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Solvent Polarity and Temperature Effects on Extracted Secondary Metabolite from the Fruit of *Tetrapleura Tetraptera* and its Antibacterial Potential on Uropathogens

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Abstract- Odoriferous medicinal plants are known to be used as natural therapeutic agents, as they are rich sources of terpenoids and polyphenols. This study was aimed at evaluating the solvent polarity, temperature effects and synergistic potential of the phytochemicals in the fruit extract obtained from the fruit of *Tetrapleura tetraptera* on clinically isolated uropathogens. *T. tetraptera* has being used locally in treating some ailments. The sample was extracted using methanol, hot water and cold water respectively. The quantitative and qualitative compositional analysis of the secondary metabolites of the fruit extract was carried out using Gas chromatography-mass spectrometry (GC-MS). The antibacterial screening was carried out using agar-well diffusion assay. The GC-MS analysis of the fruit extract led to the identification of thirty-five (35) constituents amounting to 96.28% of the extract.

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Abstract- Odoriferous medicinal plants are known to be used as natural therapeutic agents, as they are rich sources of terpenoids and polyphenols. This study was aimed at evaluating the solvent polarity, temperature effects and synergistic potential of the phytochemicals in the fruit extract obtained from the fruit of *Tetrapleura tetraptera* on clinically isolated uropathogens. *T. tetraptera* has being used locally in treating some ailments. The sample was extracted using methanol, hot water and cold water respectively. The quantitative and qualitative compositional analysis of the secondary metabolites of the fruit extract was carried out using Gas chromatography-mass spectrometry (GC-MS). The antibacterial screening was carried out using agar-well diffusion assay. The GC-MS analysis of the fruit extract led to the identification of thirty-five (35) constituents amounting to 96.28% of the extract. Alletone (16.9%), 3-hydroxydihydro-2(3H)-furanone (10.0%), 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (9.3%), 2,3-dihydothiophene (4.5%), 5-hydrxoymethylfurfural (9.0%) and (4E)-4-methyl-4-hepten-3-one (9.0%) were the most abundant components in the fruit extract. These secondary metabolites greatly showcased the antimicrobial potential of the fruit of *T. tetraptera.* The findings of this study showed that there was inhibitory effect of *T. tetraptera* extracts on all the tested organisms. The sample exhibited antibacterial properties against Gram positive and Gram negative organisms with the methanol extract showing the highest inhibitory effect. Hot water and cold water showed similar inhibitory effects. The zones of inhibition ranged from 8-21 mm. This study affirms the traditional application of the sample since it revealed that it possesses antimicrobial properties which can be used for the treatment of a wide range of diseases.

Keywords: tetrapleura tetraptera, fruit extract, phytochemical, pathogen, natural antibiotic.

I. Introduction

Medicinal plants have been an important source of natural drugs and play essential role in healthcare. A wide range of medicinal plants used as traditional medicine have been found to cure various human diseases, which are associated with microbial infections (Dar *et al.,* 2017; Oyedemi *et al.,* 2018; Ololade and Anuoluwa, 2020; Ugboko *et al.,* 2020). Phytochemicals have therefore provided the best alternative method for disease treatment and management (Oyelese *et al.,* 2020). It was discovered long ago that some plant materials exhibit antibacterial properties. Recently, there is a growing demand globally by consumers in minimizing artificial preservation that can be detrimental to human health. Consequently, spices, herbs and naturally occurring phenolics from various plants sources are being studied in detail in response to consumer requirements for fresher and more natural additive-free products (Asif, 2015; Lourenco *et al*., 2019; Ulewicz-Magulska and Wesolowski, 2019; Adesina *et al*., 2021). Plants derives medicines are of immense benefits since they are relatively safer than synthetic alternatives, they offer profound therapeutic benefits and are more affordable source of treatment (Atanasov *et al*., 2015; Anand *et al*., 2019; Ololade *et al*., 2021). Plant based antimicrobials therefore represent a vast untapped source of medicines.

