

ANTIMICROBIAL POTENTIAL OF EXTRACTS OF *Hyptis suaveolens* (L) POIT. LEAVES ON SOME GRAM POSITIVE BACTERIAL ISOLATES

ABSTRACT

This study investigated the antibacterial potential of the methanolic extract and other different fractions obtained from the crude extract of *Hyptis suaveolens* leaves on some selected Gram positive bacterial isolates. It also assessed the antibiotic susceptibility patterns of the test isolates.

Powdered sample of *Hyptis suaveolens* was extracted with methanol and water in ratio 3:2 and partitioned with n-hexane, chloroform, ethyl acetate and butanol in ascending order of their polarity. The filtrates were concentrated with rotary evaporator and kept for further use. All extracts were screened for antibacterial activity using agar well diffusion method. The minimum inhibitory and bactericidal concentrations of the extracts were also determined. Antibiotics sensitivity test was performed using Kirby Bauer method of antibiotics susceptibility test.

The results showed that the extract had activity at a concentration of 35 mg/ml on 8 of the test bacteria with zones of inhibition ranging between 14.50 ± 0.71 mm and 18.00 ± 1.41 mm for test bacteria while the zones of inhibition for the standard antibiotics ranged between 16.00 ± 0.00 mm and 23.00 ± 1.41 mm. The zones of inhibition of the active fraction (ethyl acetate) ranged between 12.00 ± 0.00 mm and 17.00 ± 2.83 mm. The MICs of the extract ranged between 1.09 mg/ml and 35.00 mg/ml while that of the fraction ranged between 0.16 mg/ml and 5.00 mg/ml. The MBCs of the extract ranged between 2.19 mg/ml and 35 mg/ml while that of the active fraction ranged between 0.31 mg/ml and 5.00 mg/ml. All the test bacteria showed 100% resistance to amoxicillin and had multiple

antibiotics resistance to other commercially available antibiotics. The study concluded that the plant possessed antimicrobial properties at higher concentrations and could be used in the treatment of various infections. Although, ethyl acetate showed highest antibacterial activity, its concentration was much less than the crude extract. Hence, the study also concluded that ethyl acetate; among other solvents was the most suitable for the extraction of antimicrobial compounds from the leaves of *Hyptis suaveolens*.

Introduction

The increasing interest in the investigation of natural products as sources of new antibacterial agents has been overwhelming over the past two decades, especially now that the increasing incidence of resistance to antimicrobials is of growing concern to medical scientists (Tenover, 2006a; Simões *et al.*, 2007). To avert this nuisance of antibacterial resistance by microorganisms, attention has been focused on discovering and development of new bioactive compounds with antimicrobial property (Brehm-Stecher and Johnson, 2003; Røssland *et al.*, 2005).

Most plants have rich secondary metabolites including alkaloids, flavanoids, tannins, anthraquinones, and phenolic compounds which are all pharmacologically active and contain antimicrobial agents (Lewis *et al.*, 2006; Edeoga *et al.*, 2005; Nawrot *et al.*, 2007). These pharmacological properties present in some of these secondary metabolites have been utilized to combat disease-causing pathogens (Kamali and Amir, 2010; Lalitha *et al.*, 2010; Hussain *et al.*, 2011).

The associated risks and side effects of antimicrobials of plant origin with enormous therapeutic potential in the cure of many infectious diseases are minimal compared to

those of synthetic drugs (Iwu *et al.*, 1999a). Another major benefit of herbal drugs is that they are relatively cheaper than the synthetic alternatives (Aiyegoro and Okoh, 2009).

Previously, infectious diseases were the most significant contributor to the high rate of human mortality until relatively recent times. Currently, infectious diseases still account for a large portion of death and disability worldwide and in certain region remains the most important cause of sicknesses (Saker *et al.*, 2004)

Infections with exogenous microbes may develop from microbial colonizing infections that lead to long-term carriage without disease (Kane *et al.*, 2011; Kuss *et al.*, 2011).

