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IN VITRO EVALUATION OF THE ANTIBACTERIAL POTENTIAL OF EXTRACTS OF THE AERIAL PARTS OF *CASSYTHA FILIFORMIS* AGAINST UROGENITAL CLINICAL GRAM POSITIVE ORGANISMS

[1]ADONU Cyril C.,[1] ESIMONE, C.O., [2]UGWU Okechukwu P.C*., [3]BAWA Abubakar and [2]OSSAI Emmanuel C

[1]*Department of Pharmaceutics, University of Nigeria,Nsukka, Enugu State, Nigeria.*

[2]*Department of Biochemistry, University of Nigeria,Nsukka, Enugu State,Nigeria.*

[3]*Department of Preliminary and Remedial Studies, Federal Polytechnic Mubi, Adamawa State, Nigeria.*

***Corresponding Author Email:** adonailab@yahoo.com, oky9992000@yahoo.com and +2348063889880 and +2348030799187.

ABSTRACT:

The antimicrobial properties of extracts from aerial parts of *Cassytha Filiformis Linn* were evaluated. The aerial parts were shade-dried, pulverized and extracted with hot-water, methanol and n-Hexane. Hot-water extraction was by 24 Hrs Maceration while extraction with methanol and n-Hexane was done in a Soxhlet apparatus. The extracts were, then, concentrated by evaporation to dryness in an oven at temperature of 40-45^{0}C to avoid denaturation of the active ingredients. Preliminary antimicrobial screening of the extracts were carried out using agar-well diffusion techniques. Zero inhibition zone diameter (IZD) was recorded with *Cassytha Filiformis Linn* extracts against Enterococcus faecalis. Nystatin (Nilstat ^{R}) and Gentamicin (Gentalek ^{R}) were used as reference standard antibiotics. Antibiograms of all the clinical isolates were prepared using multidisc antibiotics and extracts. Minimum Inhibitory concentrations (MIC) of the extracts were determined using agar dilution technique. The results of statistical analysis at $P< 0.05$ showed a significant anti-microbial activity for the extracts of the *Cassytha Filiformis Linn.*

KEYWORDS:

Cassytha Filiformis Linn, Antimicrobial, Gram+ve, Phytochemicals, Methanol, n-Hexane and Hot-water.

INTRODUCTION

People on all continents have used hundreds to thousands of indigenous plants for treatment of ailments since prehistoric time. Drugs derived from plants constitute the bulk of therapeutic agents dispensed by the herbalist. In modern medicine, plants are also recognized as important sources of drugs. Spices and herbs are generally known to posses antibacterial and antioxidant properties (Iwu, 1989). In 2001, researchers identified 122 compounds used in mainstream medicine which were derived from “ethnomedical” plant sources, 80% of these compounds were used in the same or related ethnomedical use (Fabricant *et al*., 2001).

Historically, Ancient Egyptian Medicine of 1000 B.C. are known to have used garlic, opium, castor oil, coriander, mint, indigo, and other herbs for medicines, so also, was the use of herbs such as turmeric possible as early as 1900 B.C by Indian Ayurveda medicine (Aggarwal *et al*., 2007). Moreso, the Sushruta Samhita attributed to Sushruta in the 6th century BC describes 700 medicinal plants, 64 preparations from mineral sources and 57 preparations based on animal sources (Girish *et al*., 2007).

In Nigeria, as in other African countries, several roots, leaves, fruits, and barks of plants are used for different medical purposes; some of which many researchers have discovered to be rich in secondary metabolites like tannins, alkaloids, flavonoids, phenols, steroids, and volatile oils, which are responsible for their therapeutic activities (Cowman, 1999 and Rabe *et al* ., 2000).

Cassytha filiformis Linn inhabits coastal vegetation, usually on sandy dunes, margins of evergreen gully and venerin forests, deciduous plateau and coastal woodlands, montane grassland and dambo margins and vegetation of sandy beaches or lakes. It grows on small bushes, woody shrubs and low tree, grasses and herbaceous weeds (Scot, 2008). Seeds may be dispersed by animals, man, water, strong winds, farm machinery or with crop seed. *Cassytha filiformis Linn* can survive for up to two months without a host and growing to a length of 30cm or more. *Cassytha filiformis* species are plant parasites. They parasitize other plants by

specialized attachment and penetration with feeding structures known as haustoria.