Tetrapleura tetraptera (Schumach. and Thonn Taub) is from the family of Mimosaceae and commonly known as "Aridan" in Nigeria. The medicinal plant is a perennial tree with dark green leaves and thick, woody base and spreading branches. The fruit consist of a fleshy pulp with small, brownish-black seed. The fruit possess a fragrant characteristic pungent, aromatic odour and flavour which has been attributed to insect repellent property (Odesanmi *et al*., 2010; Atawodi *et al*., 2014; Nwoba, 2015; Ozaslan *et al*., 2016; Larbie *et al*., 2020; Otimanam *et al*., 2020). Medicinally, the fruit is used to prepare soup or porridge for nursing mothers from the beginning of childbirth to prevent post-partum contraction, gastro-intestinal disorders especially stomach ulceration and to aid lactation in nursing mothers (Mpody *et al*., 2019). It has also been harnessed in the management of convulsions, leprosy, inflammation, flatulence, jaundice, malaria, rheumatism onset of diabetes mellitus in adults and as a molluscide (Uyoh *et al*, 2013).

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II. Materials and Methods

a) Collection of plant material

The fruit samples were randomly obtained from Ota, Nigeria and identified by botanists as *Tetrapleura tetraptera* in the Department of Biological Science, Bells University of Technology, Ota, Ogun State, Nigeria.

b) Sample Preparation and Extraction

The fresh fruit pods of *T. tetraptera* were air dried and stored in air tight containers until required for use. The pods were cut into small sized pieces before pulverization using laboratory mortar and pestle and finally into powder with an electric blender. Pulverised sample was weighed with an analytical balance, 30 g were soaked in methanol, hot water and cold water respectively for three days with intermittent shaking. The extracts solutions were filtered and then concentrated using water bath (Ololade and Abiose, 2019).

c) GC-MS Phytochemical Screening of the Fruit Extract of T. tetraptera

The methanolic extract of *T. tetraptera* fruit was analysed using Shimadzu GC-MS-QP2010 Plus (Japan). The separations were carried out using a Restek Rtx-5MS fused silica capillary column (5%-diphenyl-95%-dimethylpolysiloxane) of 30 m× 0.25 mm internal diameter (di) and 0.25 mm in film thickness. The conditions for analysis were set as follows; column oven temperature was programmed from 60-280°C (temperature at 60°C was held for 1.0 min, raised to 180 °C for 3 min and then finally to 280 °C held for 2 min); injection mode, Split ratio 41.6; injection temperature, 250 °C; flow control mode, linear velocity (36.2 cm/sec); purge flow 3.0 ml/min; pressure, 56.2 kPa; helium was the carrier gas with total flow rate 45.0 ml/min; column flow rate, 0.99 ml/min; ion source temperature, 200 °C; interface temperature, 250 °C; solvent cut time, 3.0 min; start time 3.5 min; end time, 24.0 min; start m/z, 50 and end m/z, 700. Detector was operated in EI ionization mode of 70 eV. Components were identified by matching their mass spectra with those of the spectrometer data base using the NIST computer data bank, as well as by comparison of the fragmentation pattern with those reported in the literature.

d) Preparation of Extract Solution for Antimicrobial Test

Stock solutions of the concentrated (methanol, hot and cold) fruit extracts (2.5mg/ml, 2.0mg/ml, 1.5mg/ml, 1.0mg/ml, and 0.5mg/ml) were prepared in dimethyl sulfoxide (DMSO). The solutions were stored in the refrigerator until time for use (Alao *et al*., 2018).

e) Antimicrobial Assay

Collection of isolates: Uropathogenic organisms which were identified as Staphylococcus aureus, Staphylococcus saprophyticus, Escherichia coli, Enterococcus faecalis and Pseudomonas aeruginosa were obtained from the stock collection of the Microbiology Laboratory of Bells University of Technology Ota, Nigeria. Stock solutions of the concentrated (methanol, hot and cold) fruit extracts (2.5mg/ml, 2.0mg/ml, 1.5mg/ml, 1.0mg/ml, and 0.5mg/ml) were prepared in dimethyl sulfoxide (DMSO). The solutions were stored in the refrigerator until time for use (Alao *et al*., 2018). In vitro antibacterial potential of the crude extracts were evaluated using agar well diffusion method.

Antibiotic Susceptibility Test: Antibiotic susceptibility test was carried out on each of the pathogenic isolates to determine their susceptibility to the conventional antibiotic dics. Multi-sensitivity discs bearing eight different antibiotics Augmentin, Ceftazidime, Cefuroxime, Cotrimoxazole, Cloxacillin, Erythromycin, Gentamicin, and Ofloxacin were aseptically placed with the aid of sterile forceps on inoculated Mueller Hinton plates. The plates were incubated at 37°C for 24 hr (Hombach *et al*., 2015; Alao *et al*., 2018; Oka and Nweze, 2020).