Infectious diseases which are usually acute and unambiguous in nature are caused by microorganisms of different types. The onset of an infectious illness, unlike that of many other types of diseases, in an otherwise healthy host can be sudden and unmistakable. Because of their nature, infectious diseases are potentially preventable with personal protection, general public health measures, or immunologic approaches such as vaccination. Many of these infections can be eliminated, especially those with broad global health impact and to which there is no non-human host or major reservoir (Morens and Fauci, 2007).

Several antibiotics which are effective against bacteria, fungi, protozoa are toxic to humans and animals, even when given in therapeutic dosage whose ranges of effectiveness varies considerably. Some of these drugs may not be effective against viruses such as the common cold or influenza, and may be harmful when taken inappropriately. Many are narrow-spectrum while others are broad-spectrum. Narrow-spectrum drugs are effective only against a limited variety of pathogens

One of the most serious problems threatening the health of the public is resistance of microorganisms to available drugs especially the chemically synthesized drugs. Some microorganisms have rendered many of these drugs impotent. The rapid rate of antimicrobial resistance among both Gram positive and Gram negative bacteria is raising concerns in the tropical parts of the world. These resistances by bacteria have become worrisome worldwide especially in developing nations like Nigeria, threatening the ability to treat infections and diseases ranging from mild to severe microbial infections (Giwa and Ogunjobi, 2017).

Gram-positive bacteria like *Staphylococcus aureus* and *Enterococcus spp* usually, are important and frequent pathogens causing bloodstream and other infections in hospitalized patients in the United States and infections caused by antibiotic-resistant gram-positive bacteria are increasing (Rice, 2006). Of the numerous plants on the surface of the earth, there exist several plants possessing antimicrobial components that could compete favorably with the commercially available antimicrobials, allow minimal side effects and resistance by Gram positive bacteria. In this work, we examined the *in-vitro* antimicrobial potential of the methanolic and ethyl acetate extract of *hyptis suaveolens* leaves on some Gram positive bacterial isolates.

MATERIALS AND METHODS

Plant Collection

Fresh leaf samples of *Hyptis suaveolens* were collected from Adesanmi Area, along Ibadan road in Ile-Ife, Osun State, Nigeria. The picture of the plant is as shown in Plate 1.

Preparation of Crude Extract

The leaf samples of *Hyptis suaveolens* were air dried at room temperature and ground into powder. The powdered sample (1.8 kg) was extracted with 60 % (v/v) aqueous methanol (5 litres) for 96 h (with regular shaking) and filtered. The filtrate was concentrated *in-vacuo* on the rotary evaporator at 40°C to obtain the crude extract which weighed 108g.

Test Microorganisms

Nine Gram positive test microorganisms used were maintained at 4°C on Nutrient Agar slants and were sub-cultured into sterile nutrient broth and incubated at 37°C for 18h for each use. They include both locally isolated organisms (LIO) and typed cultures of National Collection of Industrial Bacteriology (NCIB). These organisms include: *Bacillus cereus* (NCIB 6349), *Bacillus polymyxa* (LIO), *Bacillus subtilis* (NCIB 3610), *Bacillus stearothermophilus* (NCIB 8222), *Micrococcus luteus* (NCIB 196), *Enterococcus faecalis* (LOI), *Staphylococcus aureus* (NCIB 8588) and two different strains of *Streptococcus pneumoniae* 010 (LOI) and *Streptococcus pneumoniae* 009 (LIO)