The haustoria of *Cassytha filiformis Linn* penetrates the host epidermis and extend into more interior tissues, extracting cellular nutrients and water from plant phloem and xylem. The pathogen *Cassytha filiformis Linn* does not create immediate, fatal damage to host cells and their metabolic processes. Rather, the host plants can die a long, protracted death by starvation and desiccation while *Cassytha filiformis* gets what is required for its growth, flower and produce seeds for its future generation. Among the important economic hosts are fruit plants such as citrus, mango *Mangifera indica*, Cloves *Eugenia aromatica*, nutmeg *Myristica fragrans*, and avocado *Persea Americana* (Schroeder, 1978). It also is frequently found on many other endemic tropical and subtropical species world-wide.

In medicine, *Cassytha filiformis Linn* is used traditionally for treatment of some human birthing issues. Modern midwives recommend taking the juice made from crushed vines for 4 weeks before the expected date of birth in order to ease labour pains and to quicken labour time and lubricate the birth canal (Kobayashi, 1976). *Cassytha filiformis Linn* is purported to be used by several different Polynesian cultures for treatment of Cancers. In Palau, bark of *Termianalia cata* is mixed well with a whole plant of *Cassytha filiformis Linn* and copra, crushed together, and the juice which is squeezed out is drunk for gonorrhoea.

In modern medical research, *Cassytha filiformis Linn* has a number of biologically active chemical coupounds with potential human health application. For instance, ocoteine isolated from *Cassytha filiformis Linn*, as an alpha–adrenoceptor antagonist in rat thoracic aorta, have antiplelet aggregation activity (Chang *et al*.,1997). Cassytha filiformis is used as Vasorelaxant (*Wu et al.*,1998), and adrenoceptor antagonist (Hoet *et al.*, 2004) antitrypanosomal agent (Chang *et al.*, 1997) and diuretics in traditional medical practice (Kirtikar and Basu, 1991).

Urinary tract infection is the most common bacterial infection managed in general medical practice and accounts for 1-3% of consulations. (Arora, 1999) gave a precise definition of UTI as "the presence of bacteria undergoing multiplication in urine within the urinay drainage system. (Kass ,1956) gave a criterion of active bacteria infection of urinary tract according to which a count exceeding 10^5 organisms/ml denotes significant bacteriuria and indicate UTI. UTI is generally caused by one specie while contaminants are generally of mixed species (Arora, 1999). UTI occurs more often in females than in males. This is due to short urethra and pregnancy. Infrequent voiding and sexual intercourse which may lead to 'honey-moon' cystitis. Shorter and wider female urethra appears to be less effective in preventing access of the bacteria to the bladder. Relative infrequency of UTI in man may be due to longer male urethra and the bactericidal activity of the prostatic fluids. Other causes of urine stagnation that may predispose to UTI include enlarged prostate, urinary calculi, congenital malformation and cystoscopy which may introduce endogenous or exogenous bacteria into the bladder leading to infection. These risk factors are presented in the Box below.

Risk factors for urinary tract infection

- Incomplete bladder emptying
- Bladder outflow obstruction
- Neurological problems (eg. multiple sclerosis, neuropathic diabetic)
- Vesico-uretic reflux
- Foreign bodies
- Urethra catheter

(Adapted from Davidson Principle and practice of medicine 20th edition page 467.)

Symptoms and signs associated with infection of the urinary tact include purulent discharge from the urethra, pain during urination (dysuria), urge to urinate (urinary frequency). Pain in the lower back, fever and chills. Some of them may occur as cystitis urethritis and/or pyelonephritis. When the bacteria are present in the blood, the infection can begin in the kidneys.

Anitibiotics are recommended in all cases of proven UTI. Treatment for 3 days is the norm and is less likely to induce antibiotic resistance than more prolonged therapy (Goddard *et al* ., 2006). Trimethoprim, nitrofurantion, quinolones antibiotics and cephalexin are generally effective in the treatment of UTI. Treatment failure, with persistence of the causative organism in repeat culture suggests that an underlying cause is present. Recurrent UTI, particularly in the presence of an underlying cause may result in permanent renal damage whereas, uncomplicated UTI rarely do so. If the underlying cause cannot be removed, suppressive antibiotic therapy can be used to prevent recurrence and reduce the risk of septicaemia and renal damage. Urine is cultured at regular intervals, a regime of two or three antibiotics in sequence, rotating every 6 months, is used to reduce emergence of resistant orgainism (Goddard *et al* .,2006).