III. Results and Discussion

a) Phytochemical Composition of the Fruit Extract of T. tetraptera

In this study, the fruit of *T. tetraptera* was investigated for its chemical constituents. The colours were dark green and brown, respectively. The concentrated extract was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis for detailed identification of its components. Identification of the compound was also aided by comparison of their GS-MS mass spectra database. The retention indices of each identified components were also calculated based on their retention time in order to confirm the identification. The GC-MS analysis of the fruit extract of *T. tetraptera* led to the identification of 35 constituents representing 96.28% of the extract. The compound, retention indices and percentage composition are given in Table 1, where the identified components were listed in order of their retention indices. The GC-MS analysis of the fruit extract of *T. tetraptera* led to the identification of 35 constituents representing 96.28% of the extract. Alletone (16.9%), 3-hydroxydihydro-2(3H)-furanone (10.0%), 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (9.3%), 5-Hydrxoymethylfurfural (9.0%), (4E)-4-methyl-4-hepten-3-one (9.0%) and 2,3-dihydothiophene (4.5%) were the most abundant components in the fruit extract of *T. tetraptera*. These compounds contribute greatly to the antimicrobial effects of *T. tetraptera*. The above results showed that the fruit extract of the sample grown in Nigeria and other West African countries has various medicinally active compounds and properties that have been used to treat a great variety of human diseases such as convulsions, leprosy, inflammation, flatulence, jaundice, malaria, adult onset of diabetes mellitus,

rheumatism and as a molluscide. The findings of this study showed that *T. tetraptera* can be used in developing antibacterial substances in combating multidrug resistant bacteria.

Table 1: Chemical Composition of the Fruit Extract of *Tetrapleura tetraptera*

Compound	Retention Index	% Composition
3-methyl-4-(phenylthio)-2-prop-2-enyl-2,5-dihydrothiophene	0	0.6
3-methyl-2-heptanol	130	1.6
sec-butyl nitrite	544	0.2
tutane	598	1.0
sec-butylamine	598	1.0
N-methylisobutylamine	653	1.0
2,3-dihydothiophene	723	4.5
3-methyl-3-ethylpentane	732	2.5
N-methyl-N-(4-pentenyl)amine	806	1.0
propylene Carbonate	875	0.2
pimelic ketone	891	6.3
(4E)-4-methyl-4-hepten-3-one	938	9.0
3-hydroxydihydro-2(3H)-furanone	1013	10.0
alletone	1022	16.9
1,3-butylene glycol diacetate	1087	0.03
octylmegthylamine	1114	1.0
5-hydrxoymethylfurfural	1163	9.0
(+/-)-citronellol	1179	0.03
3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	1269	9.3
(2E)-2-undecenyl acetate	1489	0.03
decane-1, 10-diol	1501	13.0
1-ethyldecyl acetate	1516	0.03
D-glucitol, 1,4:3,6-dianhydro-, dinitrate	1678	0.2
myristic acid	1769	3.2
methyl 14-methylpentadecanoate	1814	0.1
palmitic acid, methyl ester	1878	0.1
methyl 15-methylhexadecanoate	1914	0.1
palmitic acid	1968	3.2
phytol	2045	0.03
trans-phytol	2045	0.03
methyl elaidate	2085	0.1
methyl (10E)-10-octadecanoate	2085	0.1
methyl cis-octadec-11-enoate	2085	0.1
linolelaidic acid, methyl ester	2093	0.6
1,4-diacetyl-3-acetoxymethyl-2,5-methylene-1-rhamnitol	2105	0.2
Percentage Total		**96.28**

