus, Streptococcus pneumonia, Streptococcus pyrogens, Klebsciella pneumonia, and Candida krusei*. The minimum inhibitory concentration (MIC) of the crude stem bark extract was recorded at 0.625 mg/ml, while that of the leaf was between 0.625 and 1.25 mg/ml. The results obtained suggested that both the crude ethanol extracts of the stem bark and leaf possess strong anti-bacterial and anti-fungal activities.
Conclusion: The susceptibility of microorganisms such as *Staphylococcus* sp*.* and *Klebsiella* sp*.* to certain phyto-compounds infers that the bioactive compounds in plants can be further developed for the management of several disease conditions thereby authenticating their use in traditional medicine. The relative potency of the studied plant extracts in comparison to the standard drug ciprofloxacin suggests that possible new drug candidates can be harnessed from the plant, *Tetrapleura tetraptera.* From the results obtained in this study, further work will now be done to isolate and identify the active principles.

Keywords: Tetrapleura tetraptera; leaf; stem bark; phytochemicals; anti-microbial activity; antioxidant activity.

1. INTRODUCTION

Respiratory tract infections are widespread globally, affecting both men and women. This is further exacerbated by the insurgence of drug resistant microbial pathogens, a dwindling gamut of therapeutic anti-microbial agents, increasing side-effects emanating from the use of synthetic drugs and the re-emergence of opportunistic infections, particularly in the rural areas of developing countries where host-pathogen interaction is influenced by a range of factors [1,2]. With advances in medical diagnostics, identification of the etiological agents causing specific medical conditions is no longer a major cause for concern in developing countries. However, the lack of suitable antibiotic therapies has been suggested to be the principle cause of difficulty in managing such infections [3].

Research into upper respiratory tract infections has recognized certain groups of bacteria as the primary culprits behind up to 10% of reported cases of bacteremia leading to secondary pneumonia and endocarditis, bronchitis and pharyngitis in adults which are easily transmitted in crowded areas [4,5]. These microbial pathogens include but are not limited to beta hemolytic *Streptococci* sp*, Corynebacterium diphtheriae, Neisseria gonorrheae, Bordetella pertusis, Bordetella parapertusis,* and *Mycoplasma pneumonia* [4]. Treatment of upper respiratory tract pathogens, coupled with the

advent of drug resistant strains has diminished the effective capacities of the commonly used drugs [6,7]. Principally, antibiotics such as β-lactam and β-lactamase inhibitors (ampicillin, amoxicillin), cephalosporins (ceftazidines), macrolides (erythromycin), aminoglycosides (Gentamycin, streptomycin), quinolones (ciprofloxacin), rifampicin, are usually administered for treatment. As a result, exploring the use of unconventional agents in the form of chemical compounds or formulations that possess better antimicrobial effectiveness towards treatment of upper respiratory tract infections warrants adequate consideration [8].

Medicinal plant research proffers novel and significant front runners within drug discovery development [9]. Their range of pharmaceutical intermediates which has been exploited for therapeutic endeavors includes rifamycin, obtained from *Amycolatopsis mediterranei* [10]. This compound is an intermediate involved in the synthesis of rifampicin, the primary antibiotic administered for the treatment of tuberculosis. Other notable drugs of plant origin traded globally include atropine, ephedrine, digoxin, morphine, quinine, reserpine and tubocurarine [11].

The pool of bioactive compounds within selective plants is considered to be a cost effective substitute for synthetic drugs particularly in the treatment of several bacterial and fungal infections in developing countries [12,13]. In sourcing for plants with promise towards the treatment of upper respiratory tract infections, extracts from *Garcinia mangostana* were reported to exhibit strong activity against vacomycin resistant *Enterococci* [14], that of *Caesalpina coriaria* against *Klebsiella pneumonia* [15] while *Acacia nilotica, Cinnamomum zeylanicum and Syzygium aromaticum* extracts all inhibited the growth of *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans* [16].

In Nigeria, therapeutic information for a lot of such plants is based on folklore. Therefore, there is need to establish a scientific rationale for their use in treatment by performing *in vitro* and *in vivo* testings.

