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Comparing the phytochemical composition and antibacterial activity of the wild and *in-vitro* generated *Tetrapleura tetraptera* (Schumach and Thonn.) Taub.

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Abstract: **The efficacy of Distilled water, ethanol, and acetone extracts of wild and *in-vitro* plants of *Tetrapleura tetraptera* on some selected Bacteria was investigated using standard biological methods revealed significant (P<.0.05) inhibitory effect of plant extracts, on the test bacteria with ethanol extract having the highest antibacterial activity followed by acetone and distilled water extract. Ethanol extract had higher zones of inhibition against *Staphylococcus aureus* (14.31mm) but lowest against *Pseudomonas oryzae* (8.37mm) for *in-vitro* plant extract, while the maximum inhibitory activity against *P. oryzae* (10.31mm) and a minimum inhibitory activity against *E. coli* (5.53mm) were recorded for acetone *in-vitro* extract of plant. Also, the *in-vitro* leaf extract of distilled water showed the least activity against *Staphylococcus aureus* and *E. coli* with 8.40mm and 2.75mm respectively. Similarly, for the wild plant leaf, ethanol extract produce higher zones of inhibition against *S. aureus* (17.25mm) and lowest against *Ralstonia solanacearum* (9.34mm), while higher inhibitory activity reflect against *Bacillus cereus* (10.95mm), and a minimum inhibitory activity against *E. coli* (7.34mm) were shown for acetone extract. Distilled water extract of wild leaf showed highest activity against *Enterobacter aerogenes* (6.91mm), but lowest activity against *P. aeruginosa* (4.41mm). For sensitivity of Bacteria to different concentrations of wild, *in-vitro* plant and antibiotic disc, Ofloxacin antibiotic disc gave higher inhibitory effect against *Escherichia coli*, and *Pseudomonas aeruginosa.* In 100mg and 80mg concentration *Escherichia coli, Pseudomonas aeruginosa, and Pseudomonas oryzae* were highly susceptible at the range of 10.93mm to 15.91mm for 80mg and 100mg concentration. *P. oryzea* was found to be more susceptible to acetone than ethanol extract. However, the *in-vitro* leaf extract of acetone was more susceptible compared to wild plant extract.**

Keywords: **Key words: Antimicrobial, plant leaf extract, phytochemical and *Tetrapleura tetraptera*.**

1. INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and impressively there have been a great number of developed drugs gotten from nature natural sources (Cragg and Newman 2001). Indigenous medicine is now recognized worldwide both by the rural populace and the urban elite as an important healthcare resource. The World Health Organization (WHO) has always pointed out the contribution of indigenous medicine to health goals. There are considerable economic benefits in the development of indigenous medicine, and the use of medicinal plants for the treatment of various diseases (WHO, 2003).

Tetrapleura tetraptera, locally known as Aridan in southwest Nigeria is a medicinal plant with many folkloric uses. Some of the uses of *Tetrapleura tetraptera* have been authenticated in laboratory and field experiments. The plant is claimed to be therapeutically useful in the management of different ailments such as convulsion, leprosy and rheumatoid pains (Dalziel 1948).

Medicinal plants have also been of importance in the health care of man, an ethnobotanical survey in Ghana confirmed the use of *tetrapleura tetraptera* is a good source for treating hypertension. In the eastern part of Nigeria, the strong aroma from the fruit of *tetrapleura tetraptera* is used to repel crawling reptiles like snakes from households, has low sodium content and very rich in vitamins (Adewumi *et al.*, 2001).

Importantly, the inhibitory effects of *Tetrapleura tetraptera* extracts against some human pathogens have been reported. Currently, there is a growing demand worldwide of consumers for minimizing chemical preservation that can be detrimental to human health consequently, spices, herbs and naturally occurring phenol from various plants sources are being studied in detail in response to consumer requirements for fresher and more natural additive-free products. The tree sweet fragrance is highly valued. The fruit pulp is rich in sugar and may be used in flavouring food. Timber is reddish to brown fairly hard heartwood and white sapwood. Leaves, bark, roots and kernels are used for medicinal purposes. Fruit and flowers are used as perfumes and in pomades prepared from palm oil. Extracts obtained from *Tetrapleura tetraptera* has ethno-medicinal significance and its used in the management of anti-ulcer activity, gastro-intestinal disorders especially stomach ulceration (Noamesi *et al.*, 1992). Traditional practice has resulted to high consumption and destruction of large amount of *Tetrapleura tetraptera* plant. *In-vitro* cultivation method has proven to be an efficient regeneration method for mass propagation of different crops to produce uniform and disease free propagules for potential and quality production both in the tropical and temperate region. In order to ensure highest possible yield and quality of field crops, the disease free stock can be extended to farmer by using this technique (Aamir *et al* 2008). About 80% of the world population make use of plant active constituents as folk medicine in traditional therapies and Over 50% of modern drugs used clinically are of natural plant product origin (Baker *et al*., 1995). According to World Health Organization medicinal plants is the best source to obtain vast variety of drugs. A good number of earth populations particularly the indigenous people depend largely on herbal remedies for the treatment of different types of diseases.