Plate 1: *Hyptis suaveolens*

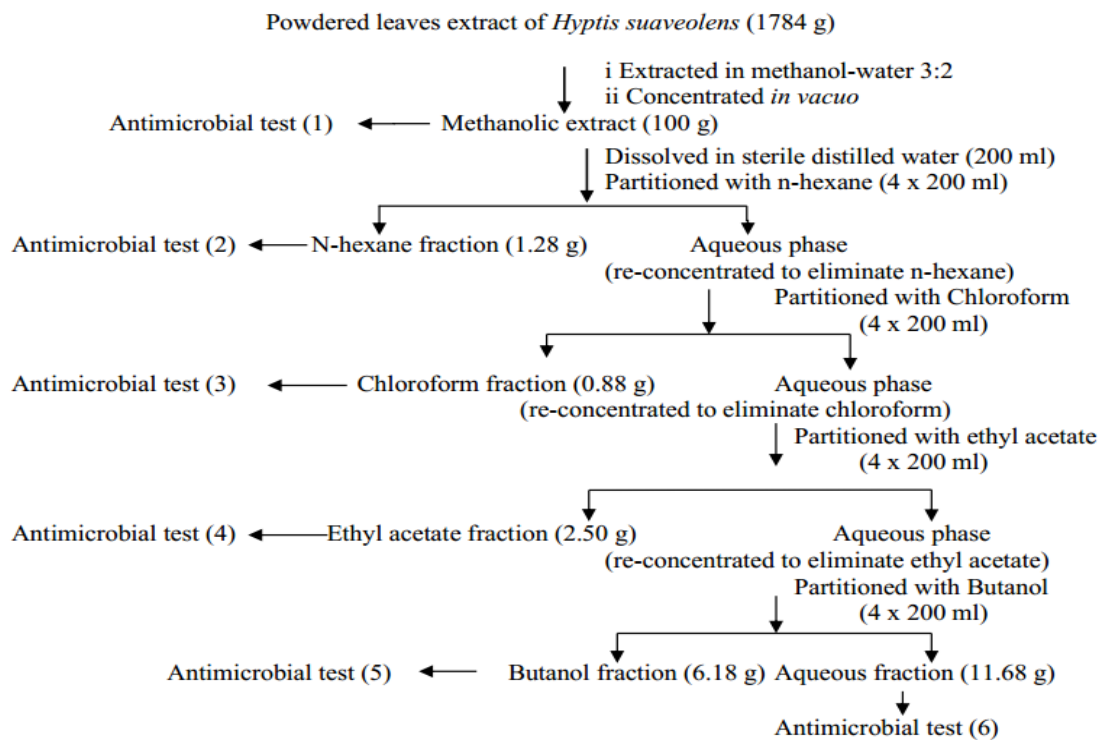


Figure 1: Extraction and Partitioning Scheme of *Hyptis suaveolens* Plant Extract

Solvent Partitioning of Crude Extract

The partitioning scheme for the crude extract is as shown in Figure 1. Exactly 100 g of the crude extract was suspended in 300 ml of sterile distilled water and was successfully partitioned with n-hexane (200 ml x 4), chloroform (200 ml x 4), ethyl acetate (200 ml x 4) and butanol (200 ml x 4). Each fraction was concentrated to dryness on the rotary evaporator to obtain four different fractions with the aqueous phase. These were kept in the desiccator until when they were used for analyses.

Sensitivity of *Hyptis suaveolens* Extract on Test Bacteria

The Agar well diffusion method of Mbata *et al.*, 2006 was used. Eighteen hour old microorganisms grown on nutrient broth were standardized to 10^8 cfu/ml while utilizing 0.5 the McFarland standards (McFarland, 1907). A volume of 0.1 ml was sub-cultured onto sterile molten Mueller-Hinton agar (Mueller and Hinton, 1941) and poured into plates. After solidifying, wells were bored with a 6mm cork borer. Each well was filled with 35 mg/ml of the crude extract while streptomycin (1mg/ml) was added to adjacent well in each case as positive control. The cultured plates were allowed for 1-2h for proper diffusion of the antimicrobial agents before incubating at 37 °C for 24 hours. Zones of inhibition produced by the activity of the extract and the control were measured and recorded.