One of such plants suggested by folklore for the treatment of different pathogens that trigger upper respiratory tract infections is *Tetrapleura tetraptera* (Schum. & Thonn.) Taub. *C*ommonly called “Aidan tree” in South-West region of Nigeria it is from the family *Mimosaceae* and is a native of tropical regions of Africa. In Nigeria it is known in Hausa language as *Dawo* and *Uyayak* in Ibibio. Its fruits consist of a fleshy pulp with small, brownish- black seeds and its ethno botanical uses include but are not limited to the management of convulsions, leprosy, inflammation and rheumatism [17]. Its sweet fragrance is highly valued and its fruit is used to spice traditional dishes such as *Banga* soup and its bark is used for other medicinal purposes. Traditional medical practice in Nigeria suggests that treatment of medical conditions emanating from certain microbial vectors such as *Escherichia coli, Streptococcus* sp*, Klebsciella* sp*, Pseudomonas sp, Staphylococcus* sp*, Aspergillus* sp and *Candida sp* is possible by using this plant.

In order to provide scientific evidence for the ethno-medical claims, this study was conducted to determine the phyto-constituents, antimicrobial activity and the antioxidant activity of the crude leaf and stem bark extracts of *Tetrapleura tetraptera* (Schum. & Thonn.) Taub.

Fig. 1. Sprouts *of Tetrapleura tetraptera*

2. MATERIALS AND METHODS

The leaves and stem bark of *Tetrapleura tetraptera* were collected from Owo area of Ondo State, Nigeria. Taxonomical identification was done at the Forest Reserve Institute of Nigeria (FRIN), Ibadan, Nigeria and herbarium number was given as FHI110372. The stem

bark was then cut into pieces and alongside the leaves were washed, air-dried and pulverized to powder with a mortar and a pestle. The powdered leaves and stem bark were then kept in airtight containers until required for further work. Each of the ground plant samples (1 Kg) was exhaustively extracted with ethanol using Soxhlet extraction method [18]. The extracts were concentrated at 40℃ to dryness using a rotary evaporator to give the crude extracts. Stock solutions of reference antibiotics (fulcin, fluconazole and ciprofloxacin) were prepared.

2.1 Phytochemical Screening

Phytochemical screening was carried out for the qualitative determination of major constituents using methods previously described by Trease and Evans [19].

2.2 Antimicrobial Screening

All the media were purchased from Sigma-Aldrich and were prepared in accordance with the manufacturer's instructions. The clinical bacterial and fungal isolates were obtained from Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Kaduna state, Nigeria. The identities of all isolates used were confirmed using standard biochemical tests [20]. Agar diffusion method adopted from EUCAST [21] was employed.

2.3 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined on the test organisms that were sensitive to the extracts and was done by broth dilution method [22]. Mueller Hinton broth was prepared, dispersed into test tubes and sterilized at 121℃ for 15 mins. The broth was then allowed to cool. Normal saline was prepared and 10 ml was dispersed into a sterile test tube and the test organisms were inoculated and incubated at 39℃ for 6hrs. Dilution of the test organisms was done in the normal saline until the turbidity marched that of the McFarland's standard scale by visual comparison (at a concentration of about 1.5 x 10^8 CFU/ml). Two fold serial dilution of the extract in sterilized broth was made to obtain the concentrations of 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml and 0.313 mg/ml. The initial concentration was obtained by dissolving 0.05 g of the crude extract in 10 ml of DMSO to obtain a concentration of 5 mg/ml from which subsequent dilutions were made. Having obtained the different concentrations of the extracts in the sterile broth they were observed for turbidity (growth), the lowest concentration of the extract in the broth which shows no turbidity was recorded to be the MIC.

2.4 Determination of Minimum Bactericidal / Fungicidal Concentrations

Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were evaluated by plating the bacterial suspensions from individual well at the beginning and at the end of the experiments on Mueller Hinton agar medium for estimation of MBC [22]. The culture from MIC well was taken and streaked on the surface of fresh Mueller Hinton agar in a 90-mm plate with division and incubated at 37℃ for 24 hours (bacteria) and 30℃ for 1-7 days (fungi) after which the plates of the medium were observed for colony growth. The MBC/MFC values were the plates with lowest concentrations of the extract without colony growth.

2.5 DPPH Radical Scavenging Assay

The effect of extracts on DPPH radical was determined using the method of [23]. The principle is based on the measure of the absorbance at 517nm of a violet color which is reduced to yellow colour in the presence of antioxidants. The following concentrations of the extracts were prepared: 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 mg/ml in methanol. Vitamin C (ascorbic acid) was used as the antioxidant standard at concentrations of 0.05, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/ml. The extract (1ml) was placed in a test tube and 3 ml of methanol was added, followed by 0.5 ml of 1mM DPPH in methanol and thereafter the decrease in absorption was measured on a UV-Visible Spectrophotometer 10 minutes later. A blank / control solution was prepared containing the same amount of methanol and DPPH. The actual decrease in absorption was measured against that of the control and the percentage inhibition was calculated. All tests and analyses were run in triplicates and the recorded final results were the average.