b) Antibacterial Screening of the Fruit Extract of T. tetraptera

In this study, different concentrations of the methanolic, hot water and cold water extracts of the fruit of *T. tetraptera* (2.5, 2.0, 1.5, 1.0, 0.5 mg/ml) were prepared) and tested on six pathogens (*Staphylococcus aureus, Staphylococcus saprophyticus, Enterococcus faecalis, Serratia marcescens, Proteus mirabilis and Pseudomonas aeruginosa*). Inhibition zones were observed for the tested organisms. The results obtained for each organism were shown in figure 1-6. Antibiotic sensitivity and resistance patterns of the isolates to standard antibiotic disc were shown in table 2. In this study, the leaves and fruit of this plant were used to determine the antimicrobial activity. The plant extracts were prepared using methanol, hot water and cold water by solvent extraction procedures and their antimicrobial properties were assessed using agar well diffusion method. The sample exhibited antibacterial properties against Gram positive and Gram negative organisms. The methanol extract showed the highest inhibitory effect. Then, hot water and cold water had similar inhibitory effects. The fruit had similar zone of inhibition ranging from 8-21 mm. However, fruit extract had wider range of activity at different concentrations. *P. aeruginosa* showed the highest zone of inhibition among the tested bacteria with the fruit extract was with a maximum zone of inhibition of 20 mm. For *Staphylococcus aureus*, the highest inhibitory effect was observed in methanol extract as depicted in figure 1. This ranged between 10-19 mm at various concentrations used in this study.

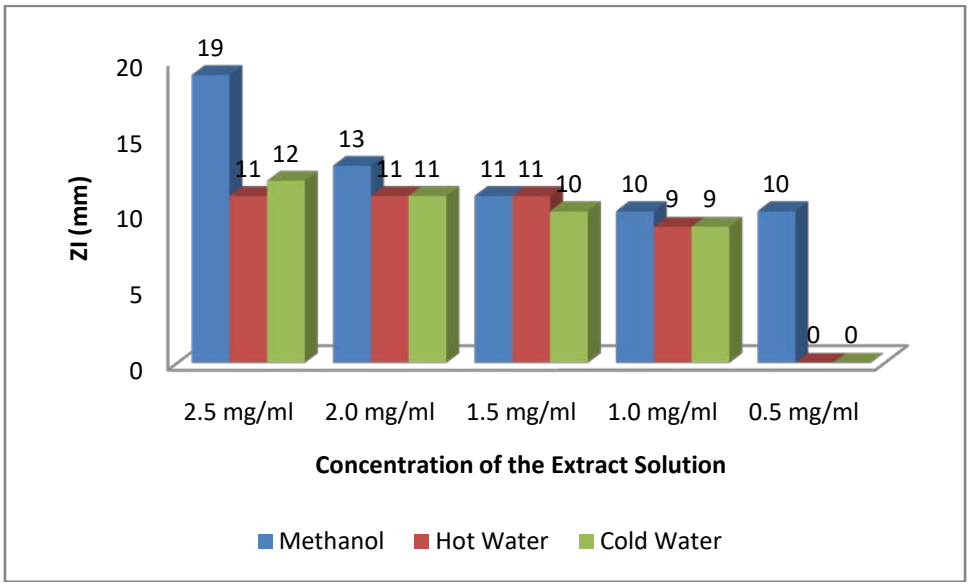

Figure 1: Antibacterial Activity of *T. tetraptera* Fruit Extract on Staphylococcus aureus

For *Staphylococcus saprophyticus,* the highest inhibitory effect was observed in hot water extract as shown in figure 2. This was ranged between 09-15 mm at various concentrations used in this study.

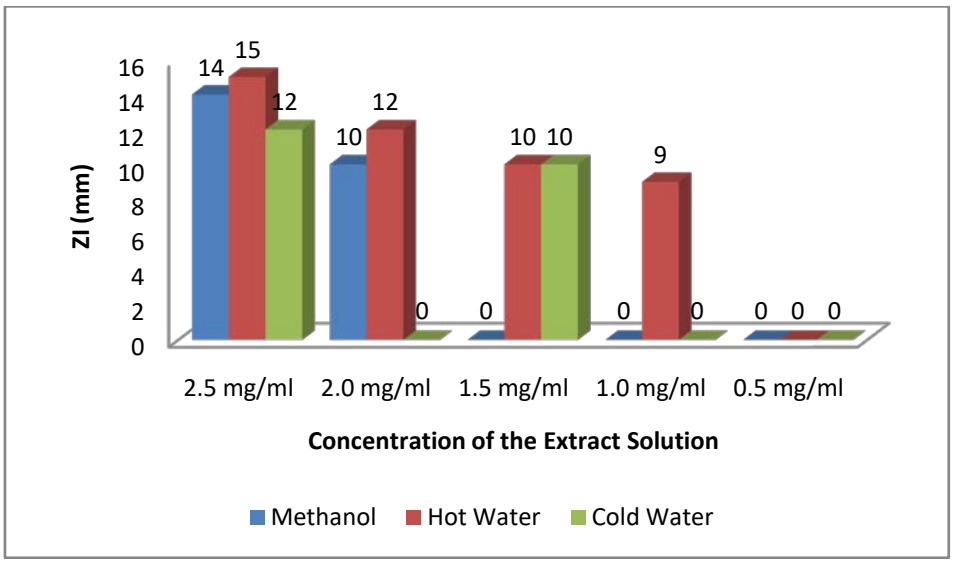

Figure 2: Antibacterial Activity of *T. tetraptera* Fruit Extract on Staphylococcus saprophyticus