Minimum Inhibitory Concentrations (MICs) of the Extract and Antibiotics on Test Bacteria

The method of Silva *et al.* (2005) was utilized. Here, two-fold dilution of the extract was prepared to obtain different concentrations. Exactly 2 ml from each concentration was

added to 18 ml of pre-sterilized molten nutrient agar to obtain a final concentration range of 0.547 to 35.0 mg/ml for the extract and 0.016 to 1.0 mg/ml for streptomycin (positive control). The mixture of nutrient agar and different concentrations of the extract and antibiotics obtained were then poured into sterile petri dishes and allowed to dry before streaking with 18 hour old standardized broth cultures. Incubation was done for 24 h after which the plates were observed for any growth. The MIC of the extract and antibiotics for each isolate was taken as the lowest concentration of the extract that inhibited the growth of each bacterial isolate.

3.2.9 Determination of Minimum Bactericidal Concentrations (MBCs) of the Extract and Antibiotics on Test Bacteria

A modification of Spence and Spencer (2004) method was used. It was determined based on the MIC plates. Inoculum was carefully taken from the line of streaks with no visible growth on MIC plates and sub cultured onto freshly prepared nutrient agar plates. The plates were then incubated at 37 °C for 48 h. The MBC of both the extract and antibiotics was taken as the lowest concentration that prevented any form of growth of each test bacteria after the incubation period.

Sensitivity Testing of N-Hexane, Chloroform, Ethyl Acetate, Butanol and Aqueous Fractions on Test Bacteria

The fractions obtained from the crude extract were tested for antimicrobial activity using a lower concentration of 10 mg/ml on the test microorganisms as earlier described.

Minimum Inhibitory Concentrations (MICs) of the Ethyl Acetate Fraction on Test Bacteria

151 The MIC for the active fractions were determined as earlier described using a
 152 concentration range of 0.156 to 10 mg/ml.

153 **Minimum Bactericidal Concentrations (MBCs) of the Ethyl Acetate Fraction on Test** 154 **Bacteria**

155 The MBC were also determined as earlier described.

156 **Antibiotics Sensitivity Test**

157 All the isolates were subjected to antibiotics susceptibility test using modified Kirby-Bauer
 158 agar disk diffusion method (Bauer *et al.* 1966) on Mueller-Hinton agar. The isolates where
 159 tested against ten commonly used antibiotics namely; Amoxicillin (25µg), Ofloxacin (5µg),
 160 Streptomycin (10µg), Chloramphenicol (30µg), Ceftriazone (30µg), Gentamycin (10µg),
 161 Pefloxacin (5µg), Cotrimoxazole (25µg), Ciprofloxacin (10µg) and Erythromycin (5µg), all
 162 of Abtek Biologicals, England. Standard bacterial suspensions (0.5 McFarland) of the test
 163 organisms were made. The diameter of the zone of inhibition of 24 hour growth of each
 164 isolate was carefully measured and compared to the Clinical and Laboratory Standards
 165 Institute (CLSI), 2013 standard. The isolates were reported as susceptible, intermediate
 166 or resistant according to interpretative chart of complete growth inhibition zone diameter
 167 sizes for bacteria following the CLSI, (2013). Data obtained were used to construct the
 168 phenotypic pattern of antibiotic resistance for respective organism.

169 **RESULTS**

170 **Antimicrobial Activities of Extract**

171 The sensitivity patterns of the test bacteria to the crude extract are as shown in Table 2.

172 Eight bacteria were susceptible to the crude extract when the extract was increased to 35

mg/ml while only one showed resistance to the extract. The zones of inhibition for susceptible test microorganisms ranged between 14.00 mm and 18.00 mm. *Bacillus cereus* and *Streptococcus pneumoniae* showed the highest zones of inhibition to the extract while *Enterococcus faecalis* and *Staphylococcus aureus* had the least. All the test bacteria were susceptible to streptomycin. *Bacillus subtilis* had the highest zone of inhibition (22.00 mm) while *Staphylococcus aureus* had the least (16.00 mm).