Bearing in mind the medicinal values of the plant *Tetrapleura tetraptera* and the significance attached to it by its consumers, there is need for continuous work on its probable adverse effects for consumers and also for better understanding of its effect its target microorganisms, and to ensure its continuous production for regular and mass production using a method that will ensure the production of genetically identical species to the parent plant in a short period of time thus aiming at the *in-vitro* propagation of *Tetrapleura tetraptera* and also ensuring an *ex-situ* means of germplasm conservation. The bacterial activity of the plant extracts was evaluated based on the inhibition of growth and changes in the structure of the bacteria. Other aspects regarding the chemical constituents and toxicity of the active extracts and fractions are also discussed.

2. MATERIALS AND METHODS

The study was carried out in the Biotechnological laboratory and the pathology laboratory of National Centre for Genetic Resources and Biotechnology (NACGRAB) moor plantation Ibadan. The plant material used in this study is *Tetrapleura tetraptera*; and microorganisms used are, *Enterobacter aerogenes, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus, Bacillus cereus, Ralstonia solanacearum, Erwinia carotovora, Xanthonmona axonopodis* pv *vignicola, Pseudomonas oryzae.*

The taxonomic identification was determined by the herbarium unit of the Department of Botany, University of Ibadan. Collective samples of the seed from *Tetrapleura tetraptera* grown *in-vitro* and acclimatized in an acclimatization chamber. The acclimatized plants were transferred into a screen house (with control watering and shed from direct sunlight) within the National Center for Genetic Resources and Biotechnology (NACGRAB) district in moor plantation Ibadan. Plant where harvested in after seven months (of acclimatization). The test human bacteria pathogens were sourced from Institute of Animal Research and Training (IAR&T) moor plantation Ibadan, and then plant bacteria were sourced from Nigeria Agricultural Quarantine Service (NAQS). The test organisms were further identified and the biochemical and morphological characteristics were confirmed by standard methods (Cheesebrough, 2000).The experimental design used was a Completely Randomized Design (CRD) with eight treatments and four replicates.

2.1 Sterilization of explants

Instruments to be used were washed with water, commercial bleach and liquid detergents. Rinsed with tap water and then with distilled water and later dry in the oven at 250^{o}C. All glass ware and dissecting tools where later sterilized in autoclave at 121^{o}C and 1.06kg/cm^{2} pressure for 30 minutes.

2.2 Seed disinfection

Tetrapleura tetraptera seed were washed in liquid detergent and rinsed thoroughly to remove any residual foam from the detergent, soaked with concentrated H_2SO_4 to break the dormancy and rinsed with distilled water after 20 minutes, the seed was left overnight. The next day, water was decanted and the seed taken to the transfer chamber under the laminar flow hood. Seed was then soak in 70% ethanol for 5 minutes and was decanted finally seed was immersed in 20% sodium hypochlorite solution (Clorox) for 20 minutes and followed by two rinses in sterile distilled water and left in water until use to prevent it from drying prior to culture.

2.3 Preparation of media

All media used for this work were prepared according to manufacturer's instructions. Seeds were inoculated on Murashige and Skoog (1962), medium in test tubes and transferred into the growth room. 28 gram of Nutrient agar (NA) was weighted into 1 liter of deionized water and the pH was made up to 7.3±2)

2.4 Embryo culture

Embryos were excised from the seeds and immersed into the media in the laminar flow and the entire cultured tubes were sealed with Parafilm M tape, labeled and transferred to the incubation room where they were subjected to temperature of $23^{O}C \pm 2$, 16 hours photoperiod and 3,000-4,000 lux light supplied by fluorescent lamps.

2.5 Preparation of inocula

Stock cultures were maintained at $4^{o}C$ on nutrient agar slants for bacteria. Active cultures for experiments were prepared by transferring a loopful of culture to 5 ml of Normal saline solution and incubated at $37^{o}C$ for 24 hours.

2.6 Plant Sample Preparation

The dry *Tetrapleura tetraptera* leaves were prepared and extracted based on the methods described by Adeshina *et al*., (2010) and Awofisayo *et al*., (2010). The leaves were first washed with distilled water and the residual moisture removed by air drying under $21^{O}C \pm 3$ for 24hours. The dried sample was cut to small bits and reduced to coarse powder using a mortar with pestle. This was finally ground to fine powder using a pre-washed mechanical blender, sieved through a 1.0mm mesh sieve, and used for the extraction.