This study was designed to determine *In Vitro* effects of the antibacterial potential of the extracts of aerial parts of *Cassytha filiformis* against urogenital clinical gram positive organisms.

MATERIALS AND METHODS:

Plant material

Fresh aerial parts of *Cassytha Filiformis Linn* was collected from Nsukka in Enugu State Nigeria between June to August, 2009. The aerial parts were identified by Mr. A.O Ozioko of the Bioresource development and conservation programme (BDCP) Nsukka, Enugu State, Nigeria. The fresh aerial parts were dried under the shade for 10 days, pulverized and stored at room temperature for two weeks before extraction processes.

Chemicals/Reagents

All chemicals used in this study were of analytical grade and products of May and Baker, England; BDH, England and Merck, Darmstand, Germany.

Extraction Procedure

The fresh aerial parts of *Cassytha Filiformis Linn* plant were plucked and shade dried for 10 days, after which the plant was pulverized into coarse form with a crestor high speed milling machine and stored for two weeks. Approximately, 400g of the coarse forms of *Cassytha Filiformis Linn* was macerated in 2.0L of hot water and allowed to stand at room temperature. This was left to stand for 48 hours. After that the hot water extract was filtered through muslin cloth on a plug of glass wool in a glass column. The resulting hot water extract was concentrated and evaporated to dryness using rotary evaporator at an optimum temperature of between 40 and 45^{o}C to avoid denaturation of the active ingredients. The concentrated extract was then stored in the refrigerator. The methanol and n-hexane extracts of the plant were obtained by soxhlet extraction. 300g of pulverized *Cassytha Filiformis Linn* was extracted with 1.5L of menthanol while 400g of pulverized *Cassytha Filiformis Linn* was also extracted with 1.5L n-hexane.

Determination of yield of extracts

The percentage yield of the extracts were determined by weighing the coarse *Cassytha Filiformis Linn* before extraction and *Cassytha Filiformis Linn* extracts after concentration and then calculated using the formula.

$$\text{Percentage (\%) yield} = \frac{\text{Weight (g) of concentrated extract}}{\text{Weight (g) of ground Plant extracts}} \times 100$$

Preparation and Sterilization of Media

All the media were prepared according to the manufacturer's instructions.

MIROBIOLOGICAL EVALUATION

Isolation and Characterization of test micro-organism

Clinical isolates of *Staphylococcus aureus* and *Enterococcus faecalis* were obtained from patients visiting Adonai Diagnostic and Research Lab. and Laboratory Department of Bishop Shanahan Hospital, both at Nsukka, Enugu State, Nigeria.

Isolation and Characterization of test bacteria.

Strains of the two bacteria were Isolated from samples of Urine, High Vaginal swab, urethral swab, endocervical swab and semen of patient manifesting symptoms of genito-urinary tract infections. The samples were immediately inoculated on blood agar and macConkey agar plates. These plates were incubated a 37^0C for 24hrs according to the method described by (Cheesbrough, 2000). Colonies suspected to belong to enterobacteriaecea are re-innoculated into slants of Kliglier Iron agar (KIA) and incubated aerobically at 37^0C for more 24 hours for double sugar fermentation and hydrogen sulphide production. After the first 24 hours, the bacterial colonies from cultures indicating infection or significant bacteriuria (Kass, 1956) were isolated, gram-stained and examined microscopically. All such isolates were also subjected to the following standard biochemical tests for micrococci and enterobacteriaecae (Cowan and Steel, 1993; Cheesbrough, 2000) (Arora and Chugh, 1977). Catatase test, Coagulase test, Nitrate test, Indole, Use of KIA (for double sugar fermentation and H_2S Production).

Maintenance and Standardization of Stock Cultures.

The stock culture of each clinical isolate was stored in nutrient agar slants at 4^0C. Prior to use, the cultures were activated by successive daily sub-culturing first into Blood agar and MacConkey agar plates and then into nutrient agar slant and SDA plates before sub culturing into SDA Slant for a period of 3 days.