Antimicrobial Activities of N-Hexane, Chloroform, Ethyl Acetate, Butanol and Aqueous Fractions on Test Bacteria

Table 3 shows the antimicrobial sensitivity of the selected test bacteria to the crude fractions at a reduced concentration of 10 mg/ml. Ethyl acetate fraction showed the highest antimicrobial activity of all the fractions used with only one organism (11.1%) out of the total test bacteria showing no inhibition. The zones of inhibition exhibited by the ethyl acetate fraction ranged between 12.00 mm and 17.00 mm while the zones of inhibition by the other fractions ranged between 12.00 mm and 16.00 mm.

4.6 The Minimum Inhibitory and Bactericidal Concentrations of the Extract, Streptomycin and the Ethyl Acetate Fraction against Susceptible Test Bacteria

The minimum inhibitory and bactericidal concentrations of the extract, standard antibiotics and the ethyl acetate fraction against susceptible test bacteria are as shown in Table 4. The extract had a MIC ranging between 1.09 and 35.00 mg/ml, standard antibiotics ranged between 0.03 and 0.25 mg/ml while the ethyl acetate fraction ranged between 0.16

and 5.0 mg/ml. The MBC of the extract against the susceptible test microorganisms ranged between 2.19 and 35.00 mg/ml, standard antibiotics ranged between 0.03 and 0.5 mg/ml while the ethyl acetate fraction ranged between 0.31 and 5.0 mg/ml.

4.7 Antibiotics Susceptibility Pattern

Table 5 indicates the susceptibility patterns of 9 test bacterial strains against 10 commercially available antibiotics while figure 1 shows the frequency of antibiotics resistance and susceptibility pattern among the test bacteria. All the test bacteria showed susceptibility to some of the antibiotics with various zones of inhibition when compared to the CLSI standard of Interpretative chart. Resistance to antibiotics was high among the test bacteria. All the bacteria used in this study were all resistant to amoxicillin and cotrimoxazole (100%). The resistance of test microorganisms to ceftriaxone, erythromycin and streptomycin was 77.8% while resistance to gentamycin, chloramphenicol, ciprofloxacin, pefloxacin and ofloxacin was 66.7%, 55.6%, 44.4%, 33.3% and 22.2% respectively. Susceptibility to ofloxacin was highest with 77.8%, followed by pefloxacin (55.6%), gentamycin and chloramphenicol (33.3%), ceftriaxone and ciprofloxacin (22.2%) while streptomycin (11.1%) had the least susceptibility. Intermediate resistance to pefloxacin, chloramphenicol and streptomycin was least with 11.1% while ciprofloxacin and erythromycin had 33.3% and 22.2% intermediate resistance respectively.

Multiple Antibiotic Resistance Patterns

The multiple antibiotic resistance patterns of the test bacteria are as shown in Table 4. Multiple antibiotic resistances were observed in all the test bacteria. Resistance ranged

from 3(30%) of *Bacillus cereus*, 4 (40%) of *B. stearothermophilus*, 5 (50%) of *S. pneumoniae* to 7 (70% each) of *E. faecalis*, *S. aureus* to all the antibiotics.

Table 1: Sensitivity Patterns of the Extract against the Test Bacteria

Test Microorganisms	Zones of Inhibition (mm)**		
	Crude Extract (35 mg/ml)	Streptomycin (1 mg/ml)	DMSO (5 %)
<i>Bacillus cereus</i>	18.00±1.41	20.00±0.00	0
<i>Bacillus polymyxa</i>	16.00±0.00	21.00±1.41	0
<i>Staphylococcus aureus</i>	0.00±0.00	16.00±0.00	0
<i>Bacillus stearothermophilus</i>	15.50±0.71	17.00±0.00	0
<i>Streptococcus pneumoniae</i> (009)	17.00±0.00	21.00±0.00	0
<i>Micrococcus luteus</i>	15.50±0.71	19.50±3.54	0
<i>Bacillus subtilis</i>	16.00±0.00	22.00±0.00	0
<i>Streptococcus pneumoniae</i> (010)	18.00±1.41	17.00±0.00	0
<i>Enterococcus faecalis</i>	14.50±0.71	19.00±0.00	0