2.7 Extraction

The extraction method used in this study was a modification of Eze *et al* (2010). The collected parts were air-dried and then powdered using blending machine. Each powder (25 g) was soaked with 250 ml absolute Distilled water, acetone and ethanol with intermittent shaking for 48 h. Each extract was then filtered through a Whatman number 1 filter paper. The filtrate was evaporated to concentrate in a steady air current for about 24 h. The extract was sterilized by filtering with a 0.22µm micropore filter. Sterility was checked by streaking the extract on sterile nutrient agar plates. All extracts were stored as in the refrigerator at $4^{o}C$ until needed.

2.8 Preparation and reconstitution of extracts

For the preparation of dilution of crude extract for antibacterial assay, the extract was reconstituted using the solvents to obtain 100mg/ml, 80mg/ml, 66.6mg/ml, 57.1mg/ml, and 50mg/ml concentrations. These were obtained by dissolving 20mls of the extract into 0mls, 5mls, 10mls and 15mls of the various solvents used for the extraction. The reconstituted extracts were then stored at $4^{o}C$ in sample bottles until required.

2.9 Sensitivity Test

Preparation of antibacterial sensitivity discs this was done with the aid of an office perforator, circular discs were cut out of Whatman No.1 filter paper (5mm). The discs were socked in hot distilled water for an hour to remove any preservative, which may contain antibacterial substances. The discs were transferred to Aluminum foil and dried with oven at $100^{o}C$ to remove the water. They were wrapped well in a foil and sterilized by autoclaving. The sterile discs were used for the tests.

2.10 Antibiotics activity assay

Antibiotic discs were placed on the surface of a Nutrient agar that has been inoculated with test microorganisms. During incubation, the antibiotics diffuse outward from the discs creating a concentration gradient. After 18-24 hours, the zone

diameter of inhibition is measured and reference tables are used to determine if the bacteria are Sensitive (S), Intermediate (I) or Resistant (R) to the antimicrobial drugs (Sockett, 2006).

2.11 Disc Sensitivity Screening of Extracts

The antibacterial sensitivity testing of the extracts were performed in triplicates using disc diffusion method on Nutrient agar the mean zones of inhibition were determined and expressed in millimeter. Two additional batches of each isolate in triplicate were treated with standard antibiotic discs as positive controls, while discs impregnated with the pure solvents at the exact percentage used for the extraction of the plant materials served as negative controls. The mean zones of inhibition of each were determined and expressed in millimeter.

2.12 Test on Bacterial Sensitivity Extract

The susceptibility of each test bacteria (pathogen) to the effect of the various extracts was determined through sensitivity test .The disc diffusion technique (Cheesbrough, 2000). A loop full of the inoculum was aseptically collected from a pure culture plate of each test bacteria placed on the surface of sterile agar plate. A swap stick was used to spread the inoculum evenly over the surface of the plate. Then very carefully, the extract embedded paper discs were picked with sterile forceps and placed at some distance from one another. The plates were allowed to stand for a few minutes to enable the extract diffuse into the medium. The plates were incubated at 37^{o}C in an incubator for 24hours. This was observed for growth and the presence of inhibition zones as a mark of sensitivity to the test extract. Extent of sensitivity was determined by the diameter of the inhibition zone as measured with a transparent ¼ meter rule.

2.13 Qualitative and quantitative analysis of phytochemical screening

The processed sample was subjected to screening to establish the presence or absence of some specific active principles.

2.14 Tests for Tannins

The ferric chloride test described by Harborn (1973) was used. An aqueous extract of the sample was obtained by shaking 10g of the powdered sample in 100ml of distilled water for 30minutes.After filtration; the extract was used for the test as described below. 2mls of the aqueous extract mixed with equal volume of distilled water in a test tube and drops of diluted ferric chloride solution. The presence of dark precipitate gave an indication of the presence of tannin in the extract.

2.15 Test for saponins

The combined froth and emulsion test was used to test for the presence of saponin in the sample. 2mls of the aqueous extract was mixed with 5mls of distilled water in a test tube. The mixture was shaken well and observed. The formation of a stable froth (foam) gave a positive result. However, this was confirmed by the addition of few drops of olive oil and shaken again. The formation of an emulsion confirmed the presence of saponin in the test sample. (Harborn, 1973 and Sofowora, 1978).

2.16 Test for Flavonoid

The alkaline acid test (Harborn, 1973) was used. A drop pf bench ammonia solution was added to 3mls of the aqueous extract of the sample in a test tube. The formation of yellow coloration which clears on the addition of concentrated acid solution was taken as a positive result for the test.