The standardization of innoculum was carried out according to the method described by (Arora, 1999). The tops of 5-10 similar appearing, well isolated colonies on an agar plate were touched with a sterilized straight wire and then, inoculated in a nutrient broth medium. These broth bottles were incubated at 37^0C for 4 – 6 hours to obtain the growth at logarithmic phase. The density of the organisms (bacteria) was adjusted to approximately 10^8 colony – forming units (CFU)/mL by comparing its turbidity with that of 0.5 McFarland Opacity standards.

Preparation of turbidity standard equivalent to O.5 McFarland

The standard was prepared by using the techniques described by (Cheesbrough, 2000): A 1 % v/v solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of water. A 1% w/v solution of barium chloride was prepared by dissolving 0.5 g of dihydrate barium chloride in 50ml of distilled water. Then, 0.6ml of the 1% barium chloride solution was mixed with 99.4 ml of the 1% sulphuric acid. A small volume of this turbid solution (barium sulphate) was transferred to a capped tube of the same type as the tubes used for preparing the test organisms.

Establishment of Susceptibility – Resistance Profile of Commonly used antibiotics Compared with the extracts.

The anitbiogram of the bacteria isolates were established by the disc sensitivity test using mulitidisk containing the following antibiotics Ciprofloxacin 10µg

(Cpx 10), ofloxacin 10µg, Norfloxacin (NB 10µg), Gentamicin (10µg), Rifampicin (RD 20µg), Chloramphenicol (CH30µg), Levofloxacin (LEV 20µg), Augmentin (Au 25µg), Septrin Sxt (30µg) Ampicillin (30µg).

The method used was described by (Bauer *et al* .,1996). With 10 – 20 minutes after adjusting the turbidity of the innoculum suspension to that of standard, a sterile nontoxic cotton swab was dipped into the innoculum and rotated several times with firm pressure on the inside wall of the tube to remove excess fluid. The dried surface of Nutrient agar plate 100mm in diameter containing 20 ml nutrient agar was inoculated by streaking the swab three times over the entire agar surface. The lid of the dish was then replaced and the dish was allowed to stand at room temperature for 3 – 5 minutes to allow the surface of the agar to dry before the antibiotics discs were applied using sterile forceps. After placement, the disc on the surface of medium was pressed to provide uniform contact. The plates were incubated aerobically at 37^0C for 24hours and the zones of inhibition developed were measured and recorded. The zones of inhibition (IZDs) of all the antibiotics in the discs measured and recorded were used to establish the antibiogram of the clinical isolates by comparing their IZDs with the IZD breakpoints already established by European Committee on Antimicrobial susceptibility testing (EUCAST, 2009).The IZDs of 15mg/ml extracts against the isolates were measured simultaneously and compared with those of the antibiotics. The percentage susceptibily – resistance recorded for the isolates against each antibiotic and extract were determined and tabulated

Antimicrobial Screening Test.

Preliminary Sensitivity test.

Preliminary antimicrobial screening of the extracts of *Cassytha Filiformis Linn* and standard antibiotics against the bacteria was done by the method of the cup-plate agar diffusion (Mirjana *et al* ., 1979), Boakye – Yiado , 1979 and Rios *et al* .,1988). 120mg/ml of each of the extracts in DMSO was further two fold-diluted serially with sterile distilled water. Molten nutrient agar and SDA (20ml each) were seeded with 0.1ml of standardized broth cultures of bacteria. A total of 6 wells, 8mm in diameter were made in the agar using a sterile cork borer. 0.06ml each of the two-fold dilutions was added into each labeled hole using a sterile pipette. As a procedural control, 0.06ml DMSO was put in the centre well. Similarly, one fold dilution of 120 µg/ml of Gentamicin (Gentalek) was added into respective agar-wells for comparison. The plate was left for 1hour at room temperature for diffusion) after which they were incubated at 37^0C for 24 hrs for bacteria. Diameters of the zones of inhibition (IZD) were measured at the end of the incubation period. The mean of duplicate determinations was taken.

Evaluation of Minimum inhibitory concentration (MIC) of extracts and the standard antibiotics.