Legend: mm** represents mean of two replicates, 0 = not sensitive

Table 2: Sensitivity Patterns of the N-Hexane, Chloroform, Ethyl Acetate, Butanol and Aqueous Fractions (10 mg/ml each) on Test Bacteria

Zones of Inhibition (mm)

Microorganism	Aqueous Fraction	Chloroform Fraction	Ethyl acetate Fraction	N-hexane Fraction	Butanol Fraction
<i>Bacillus cereus</i>	0	0	14.50±0.71	0	0
<i>Bacillus polymyxa</i>	13.00±0.0	0	14.00±0.00	0	14.50±0.7
	0				1
<i>Staphylococcus aureus</i>	0	0	0	0	0
<i>Bacillus stearothermophilus</i>	12.00±0.4	0	14.00±0.00	0	12.50±0.7
	1				1
<i>Streptococcus pneumoniae</i>	0	0	13.50±2.12	16.00±0.00	0
(009)					
<i>Micrococcus luteus</i>	0	0	12.00±0.00	11.50±0.71	0
<i>Bacillus subtilis</i>	0	12.50±0.71	12.50±0.71	0	0
<i>Streptococcus Pneumoniae</i>	0	0	17.00±2.83	15.00±00	0
(010)					
<i>Enterococcus faecalis</i>	0	0	15.50±0.71	0	11.50±0.7
					1

225 KEY: mm** represents mean of two replicates, 0 = not sensitive

226 **Table 3: Minimum Inhibitory Concentrations and Minimum Bactericidal**
 227 **Concentrations of the Extract, Standard Antibiotics and the Ethyl Acetate (mg/ml)**
 228 **Fraction against Susceptible Test Bacteria**

Microorganisms	Extract		Streptomycin		Ethyl Acetate	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>Bacillus cereus</i>	1.09	2.19	0.03	0.03	0.16	