2.17 Test for Cyanogenic glycoside

An alkaline picrate colorimetric method was used. One gram of the processed sample was dispersed in 150mls of distilled water in a conical flask. An alkaline picrate paper was suspended over the mixture and held in place by the rubber bung (stopper).Care was taken to avoid the paper touching the surface of the mixture. The arrangement was allowed to stand for 18hours (over-night) at room temperature. The picrate paper was examined for colour change from yellow to orange as a positive test for the presence of cyanogenic glycoside (HCN) (Harborn, 1973 and Sofowora, 1978).

2.18 Test for Alkaloid

The Mayors and Haglens test (Harborn, 1973) was used to test for alkaloid in the sample. 2mls of an ethanolic extract was mixed with equal volume of Mayors and Haglens reagents in separate test tubes. The formulation orange-brown precipitate indicated the presence of alkaloid.

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2.19 Test for Steroids/Triterpens

The Dragendert's test was used. A portion of the ethanolic extract was mixed with acetyl anhydride in a test tube. The presence of a brown colouration at the interface between the two layers indicated a positive result for steroid (Harborn, 1973 and Sofowora, 1978).

2.20 Data collection and analysis

Data were collected by measuring the zones of inhibition by each extracts on the test organisms. The data generated were subjected to analysis of variance (ANOVA) using the statistical software (SPSS) version 20 and means were separated using the Fisher's least significant difference (DMRT) at both 95% and 99% confidence intervals.

3. RESULTS

3.1 Phytochemical composition of *Tetrapleura tetraptera* leaf extracts

The qualitative phytochemical screening in Table 1 revealed the presence of some biologically active constituents analysed. The result showed that saponin, alkaloid, flavonoid, glycoside, tannins, steroids and triterpens were present in all solvent of *Tetrapleura tetraptera* leaf extract. In the wildly grown *Tetrapleura tetraptera*, alkaloids of both the water and ethanol leaf extract were stronger than the *in-vitro* generated once. The same was observed for tannins and steroids of wild type ethanol leaf extract were stronger than the *in-vitro* generated ethanol leaf extract. Though on the contrary the flavonoid content of the in-vitro water leaf extract which showed stronger presence than the wild type.

3.2 Phytochemical (quantitative) composition of the wild *Tetrapleura tetraptera* leaf and *in-vitro* generated *Tetrapleura tetraptera* leaf.

The quantitative phytochemical screening in Table 2 shows the biologically active constituents analysed, with significant difference among the composition of phytochemicals. The result showed that the matured wild type plant of *Tetrapleura tetraptera* (leaf) had a higher biological active constituents more than all the *in-vitro* leaf, though the *in-vitro* leaf of the 1st month gave a higher flavonoid constituent. However there was no significant difference in the wild plant of *Tetrapleura tetraptera* and the 6th month *in-vitro* plant on Tannins and also Terpenoids showed no significant difference among the 5th and 6th month *in-vitro Tetrapleura tetraptera* plant leaf.

3.3 Phytochemical (quantitative) composition of the wild *Tetrapleura tetraptera* root and *in-vitro* generated *Tetrapleura tetraptera* root.

The quantitative phytochemical screening in Table 3 shows that there was significant difference among the composition of phytochemicals with the wild *Tetrapleura tetraptera* (root) having higher phytochemical constituent except for Flavonoids which the *in-vitro* plant of the 1st , 2nd and 3rd month showed higher phytochemical constituent with no significance. There were no significance among the phytochemical of Phenolics and Alkaloids between the wild plant type of *Tetrapleura tetraptera* and the 6th month in-vitro generated *Tetrapleura tetraptera* plant root. However the Saponin of the 1st month in-vitro plant of *Tetrapleura tetraptera* (roots).

TABLE 1: Phytochemical (qualitative) composition of the wild and in-vitro generated *Tetrapleura tetraptera* leaf extracts

Phytochemicals	Extractants					
	Water		Ethanol		Acetone	
	In-vitro	wild type	*In-vitro*	wild type	*In-vitro*	wild type
Alkaloids	+	++	+	++	+	+
Saponins	+	+	+	+	++	++
Cardiac glycosides	++	++	++	++	-	-
Tannins	+	++	+	+++	+	+
Flavonoids	+++	++	++	++	+	-
Steroids	+	+	+	+++	+	++
Triterpens	-	-	+	+	+	+

Key : + = Present in trace amount, ++ = Present in large amount, +++ = Present in very large amount, - = Absent.

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TABLE 2: Phytochemical (quantitative) composition of the wild *Tetrapleura tetraptera* leaf and *in-vitro* generated *Tetrapleura tetraptera* leaf.