For *Enterococcus faecalis,* the highest inhibitory effect was observed in cold water extract, followed by hot water extract and least by the methanol extract as shown in figure 3. This was ranged between 08-21 mm at various concentrations used in this study.

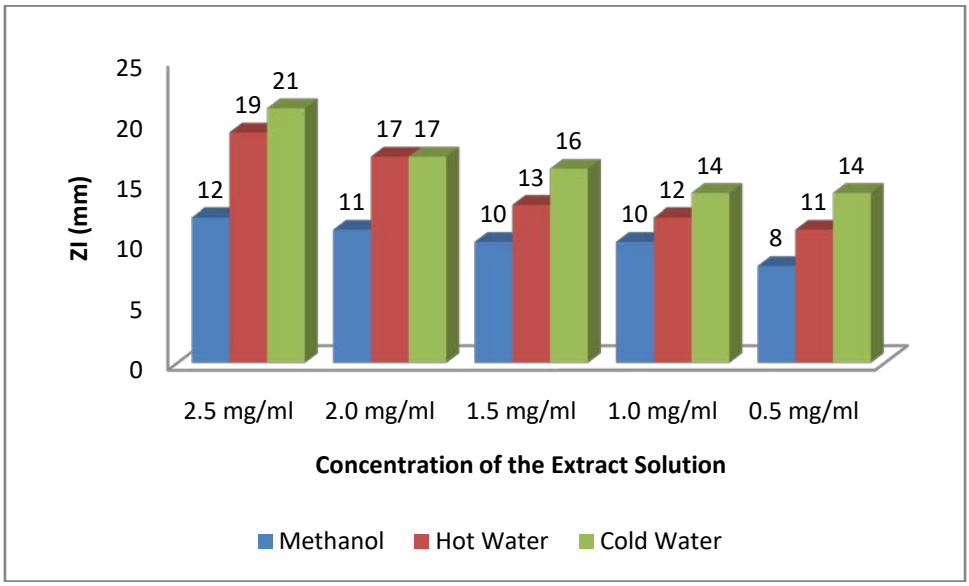

Figure 3: Antibacterial Activity of *T. tetraptera* Fruit Extracts on Enterococcus faecalis

For *Serratia marcescens,* the highest inhibitory effect was observed in methanol extract, followed by hot water extract and then cold water extract as shown in figure 4. The value of zones of inhibition was ranged between 09-21 mm at various concentrations considered in this study.

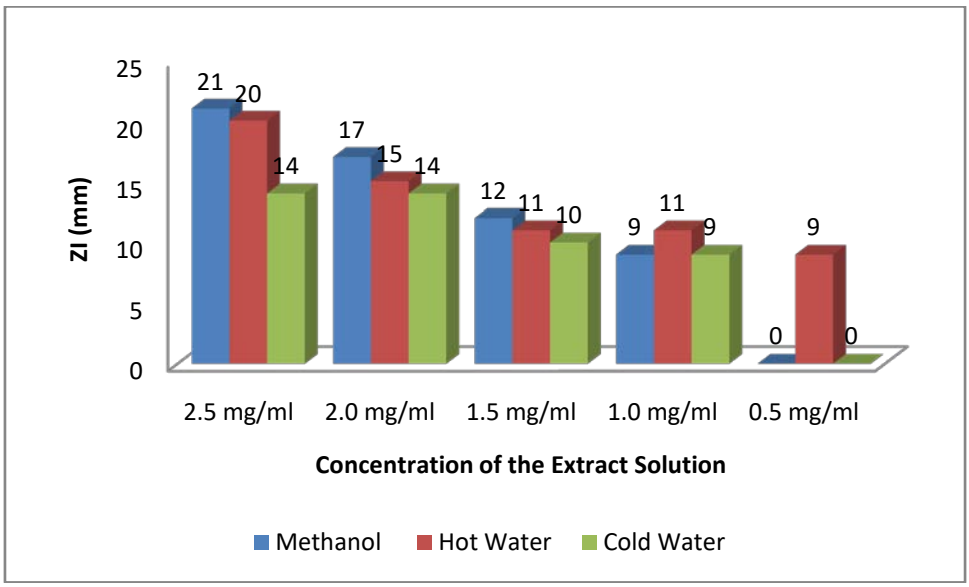

Figure 4: Antibacterial activity of *T. tetraptera* Fruit Extract on Serratia marcescens