3. RESULTS AND DISCUSSION

The results of phytochemical analysis of ethanol extracts from the leaves and stem bark of *Tetrapleura tetraptera* are shown in Table 1. The results indicated that the principal classes

of natural products present in both plant parts were alkaloids, flavonoids and tannins. Other compounds present were phlobatannins and the volatile oils. The presence of these well known classes of bioactive phyto-compounds may be responsible for the activity of the extracts against several pathogens [24,25]. Further analysis of the data obtained from Table 1 suggests that the stem bark extract contained certain specific classes of compounds including steroids and saponins, while the leaf extract contained only terpenoid, resins and balsams. This result is similar to those obtained from study by other researchers who investigated the chemical components of the plant's fruit extract [26].

It was observed that none of the crude extracts contained cardiac glycosides, glycosides and triterpenoids and therefore it was expected that the plant would not exhibit their full antioxidant potential that could enhance the body defense against pathology induced free radical generation [27]. However, antioxidant activity screening gave an expected result due to the presence of flavonoids, tannins and phenols. The data obtained (Table 2 and Fig. 2) suggest that absence of these phyto-compounds did not necessarily affect the antioxidant activity of this plant, thereby indicating that anti-oxidant potential of a plant may also be contributed by other classes of natural products such as phenols which are present in this plant. The free radical scavenging activities of the samples were determined as 69.81%, 71.54% , 71.54%, 70.00%, 69.23%, 68.08%, 67.31% and 62.31% for the crude stem bark and 63.46%, 63.85%, 64.04%, 69.23%, 64.04%, 47.31%, 19.81% and 16.15% for the crude leaf, respectively (Fig. 1). This result is high for plant extracts in comparison to the synthetic standards of vitamin C and Tocopherol which gave 85.00%, 89.51%, 90.01%, 90.41%, 91.33%, 91.32%, 90.47%, 90.19% and 65.45%, 69.21%, 69.61%, 68.15%, 69.55%, 71.29%,73.60%,73.20% respectively, all at 5, 4, 3, 2, 1, 0.5, 0.1 and 0.05 mg/ml concentrations. From the results, antioxidant activities of the extracts and vitamin C decrease at lower concentrations while that of tocopherol increases. The presence of enzymatic or non-enzymatic proxies in plant samples are of immense importance as their primary role of medical importance is to dissipate the deleterious effects to the body brought upon by oxidants. Their ability to quash free radicals before they can attack the cells and biological targets thwarts impairment to carbohydrates, DNA, enzymes, lipids and proteins [28].

In this study, non-enzymatic antioxidants, tocopherol and vitamin C, which are obtained from natural plant sources, were used as standards. From the phytochemical analysis, the role of antioxidants could be streamlined to the phenolic compounds with emphasis on the flavonoids and other phenolic compounds. The stem bark of *Tetrapleura tetraptera* shows higher promise for free radical scavenging than that of the leaf in comparison to tocopherol. Thus, the result of this work strongly supports the search for alternative sources of antioxidant from plants. The success in treating upper respiratory tract infections, attributed to *Streptococci* sp*, Corynebacterium diphtheriae, Neisseria gonorrheae, Bordetella pertusis, Bordetella parapertusis* and *Mycoplasma pneumonia* invasions could be due to the anti-allergic, anti-inflammatory and general anti-microbial properties of flavonoids, particularly the proanthocyanidins [29,30]. It has been reported that this flavonoid participates in the modulation of the arachiodonic acid pathway, essential towards the triggering of an inflammatory response to stimuli (biotic or abiotic).