Phytochemicals	*In-vitro* plant (months)						
	1st month	2nd month	3rd month	4th month	5th month	6th month	Wild plant
Carotenoids (µg/100g)	1280.00^{e}	1333.33^{d}	1368.38^{cd}	1426.66^{c}	1485.00^{c}	1543.33^{b}	1738.33^{a}
Alkaloids (mg/100g)	593.33^{d}	608.33^{cd}	620.00^{c}	646.66^{c}	646.66^{b}	683.33^{b}	740.00^{a}
Tannins (mg/100g)	383.33^{e}	381.66^{d}	405.00^{cd}	426.66^{c}	441.66^{b}	490.00^{a}	533.33^{a}
Flavonoids (mg/100g)	1010.00^{a}	986.66^{b}	978.33^{b}	970.00^{b}	983.33^{b}	963.33^{bc}	858.33^{c}
Terpenoids (mg/100g)	263.33^{c}	256.66^{c}	278.33^{b}	286.66^{b}	308.33^{a}	311.66^{a}	233.33^{d}
Cardiac Glycosides (mg/100g)	23.33^{e}	43.33^{d}	56.67^{c}	80.00^{b}	83.33^{b}	91.66^{a}	100.00^{a}
Phenolics (GAE/g)	26.26^{c}	27.66^{c}	29.46^{c}	32.57^{b}	36.23^{b}	38.80^{b}	56.50^{a}
Saponins (mg/100g)	625.00^{a}	591.66^{a}	538.33^{b}	491.66^{cb}	441.66^{c}	396.67^{d}	253.33^{e}

TABLE 3: Phytochemical (quantitative) composition of the wild *Tetrapleura tetraptera* root and *in-vitro* generated *Tetrapleura tetraptera* root.

Phytochemicals	*In-vitro* plant (months)						
	1st month	2nd month	3rd month	4th month	5th month	6th month	Wild plant
Carotenoids (µg/100g)	56.66^{c}	60.00^{d}	63.33^{b}	66.66^{b}	68.33^{ab}	73.33^{b}	85.33^{a}
Alkaloids (mg/100g)	888.33^{c}	918.33^{cd}	951.66^{bc}	1023.33^{b}	1083.33^{b}	1233.33^{a}	1351.66^{a}
Tannins (mg/100g)	566.66^{b}	586.66^{b}	616.66^{ab}	636.66^{ab}	658.33^{ab}	690.00^{ab}	813.33^{a}
Flavonoids (mg/100g)	233.33^{a}	211.66^{a}	191.66^{a}	180.00^{b}	160.00^{b}	161.67^{b}	111.66^{c}
Terpenoids (mg/100g)	40.00^{c}	56.66^{c}	68.33^{b}	71.66^{b}	85.00^{a}	91.67^{a}	83.33^{a}
Cardiac Glycosides (mg/100g)	6.66^{d}	11.66^{cd}	15.00^{c}	21.66^{b}	23.33^{b}	28.33^{ab}	38.33^{a}
Phenolics (GAE/g)	16.43^{c}	19.10^{c}	24.50^{cb}	28.33^{b}	33.43^{a}	37.33^{a}	43.30^{a}
Saponins (mg/100g)	281.66^{a}	263.33^{ab}	246.33^{ab}	226.66^{b}	193.33^{c}	188.33^{c}	123.33^{d}

The values with the same letters are not significantly different ($p<0.05$)

3.4 Comparison of inhibition zones (mm) of tested bacteria by *in-vitro* extracts of *Tetrapleura tetraptera* leaf

The result in table 4 shows that there was high significant inhibitory effects of ethanol in all test bacteria also result shows that there was significant inhibitory effects on *Escherichia coli* compare to most of the test bacteria. On the other hand ethanol and acetone had similar inhibitory performance against *Ralstonia solanacearum*. Though there was no significant inhibitory effect of acetone and ethanol against *Enterococcus faecalis*. However acetone showed highest inhibitory effect on *Pseudomonas oryzae.*

3.5 Comparison of inhibition zone (mm) of tested bacteria by extracts from the wild plant leaf

The result in table 5 shows that there was significant inhibitory affect in both acetone and ethanol leaf extracts of the wild plant of *Tetrapleura tetraptera* with ethanol having the highest significant inhibitory effect of acetone and ethanol against *Bacillus cereus*. While acetone showed higher inhibitory performance against *Pseudomonas oryzae*.