The MIC of the antimicrobial agents were determined using agar dilution method (NCCLS, 1999). Six (6) different concentrations of each of the extracts in DMSO were prepared by two-fold dilution. The ranges of the concentrations of the extracts against bacteria was 1.875 – 60mg/ml. The antibiotic concentrations range from (0.78 – 25 x 10^2 unit/ml) for Nystatin and (1.88 - 60µg/ml) for Gentamicin. With an automatic micropipette, 1.0ml each of these different dilutions (one dilution per plate) of a single agent was introduced into individual agar plates. The molten agar, at 48C and the antimicrobial agents were mixed carefully and thoroughly allowed to set. With the aid of a sterile wire hoop, the standardized test micro organism were delivered on the agar surface of the plates containing different concentrations of the agent. This was done by streaking (about six-eight different strains of the isolates per plate) on the surface of the set agar. These inoculated agar plates were incubated at 37^0C for 24 hrs (for bacteria) and 48 hrs. At the end of the incubations, the MICs were determined as the lowest concentration of the extracts and the antibiotics that allowed not more than two colony forming units (CFU) to grow in it (Baron and Finegold, 1980).

RESULTS:

Percentage yield of the extracts

Table 1: Results of percentage yield of the extracts of aerial parts of *Cassytha Filiformis Linn* .

The percentage yield of *Cassytha Filiformis Linn* showed that the yield per plant extracts increased with increasing polarity of the solvent; highest yield was noted with hot water, followed by methanol and the least was n-hexane extract.

Table 1: Results of percentage yield of the extracts of aerial parts of *Cassytha Filiformis Linn* .

Plant	Part	Percentage yield of the extracts (%)	Percentage yield of the extracts (%)	Percentage yield of the extracts (%)
		Hot water	**Methanol**	**n-hexane**
Cassytha Filiformis Linn.	aerial parts	12.5	5.8	4.5

Sources, Isolation and Characterization of Test micro organisms.

A total of 29 clinical uro-genital isolates of bacteria were selected and labeled accordingly. All the isolates chosen conformed to standard identification and biochemical tests for the test micro-organism.

Table 2: Isolates of Urogenital Pathogens/Sources

Pathogens	**Sources and number of Strains Isolated**					
	Urine	**HVS**	**ECS**	**US**	**Semen**	**Total**
Staph. aureus	7	7	1	8	1	24
E. faecalis	3	1	0	1	0	5

KEY: HVS = High Vaginal Swab; ECS = Endo Cervical Swab; US = Urethral Swab ;Semen = Seminal fluid with sperm cells

The table above showed that the greatest number of micro-organism isolated from the urine was *Staph. aureus*. It is understandable because these are the micro-organisms mostly isolated in urinary tract and genital infections.

Antibiogram of Urogenital Clinical Isolates prepared with the common antibiotics and the extracts.

The antibiograms of the isolates are presented in Table 3.

The antibiogram of the Urogenital clinical isolates of the test bacteria was prepared by disc diffusion method (Bauer *et al* .,1996) using common antibiotics with established IZD breakpoints or/and antibiotics already known to be used in the treatment of infections caused by these test bacteria (EUCAST, 2009).The extracts of *Cassytha Filiformis Linn* (Methanol, Hot water and n-hexane) that were active against the test bacteria were tested at concentration of 15mg/ml each, and used for the comparison with the standard antibiotics.

In table 3, antibiogram of *Staph aureus* was prepared with the common antibiotics. Large zones of inhibition were produced by some of the antibiotics against the clinical isolates of *Staph aureus*. However, some of these large IZDs are less than the established IZD break points. All the IZDs less than IZD breakpoints already established by the EUCAST show resistance. Susceptibility was shown by the IZD greater than the IZD breakpoint. For instance, the table shows that 15 strains of staph aureus have IZD (mm) values greater than 19mm (IZD breakpoint) for ciprofoxacin against staph aureus and thus, these strains are said to be susceptible to ciprofloxacin. The IZD values of other antibiotics against staph aureus were noted and the corresponding susceptibility- resistance profile prepared as **Table 4**, so also, was the IZD of the extracts at 15mg lml concentrations which were used for comparison with these antibiotics.