For *Proteus mirabilis,* the highest inhibitory effect was observed in methanol extract and cold water extract and then hot water extract did not show activity except at 2.5 mg/ml as shown in figure 5. The value of zones of inhibition was ranged between 09-18 mm at various concentrations considered in this study.

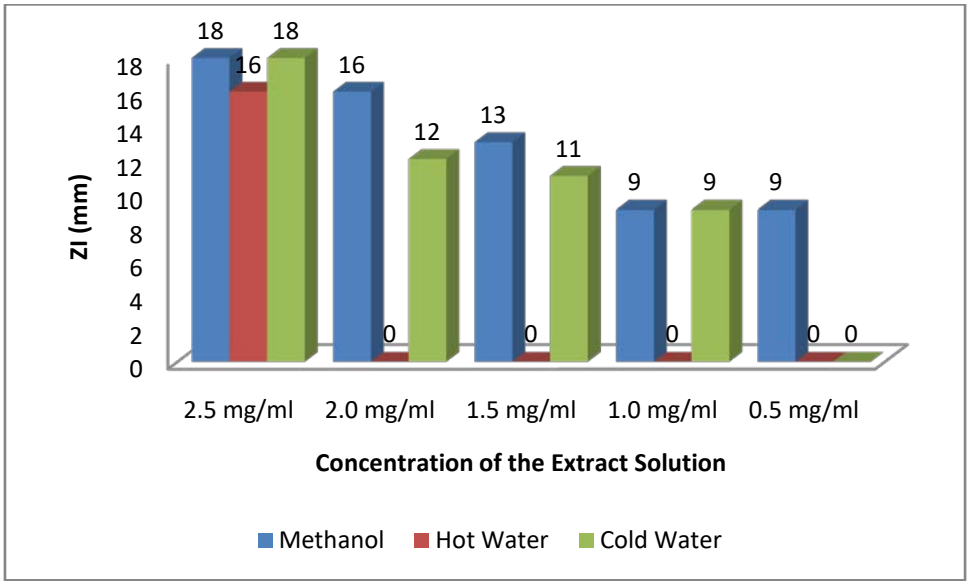

Figure 5: Antibacterial Activity of *T. tetraptera* Fruit Extracts on Proteus mirabilis

For *Pseudomonas aeruginosa,* the highest inhibitory effect was observed in methanol extract followed by hot water extract and then cold water extract as shown in figure 6. The value of zones of inhibition was ranged between 11-20 mm at various concentrations considered in this study.

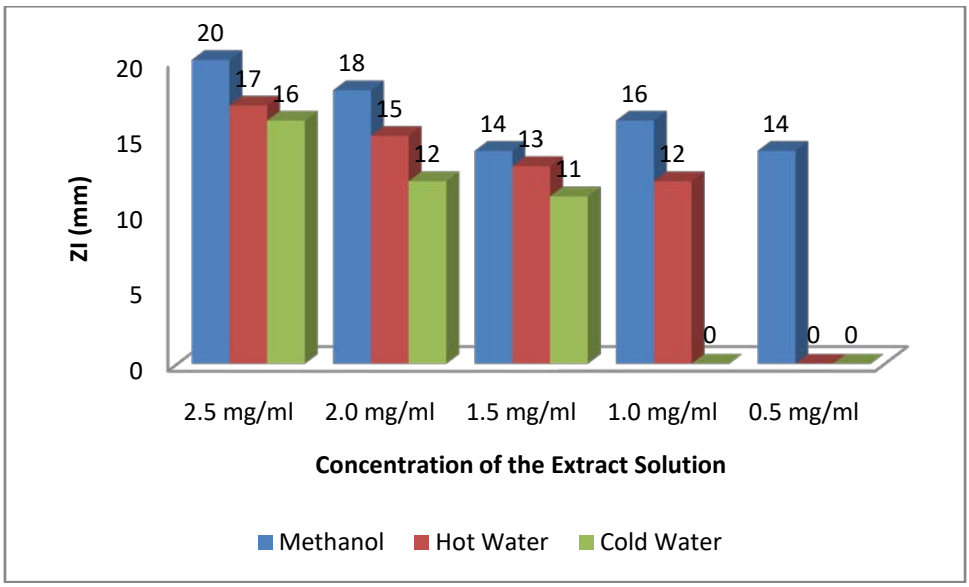

Figure 6: Antibacterial Activity of *T. tetraptera* Fruit Extracts on Pseudomonas aeruginosa