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Table 4: Comparison of inhibition zones (mm) of tested bacteria by *in-vitro* extracts of *Tetrapleura tetraptera* leaf

Method	*E. coli*	*P. aeruginosa*	*E. faecalis*	*S. aureus*	*E. aerogenes*	*B. cereus*	*R. solanacearum*	*E. carotovora*	*X. axonopodis*	*P. oryzae.*
Distilled water	2.75^{c}	2.78^{c}	4.63^{b}	8.40^{c}	7.25^{c}	4.72^{c}	5.84^{b}	4.75^{c}	5.09^{c}	8.37^{b}
Acetone	5.53^{b}	5.62^{b}	8.72^{a}	10.31^{b}	8.13^{b}	8.78^{b}	8.66^{a}	7.38^{b}	7.34^{b}	10.31a
Ethanol	8.03^{a}	10.12^{a}	9.75^{a}	14.31^{a}	10.34^{a}	10.53^{a}	8.97^{a}	10.31^{a}	10.84^{a}	8.37b

The values with the same letters are not significantly different ($p<0.05$)

Table 5: Comparison of inhibition zones (mm) of tested bacteria by extracts from the wild plants

Method	*E. coli*	*P. aeruginosa*	*E. faecalis*	*S. aureus,*	*E. aerogenes*	*B. cereus*	*R. solanacearum*	*E. carotovora*	*X. axonopodis*	*P. oryzae.*
Distilled water	4.81^{c}	4.41^{c}	5.00^{b}	9.88^{c}	6.91^{c}	5.09^{b}	4.69^{b}	4.53^{b}	4.81^{b}	6.88^{b}
Acetone	7.34^{b}	7.44^{b}	9.91^{a}	12.06^{b}	9.56^{b}	10.94^{a}	10.03^{a}	9.47^{a}	8.69^{a}	9.59^{a}
Ethanol	9.69^{a}	12.62^{a}	11.03^{a}	17.25^{a}	10.59^{a}	10.94^{a}	9.34^{a}	10.00^{a}	10.19^{a}	7.40^{b}

The values with the same letters are not significantly different ($p<0.05$)

Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus, Enterobacter aerogenes, Bacillus cereus, Ralstonia solanacearum, Erwinia carotovora, Xanthonmona axonopodis pv *vignicola, Pseudomonas oryzae*

3.6 Sensitivity of Bacteria to different concentrations of *in-vitro* plant extracts and antibiotic disc (mm).

Table 6 showed significant inhibitory performance in all concentration of plant extracts and antibiotics disc. Oflaxacin antibiotic disc showed high significant inhibitory effects on all test bacteria except *Escherichia coli* and *Pseudomonas oryzae*. However 100mg and 80mg of extracts showed significant inhibitory effects on both *Escherichia coli* and *Pseudomonas aeruginosa*

3.7 Sensitivity of Bacteria to different concentrations of wild plant extracts and antibiotic disc (mm).

Table 7 showed significant inhibitory effect on all concentration of wild plant of *Tetrapleura tetraptera* extract and antibiotic disc, with Ofloxacin antibiotic showing higher significant inhibitory performance on all test bacteria except *Escherichia coli* and *Pseudomonas aeruginosa.* Also 100mg and 80mg concentration of wild plant extract *Tetrapleura tetraptera* showed high inhibitory performance on *Escherichia coli* and *Pseudomonas aeruginosa.* However 80mg concentration of the wild plant extract showed higher inhibitory performance on *Pseudomonas oryzae.*

Table 6: Sensitivity of Bacteria to different concentrations of in-vitro plant extracts and antibiotic disc (mm).

Treatment	*E. coli*	*P. aeruginosa,*	*E. faecalis*	*S. aureus*	*E. aerogenes*	*B. cereus*	*R. solanacearum*	*E. carotovora*	*X. axonopodis*	*P. oryzae.*
100mg	11.92^{a}	11.83^{a}	9.91^{b}	10.25^{cd}	12.00^{b}	8.58^{bc}	9.03^{b}	6.86^{e}	9.83^{b}	10.33^{b}
80mg	10.42^{a}	11.58^{a}	9.25^{b}	12.08^{c}	8.58^{c}	6.75^{cd}	7.16^{c}	6.00^{c}	6.58^{b}	8.58^{c}
66mg	6.67^{bc}	8.00^{b}	6.91^{c}	9.00^{d}	7.50^{c}	6.67^{cd}	5.08^{d}	5.41^{c}	5.75^{c}	8.00^{c}
57mg	5.08^{c}	6.00^{bc}	5.50^{c}	5.75^{e}	5.50^{d}	4.75^{d}	4.16^{d}	2.67^{d}	2.67^{d}	6.41^{d}
50mg	2.42^{d}	0.00^{d}	3.00^{d}	3.83^{e}	2.25^{e}	2.33^{e}	2.08^{e}	2.17^{d}	1.83^{de}	3.83^{e}
+ Control (Gentamycin)	7.00^{b}	8.00^{b}	7.00^{c}	20.17^{b}	7.50^{c}	10.00^{b}	10.00^{b}	10.00^{b}	10.00^{b}	10.00^{b}
+ Control (Ofloxacin)	0.00^{e}	0.00^{d}	20.00^{a}	27.00^{a}	25.50^{a}	25.00^{a}	25.00^{a}	25.50^{a}	25.00^{a}	25.00^{a}
- Control	0.00^{e}	0.00^{d}	0.00^{e}	0.00^{f}	0.00^{f}	0.00^{f}	0.00^{f}	1.25^{d}	0.41^{e}	0.00^{f}