Table: 3 The anti-biogram of the clinical isolate of *Staph.aureus*.

	Ciprofloxacin	Gentamycin	Rifampicin	Chloramphenicol	Co-trimoxazole	Norfloxacin	Augmentin	Ampicillin	Hot water extract	N-Hexane extract	Methanol extract	
Disc Potency	10□g	10□g	20□g	30□g	30□g	10□g	25□g	30□g	15mg	15mg	15mg	
Break Point (IZD) mm	S > 19	> 18	>25	>18	>17	>17	S>26	S>26				
	I > 19 (S),		22-25	18	14 -17		R<26	R<26				
	R < 19	< 18	<22	<18	<14	<17						
Organism												
S_{a1}	22	16	24	12	0	6	12	6	12	17	12	
S_{a2}	8	8	25	0	0	18	20	0	12	16	12	
S_{a3}	16	8	20	0	10	6	0	0	11	14	11	
S_{a4}	16	6	26	8	6	8	0	0	12	13	12	
S_{a5}	0	10	0	8	0	20	0	0	12	12	0	
S_{a6}	20	12	26	10	18	24	12	0	12	12	0	
S_{a7}	25	24	24	10	6	18	26	0	13	11	12	
S_{a8}	18	0	26	0	0	8	10	0	11	14	13	
S_{a9}	24	19	20	12	0	0	20	0	10	14	12	
S_{a10}	10	20	18	8	6	15	10	0	12	12	12	
S_{a11}	24	4	28	14	0	19	12	0	11	12	11	
S_{a12}	24	16	16	26	0	0	10	0	0	10	16	12
S_{a13}	20	14	16	16	11	0	20	0	0	11	14	14
S_{a14}	21	19	12	26	10	0	14	0	4	14	13	11
S_{a15}	24	14	20	24	6	11	20	23	0	10	12	12
S_{a16}	22	21	14	16	11	10	10	24	8	14	14	12
S_{a17}	20	20	8	24	6	0	18	10	0	11	12	13
S_{a18}	24	20	24	25	12	18	16	12	0	11	11	13
S_{a19}	23	22	20	21	10	15	0	15	0	12	11	11
S_{a20}	10	20	20	2	15	13	16	20	0	10	16	10

	Ciprofloxacin	Gentamycin	Rifampicin	Chloramphenicol	Co-trimoxazole	Norfloxacin	Augmentin	Ampicillin	Hot water extract	N-Hexane extract	Methanol extract	
Disc Potency	10mg	10mg	20mg	30mg	30mg	10mg	25mg	30mg	15mg	15mg	15mg	
Break Point (IZD) mm	S > 19	> 18	>25	>18	>17	>17	S>26	S>26				
	I > 19 (S),		22-25	18	14 -17		R<26	R<26				
S_{a21}	20	19	2	25	10	0	0	26	4	10	14	0
S_{a22}	12	19	21	24	0	10	0	6	0	12	14	0
S_{a23}	16	20	20	14	10	18	16	0	0	13	12	0
S_{a24}	21	0	0	25	0	10	16	16)	0	11	14	11

Table 4: Percentage susceptibility-resistance profile (%) of *Staph. aureus* compared with the extracts.

Clinical Isolates		Ciprofloxacin	Gentamicin	Levofloxacin	Norfloxacin	Ofloxacin	Chloramphenicol	Ampicillin	Rifampicin	Augmentin	Co-trimoxazole	Hot Water extract	N-Hexane extract	Methanol extract
Staph.	S	63	50	NA	38	38	0	0	46	13	13	100	100	87
aureus	I	NA	NA	NA	NA	N.A	0	17	21	N.A	12			
	R	37	50	NA	62	62	100	100	33	87	75	0	0	13

KEY:
NA = IZD break point Not yet Available as at the time of this work by EUCAST, 2009. N.R=Susceptibility testing is not recommended as the species is a poor target for therapy with the drug; S = Sensitive; I = Intermediate susceptible; R = Resistant

This corresponding percentage of the isolates that are susceptible, intermediately susceptible (Is) and resistant (R) to the various antibiotics were determined by comparing the inhibition zone diameter (IZD) (mm) produced by the antibiotics against *Staph. aureus* with the antimicrobial IZD breakpoint-values as given by European Committee on Antimicrobial Susceptibility Testing (EUCAST 2009).