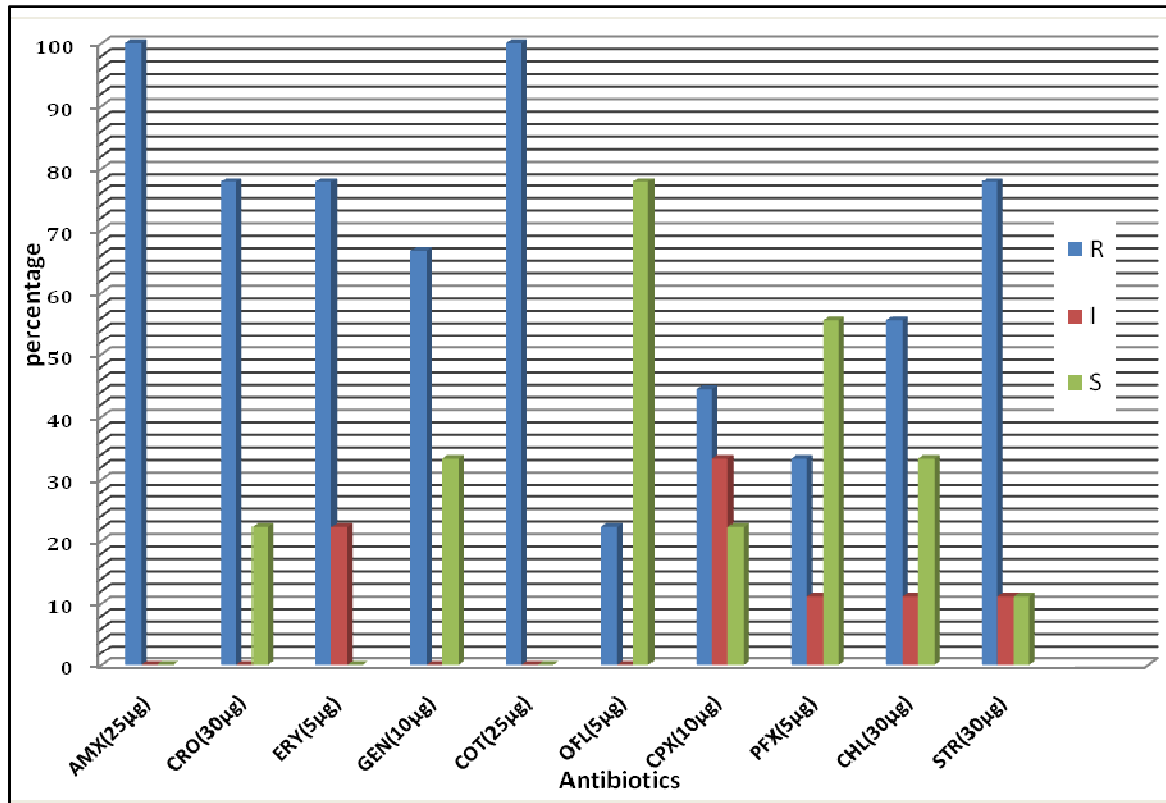
0.31

<i>Bacillus polymyxa</i>	35.00		0.03	0.03	5.00
	35.00				5.00
<i>Staphylococcus aureus</i>	0	0	0	0	0
<i>B.</i>	35.00	ND	0.25	0.25	2.50
<i>stearothermophilus</i>					5.00
<i>S. pneumoniae</i> (009)	1.09	2.19	0.06	0.13	0.31
					0.31
<i>Micrococcus luteus</i>	8.75	8.75	0.25	0.25	2.50
					2.50
<i>Bacillus subtilis</i>	8.75	8.75	0.25	0.50	0.63
					0.63
<i>S. pneumoniae</i> (010)	1.09	4.38	0.13	0.13	0.63
					0.63
<i>Enterococcus faecalis</i>	2.19	0	0.25	0.25	2.50
					2.50

229 **Legend:** 0 = no observable inhibition, MIC= Minimum Inhibitory Concentration, MBC=

230 Minimum Bactericidal Concentration

231



232

233 **Figure 1: Test antibiotics and bacterial sensitivities**

234 **Legend:** AMX = Amoxicillin; CRO = Ceftriazone; ERY = Erythromycin; GEN =
 235 Gentamycin COT = Cotrimoxazole; OFL = Ofloxacin; CPX = Ciprofloxacin; PFX =
 236 Pefloxacin; CHL = Chloramphenicol; STR = Streptomycin.

237 **Table 4: Phenotypic patterns of multiple antibiotic resistances among Test**
 238 **isolates**

Multiple Antibiotics Resistance Patterns	Name of Isolate	Number of Isolate
AMX CRO COT	<i>Bacillus cereus</i>	1
AMX CRO COT ERY	<i>B.</i> <i>stearothermophilus</i>	1
AMX CRO COT GEN	<i>S. pneumoniae</i> (010)	1

AMX CRO COT ERY STR	<i>S. pneumoniae</i>	1
	(009)	
AMX GEN CHL COT ERY	<i>M. luteus</i> , <i>B. polymyxa</i>	2
AMX GEN CHL CRO CPX ERY COT	<i>B. subtilis</i>	1
AMX CPX CRO GEN ERY CHL COT OFL STR	<i>E. faecalis</i> , <i>S. aureus</i>	2
PFX		

Total	10	9
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Legend: AMX = Amoxicillin; CRO = Ceftriazone; ERY = Erythromycin; GEN = Gentamycin COT = Cotrimoxazole; OFL = Ofloxacin; CPX = Ciprofloxacin; PFX = Pefloxacin; CHL = Chloramphenicol; STR = Streptomycin.