The values with the same letters show no significant difference ($p<0.05$)

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Table 7: Sensitivity of Bacteria to different concentrations of wild plant extracts and antibiotic disc (mm)

Treatment	*E. coli*	*P. aeruginosa*	*E. faecalis*	*S. aureus*	*E. aerogenes*	*B. cereus*	*R. solanacearum*	*E. carotovora*	*X. axonopodis*	*P. oryzae.*
100mg	15.75^{a}	15.91^{a}	12.00^{b}	15.92^{c}	11.58^{b}	11.42^{b}	8.67^{bc}	7.33^{e}	9.92^{b}	9.17^{bc}
80mg	13.33^{b}	13.67^{a}	10.42^{b}	13.33^{d}	9.58^{c}	9.25^{b}	7.50^{c}	6.33^{c}	8.92^{b}	8.42^{c}
66mg	10.42^{c}	12.50^{b}	7.25^{c}	12.01^{d}	7.66^{d}	6.66^{c}	5.08^{d}	5.58^{cd}	4.58^{c}	6.75^{d}
57mg	7.17^{d}	9.58^{c}	6.75^{c}	9.67^{e}	5.92^{e}	5.25^{cd}	4.33^{de}	4.16^{de}	2.66^{cd}	3.33^{e}
50mg	4.58^{e}	5.58^{d}	5.75^{c}	6.25^{f}	4.42^{f}	3.92^{d}	2.33^{ef}	7.33^{c}	1.67^{d}	1.00^{f}
+ Control (Gentamycin)	7.00^{d}	8.00^{cd}	7.00^{c}	20.25^{b}	7.50^{d}	10.00^{b}	10.00^{b}	10.00^{b}	10.00^{b}	10.00^{b}
+ Control (Ofloxacin)	0.00^{f}	0.00^{e}	20.00^{a}	27.00^{a}	25.50^{a}	25.00^{a}	25.00^{a}	25.16^{a}	25.00^{a}	25.00^{a}
- Control	0.00^{f}	0.00^{e}	0.00^{d}	0.00^{g}	0.00^{g}	0.42^{e}	1.25^{f}	1.67^{f}	0.47^{d}	0.00^{f}

The values with the same letters show no significant difference ($p<0.05$)

Escherichia coli, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Bacillus cereus*, *Ralstonia solanacearum*, *Erwinia carotovora*, *Xanthonmona axonopodis* pv *vignicola*, *Pseudomonas oryzae*

Fig 1: Regenerated *Tetrapleura tetraptera* in test tube after 3 weeks

Fig 2: *Tetrapleura tetraptera* in test tube after 5 weeks

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Fig 3: Acclimatization of the *in-vitro* plantlet

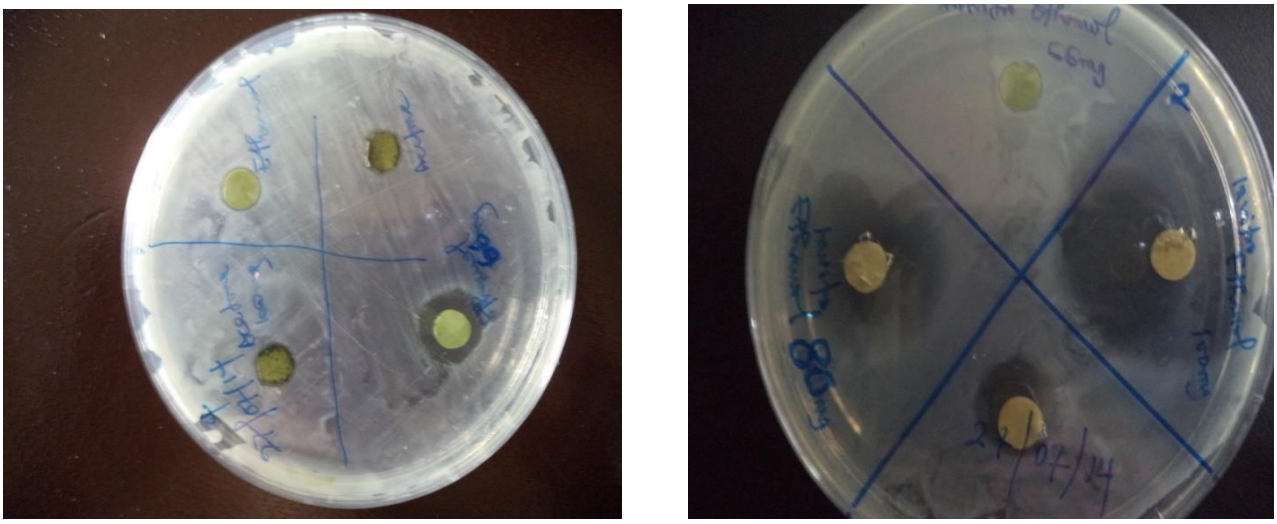

Fig 4: Zone of inhibition for *B. cereus* by crude ethanol extracts of *Tetrapleura tetraptera* leaf