In table 4, the results reveal that all the isolates of *Staph. aureus* were resistant (R = 100%, Is = 0%, S = 0%) to chloramphenicol and Ampicillin, and susceptible and intermediately susceptible in varying degrees to Ciprofloxacin Gentamicin, Levofloxacin, Norfloxacin, Rifampicin, Augmentin, Erythromycin and Co-trimoxazole. Apart from Ciprofloxacin (S= 63%), Gentamcin (S=50%) and Rifampicin(S=46%), the percentage of strain of the *Staph aureus* isolates resistant to other antibiotics are so large, which has made the treatment of *Staph aureus* infections with most of these antibiotics a failure. Comparatively the 15mg/ml each of the plant extracts: methanol extract of *Cassytha filiformis* and the hot water extract of *Cassytha filiformis* showed promising IZD (mm). Generally speaking, the drugs to which all these species are resistant to, are commonly used/abused antibiotics and thus the observed effects are understandable. In addition some of these antibiotics , veterinary pathology and such agricultural use of antibiotics also contribute to the pool of antibiotic – resistant bacteria in a community (Harvey 1990).

Preliminary sensitivity Results.

The results of sensitivity tests of the extracts (methanol, hot water and n-hexane) of *Cassytha filiformis* and standard reference drugs against various microorganisms are presented in **Table 5**. Both the methanol and hot-water extracts of the *Cassytha filiformis Linn* were effective against *Staphylococcus aureus*.

Table 5.The inhibition zone diameter (IZD) of different extracts of *Cassytha filiformis linn* and Gentalek® against twenty four strains of *Staphylococcus aureus*

Concentrations (Mg/ml)	**Means ± standard error**		
	Hot Water extract	Methanol Extract	Gentalek
60.	16.75 ± 0.45^{a}	15.83 ± 0.53^{a}	13.42 ± 0.64^{b}
30.	15.54 ± 0.53^{b}	13.50 ± 0.29^{a}	11.04 ± 0.23^{c}
7.5.	10.25 ± 0.70^{b}	4.67 ± 1.06^{a}	0.00 ± 0.00^{c}
3.75.	2.08 ± 0.85^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
1.88.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

*** Different superscripts in a row indicate significant differences between the groups ($p<0.05$).**

In table 5 the results of the activities of the extracts and Gentalek against *Staph. aureus* showed large inhibition zone diameters. This reveals high efficacy of the extracts against *Staphylococcus aureus*. There is a significant difference between methanol and hot water extracts when compared to that of Gentalek. This implies that hot water extract with higher IZD than methanol extract has higher activities against *Staph. aureus*. The hot water extract of *Cassytha filiformis linn* produced the largest values of IZDs when compared with the methanol extract of the plant.

Table 6: Table of Characterization of the Test Bacteria

Bacteria	Gram character	Catalase	Nitrate	Indole P.	H_2S. p.	Gas .P.	Lac	glc	Pyocyanin production	Swarming
Staphylococcus aureus	+	+	-	-	-	-	+	+	-	-
Enterococcus faecalis	+	-	-	-	-	-	+	+	-	-

Key: + =Present, -=absent; Nitrate =Convert nitrate to nitrite (Nitrate reducer); Indole P =Indole production; Gas P =Gas production; H_2S.P=Hydrogen Sulphide Production; Lac=Lactose Fermenter; Glc =Glucose Fermenter

CONCLUSION

The Secondary metabolites detected by the preliminary phytochemical tests could be responsible for the observed antimicrobial effects of the extracts of *Cassytha filiformis linn* . The extracts were efficacious against *Staphylococcus aureus.* It is clear from the results of this study that more pronounced antimicrobial activities could have been observed if pure compounds from these extracts were used, considering the fact that crude extracts were used. Therefore, it is of great interest to carry out a further screening of these plant extracts in order to reveal all their active ingredients by isolation and characterization of their antimicrobial constituents and carry out further pharmacological evaluations.

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***Corresponding author address:**
UGWU Okechukwu P.C
****Corresponding Authors E-mail and contact:***
adonailab@yahoo.com, oky9992000@yahoo.com
+2348063889880 and +2348030799187.