Table 1. Phytochemical screening of crude extracts of *Tetrapleura tetraptera* leaves and stem bark

Phyto-constituents	Crude leaf extract	Crude stem bark extract
Steroids	-	+
Triterpenoids	-	-
Glycosides	-	-
Saponins	-	+
Phenols	+	-
Alkaloids	+	+
Cardenolides	-	-
Terpenoids	+	-
Cardiac glycosides	-	-
Phlobatanins	+	+
Resins	+	-
Balsams	+	-
Volatile oils	+	+
Tannins	+	+
Flavonoids	+	+

(+) – Present (-) - Absent

By disruption of the pathway events, this flavonoid suppresses inflammation via a unique mechanism that also involves the inhibition of gene transcription, protein expression, the receptor binding/signaling activity of both inflammatory enzymes and mediators [31]. In addition, the rich array of bioactive compounds of phenolic origins has long been studied and reportedly exhibited antimicrobial activity against viruses, bacteria, yeasts, and fungi [32,33].

The mean zones of inhibition of the two extracts against different bacterial and fungal species are summarized in Tables 3 and 4. Notably, the stem bark extract conferred a high degree of resistance against *Streptococcus pyrogenes* (29 mm) for which there was none with the leaf extract. Also the leaf extract conferred a high degree of inhibition against *Corynebacterium ulcerans* (26 mm) and *Candida tropicalis* (26 mm) which was not observed for the stem bark extract.

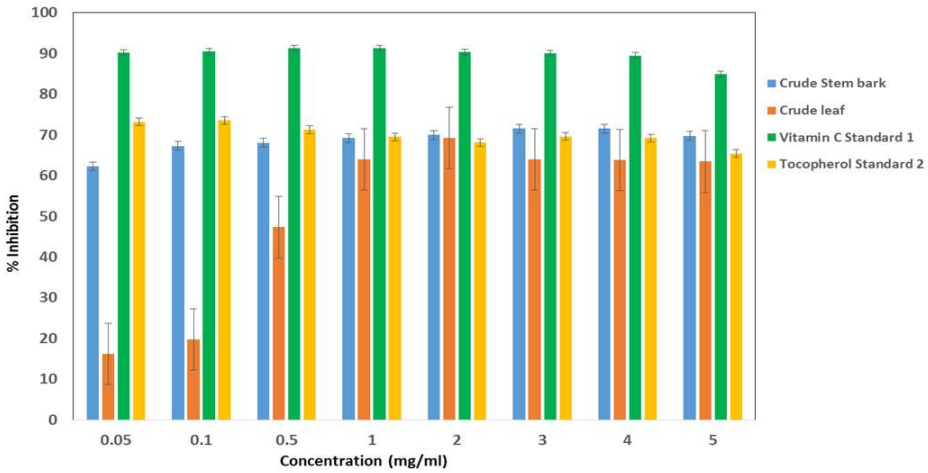

Fig. 2. Antioxidant activity of crude leaf and stem bark extracts of *T. tetraptera*

Table 2. Anti-oxidant activity (% inhibition) of crude extracts of *Tetrapleura tetraptera* leaves and stem bark

Concentration (mg/ml)	Crude stem bark	Crude leaf	Vitamin C	Tocopherol
5.0	69.81±0.000	63.46±0.000	85.00±0.001	65.45±0.000
4.0	71.54±0.001	63.85±0.003	89.51±0.001	69.21±0.005
3.0	71.54±0.003	64.04±0.000	90.01±0.000	69.61±0.001
2.0	70.00±0.000	69.23±0.000	90.41±0.003	68.15±0.002
1.0	69.23±0.005	64.04±0.001	91.33±0.001	69.55±0.000
0.5	68.08±0.000	47.31±0.003	91.32±0.000	71.29±0.003
0.1	67.31±0.001	19.81±0.000	90.47±0.003	73.60±0.000
0.05	62.31±0.007	16.15±0.000	90.19±0.001	73.20±0.000

Table 3. Antimicrobial activity of crude extracts of *Tetrapleura tetraptera* leaves and bark

Test microorganisms	Crude leaves	Crude stem bark	Control[1]	Control[2]	Control[3]
Staphylococcus aureus	S	S	R	R	S
Streptococcus pneumonia	S	S	R	R	S
Streptococcus pyrogenes	R	S	R	R	S
Klebsiclla pneumonia	S	S	R	R	S
Corynebacterium ulcerans	S	S	R	R	S
Candida albicans	R	R	R	S	R
Candida krusei	S	S	R	S	R
Candida tropicalis	S	R	S	S	R

R= Resistant, S= Sensitive

Table 4. Antimicrobial activity of crude extracts of *Tetrapleura tetraptera* ieaves and stem bark [Zone of inhibition (mm)]