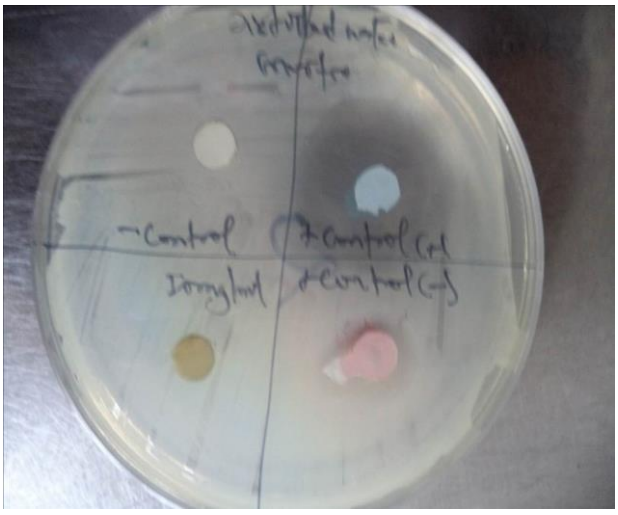

Fig 5: Effect of distilled water extract, ofloxacin and gentomycin on *Pseudomonas oryzae* (Control)

4. DISCUSSION

Plants remain one of the major sources of natural products for new therapies, because most of them are cost effective, have a wider range of antibiotic resistant on microorganisms, and have fewer adverse effects (Ozoula et al., 2010). Plant regeneration has been facilitated by the identification of appropriate explant and *in-vitro* culture conditions (Vasil, 1987, Gbadamosi *et al* 2013). The method of producing large number of identical clones by in-vitro culture is being routinely used for wide range of plant species (Aamir Ali *et al* 2008).

In this study, different concentration and in combination of auxin and cytokinins in culture Medium was successfully utilized for shoot organogenesis and root regeneration and this concurs with report by (Yutaka *et al* 1998).

As part of the general response to the public health problems associated with increasing drug resistance among bacteria, attention is more and more focused on ethno medicine and diet therapy. This work evaluated by *in-vitro* analysis the antibacterial activity of extracts of *Tetrapleura tetraptera*. Phyto-chemical analysis of the extracts revealed, among others, the presence of alkaloids, saponins, flavonoids and terpenoids in the plants. These secondary metabolites have been previously shown to have antibacterial activities (Liven *et al*., 1979; Okafor *et al*; 2001; Okafor *et al*; 2002; and Esimone *et al*; 2005).

The findings from this study revealed the presence of phytochemical compounds of *Tetrapleura tetraptera* with at least four (4) in each of the solvent extracts analysed. This finding agrees with the report of Ekwenye and Okorie, (2010). Ethanol extract was found to be more potent compared to the acetone and distilled water leaf extracts against all bacteria tested. This is with accordance with the report of Uchechi *et al,* (2010)

Only *Pseudomonas oryzea* was found to be more susceptible to acetone extract than ethanol extract. However the *in-vitro* leaf acetone extract was more susceptible compared to that of wild plant extract. Some studies have shown that the type of extract employed influences the biological activity of an extract. Mala *et al.,* (2009), observed that the acetone extract of Spirulina showed high biological activity and was better at extracting total phenols and hence recommended acetone for use in extraction of plant materials.

5. CONCLUSION

Tetrapleura tetraptera components extracted using different solvents exhibited antibacterial activity, which was dependent on the type of solvent used for the extraction, concentration of the extract applied, and the type of bacterial tested. The inhibitory activities of *tetrapleura tetraptera* against some of the selected bacteria showed that it could be used in the management of diseases caused by these microorganisms. The findings from this research justified some of the reasons for the local use of the plant in pest control and in the management of disease caused by bacterial pathogens.

It is therefore recommended that the use of *Tetrapleura tetraptera* in food preparation should not be discouraged. There is also need for further studies on the plant extract and its activity against other known pathogens. In depth biochemical studies of the implicit may be necessary to implicate the actual phytochemicals responsible for such antibacterial activity and this may by any chance increase the utilization of the plant especially in the pharmaceutical companies.

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