In addition, the effect observed was dependent on the concentration of the extracts and the extract established an interaction with the concentration used as the range of activity reduced with the decrease in concentration of each extraction solvent. Finally, the effects measured was also dependent on the extraction method and solvent (absolute methanol, hot water and cold water) used and the fruit established an interaction with the extraction method. Table 2 showed the susceptibility of the tested organisms to different antibiotics. All of them were inhibited by at least one antibiotic with no exception. They were all resistant to Augmentin, Ceftazidime, Cefuroxime, and Cloxacillin. Also, the findings from this study indicated higher resistance pattern exhibited by the organism to synthetic antibiotic in comparison to the high inhibitory effects of *T. tetraptera* extracts against these organism. Therefore, if the plant can be adequately harnessed and studied, it can be used as a natural antibacterial agent against some of the pathogens as discovered in this study.

Solvent polarities are factors that responsible for the variation in the antibacterial activity of plant extracts, permeability of cell of bacteria, concentration etc (Gonelimali *et al*., 2018; Zhang *et al*., 2020). The effect of solvent polarity on extraction yield and antibacterial properties of secondary metabolites in the fruit was studied. Solvent type and polarity index play an important role in the antibacterial activities level in the

extracts (Truong *et al*., 2019; Wakeel *et al*., 2019). Extraction in highly polar solvents resulted in high extract yield of phytochemicals. The polarity-dependent increase in antibacterial potential indicates the extraction of strong antimicrobial compounds in polar solvents. The quantities of crude extracts with different solvents were different in different extracts reported that the extracts of these solvents have significantly different antimicrobial activity (Altemimi *et al*., 2017; Nawaz *et al*., 2019). The different antimicrobial activities of these solvents and plants parts might be because of the different types and quantity of biological compounds in these extracts. The role of solvent polarity in the quantity and quality of crude extracts, secondary metabolites, and biological activities cannot be over emphasized. Differences in the antibacterial activities of the may be because of the phytochemical polarity index and their association with solvent polarity index. Similar polarity index containing solvents can dissolve phytochemicals that have similar or close related polarity index (Othman *et al*., 2019; Chassagne *et al*., 2021; Vaou *et al*., 2021).

Table 2: Antibiotic Sensitivity and Resistance Patterns of Isolates

Isolates	OFL 5µg	AUG 30µg	CAZ 30µg	CRX 30µg	GEN 10µg	CTR 30µg	ERY 15µg	CXC 5µg
E. faecalis	34	R	R	R	21	12	R	R
P. aeruginosa	21	R	R	R	15	26	R	R
S. marcescens	22	R	R	R	16	23	R	R
S. saprophyticus	16	R	R	R	15	15	R	R
P. mirabilis	21	R	R	R	15	10	R	R
K. pneumoniae	15	R	R	R	15	R	15	R
S. aureus	28	R	R	R	R	R	R	R

Key: OFL-Ofloxacin, AUG-Augmentin, CAZ- Ceftazidime, CRX-Cefuroxime, GEN-Gentamicin, CTR-Ceftriaxone, ERY-Erythromycin, CXC-Cloxacillin; R-Resistant, I-Intermediate, S-susceptible.

IV. Conclusion

This study revealed that the fruit extract of *T. tetraptera* commonly used by the local people in Africa in the preparation of herbs, has the potential of being used in the production of drugs with a broad spectrum of activity. This study also serves as an affirmation that the traditional application of sample is of great essence and that it possess antimicrobial properties which can be used for the treatment of a wide range of diseases. The antimicrobial activities of *T. tetraptera* was investigated in this study and proven that it is a potential source of antibiotics for the development of newer and more effective antibacterial agent. With respect to this study, it is recommended that clinical studies should be carried out on this plant to harness its potential for drug production.

Conflict of Interest: We have no conflict of interest.

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