Test microorganisms	Crude leaves	Crude stem bark	Control[1]	Control[2]	Control[3]
Staphylococcus aureus	25.0 ±1.10	27.0 ±0.80	0	0	32±0.25
Streptococcus pneumonia	28.0 ±0.00	30.0 ±0.40	0	0	30 ±0.15
Streptococcus pyrogenes	0	29.0 ±0.35	0	0	31 ±0.75
Klebsiclla pneumonia	29.0 ±0.30	32.0 ±0.25	0	0	34 ±0.60
Corynebacterium ulcerans	26.0 ±1.40	0	0	0	0
Candida albicans	0	0	0	35 ±0.30	0
Candida krusei	24.0 ±0.80	27.0 ±0.30	0	36 ±0.55	0
Candida tropicalis	26.0 ±0.10	0	30 ±0.35	35 ±0.45	0

Control[1] = Fulcin (5 µg/ml), Control[2] = Fluconazole (5 µg/ml), Control[3] = Ciprofloxacin (5 µg/ml)

Overall, for the studied microorganisms the stem bark extract generally displayed a greater antimicrobial activity than the leaves, thus: *Staphylococcus aureus* (27: 25 mm), *Streptococcus pneumonia* (30: 28 mm), *Klebsiclla pneumonia* (32: 29 mm) and *Candida krusei* (27: 24 mm). Against *Streptococcus pneumonia,* the stem bark extract exhibited the same level of resistance as the control, ciprofloxacin (5 µg/ml). The findings from Tables 3 and 4 compliment the results given in Table 1 and other studies which have shown that flavonoids and tannins are both active against *Staphylococcus aureus* and a range of bacterial pathogens linked with respiratory infections [34,35]. Other studies have also implicated saponins, terpenoids, flavonoids and tannins as the driving force behind the reported activity against infectious disease causing agents like *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococus pyogenes* as well as their activity against multi drug resistant strains of *M. tuberculosis* [36,37]. Interference of bacterial enzyme activity is commonly observed in the presence of phyto-tannins [38].

Table 5 shows the MIC, MBC and MFC of the crude leaf and stem bark extracts of *Tetrapleura tetraptera*. The data indicated that the crude stem extracts were more active against all tested bacteria except *Corynebacterium ulcerans* with MIC values of 1.25 mg/ml, respectively. Also, with the exclusion of *Staphylococcus aureus* (2.5 mg/ml), the MBC result was 1.25 mg/ml, respectively. No MIC or MFC values were obtained for both plant parts against *Candida albicans*, a result that supports data from Tables 3-4. Both plant parts gave the same MFC value of 2.5 mg/ml against *Candida krusei* with only the crude leaf extract exhibiting activity against *Candida tropicalis* with MFC value 2.5 mg/ml in Table 5.

Table 5. Minimum Inhibitory, minimum bactericidal and fungicidal Concentrations (MIC, MBC, MFC) of crude extracts of *Tetrapleura tetraptera* leaves and stem bark (mg/ml ± SD)

Microorganisms	Crude leaves (mg/ml)			Crude stem bark (mg/ml)		
	MIC*	MBC	MFC	MIC*	MBC	MFC
Staphylococcus aureus	1.25	2.5	-	0.625	2.5	-
Streptococcus pneumonia	0.625	1.25	-	0.625	1.25	-
Streptococcus pyrogenes	-	-	-	0.625	1.25	-
Klebsiella pneumonia	0.625	1.25	-	0.625	1.25	-
Corynebacterium ulcerans	1.25	2.5	-	-	-	-
Candida albicans	-	-	-	-	-	-
Candida krusei	1.25	-	2.5	0.625	-	2.5
Candida tropicalis	1.25	-	2.5	-	-	-

**Each value represents mean (n = 3)*

The general conception would be that a synergy of both plant parts and the whole plant would render an even more effective antimicrobial activity for the management of respiratory infections. Furthermore, the displayed activity against the selected bacteria suggests that the studied plant parts could be employed towards alleviating more thorny respiratory complications.

4. CONCLUSION

The susceptibility of microorganisms such as *Staphylococcus sp.* and *Klebsiella* sp*. to* certain phyto-compounds infers that the bioactive compounds in plants can be further developed for the management of several disease conditions thereby authenticating their use in traditional medicine. The relative potency of the studied plant extracts in comparison to the standard drug ciprofloxacin suggests that possible new drug candidates can be harnessed from the plant, *Tetrapleura tetraptera.* From the results obtained in this study, further work will now be done to isolate and identify the active principles.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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