Table 5: Antibiotics Sensitivity Pattern Showing the Zones of Inhibition of the Test Organisms

Microorganisms	Antibiotics									
Gram positive	AMX	OFL	STR	CHL	CRO	GEN	PFX	COT	CPX	ERY
<i>Bacillus cereus</i>	0 ^R	20 ^S	24 ^S	20 ^S	0 ^R	25 ^S	22 ^S	0 ^R	23 ^S	22 ^I
<i>Micrococcus luteus</i>	0 ^R	22 ^S	0 ^R	0 ^R	26 ^S	0 ^R	23 ^S	0 ^R	18 ^I	13 ^R
<i>Enterococcus faecalis</i>	0 ^R	0 ^R	0 ^R	0 ^R	0 ^R	0 ^R	0 ^R	0 ^R	0 ^R	0 ^R
<i>Staphylococcus aureus</i>	0 ^R	0 ^R	0 ^R	0 ^R	0 ^R	0 ^R	0 ^R	0 ^R	0 ^R	0 ^R
<i>Bacillus subtilis</i>	0 ^R	20 ^S	0 ^S	0 ^R	0 ^R	0 ^R	0 ^S	0 ^R	0 ^R	0 ^R
<i>Bacillus polymyxa</i>	0 ^R	20 ^S	0 ^R	0 ^R	21 ^S	0 ^R	21 ^S	0 ^R	15 ^R	0 ^R
<i>B.</i>	0 ^R	21 ^S	13 ^I	20 ^S	0 ^R	21 ^S	22 ^S	0 ^R	18 ^I	0 ^R

stearothermophilus

S. pneumoniae 0^R 28^S 0^R 19^S 0^R 22^S 26^S 0^R 25^S 0^R
(009)

S. pneumonia (010) 0^R 18^S 0^R 17^I 0^R 10^R 13^I 0^R 17^I 14^I

Legend: AMX = Amoxicillin; CRO = Ceftriazone; ERY = Erythromycin; GEN = Gentamycin COT = Cotrimoxazole; OFL = Ofloxacin; CPX = Ciprofloxacin; PFX = Pefloxacin; CHL = Chloramphenicol; STR = Streptomycin.

DISCUSSION

In this study, the antimicrobial activities of methanolic extract of *Hyptis suaveolens* leaf on some gram-positive pathogenic bacteria was investigated and it was observed that the plant leaves possess certain phytochemical constituents namely; flavonoids, saponins, steroids, triterpenes, phenolics, cardiac glycosides and alkaloids. Most of these phytochemicals have been reported in previous screening of *Hyptis suaveolens* (Ijeh *et al.*, 2007; Prasanna and Koppula, 2012). The presence of these phytochemicals has been the basis for its usage in ethno-medicine (Edeoga *et al.*, 2005, Omoyeni *et al.*, 2012). These components were found to be responsible for their biological activity including antimicrobial action on pathogenic microorganisms as was suggested by Dinda *et al.* (2006); Eldeen *et al.* (2006).

265 Researchers have previously used different solvents for the extraction of antimicrobial
266 compounds from different plant materials. Nair and Sumitra (2007); Renisheya *et al.*
267 (2011), stated the usage of ethanol as a solvent for the preparation of plant extract for
268 antibacterial studies. Johnson *et al.* (2011) determined the antibacterial efficacy of
269 chloroform, ethanol, ethyl acetate and water extracts of *Mentha arvensis* against
270 *Salmonella typhi*, *Streptococcus pyogenes*, *Proteus vulgaris* and *Bacillus subtilis*.
271 Irudayaraj *et al.* (2010) used five different extracts (Petroleum Ether, Benzene,
272 Chloroform, Ethanol and Distilled Water) of the spike-moss *Selaginella inaequalifolia* to
273 examine the antibacterial activities against the selected pathogens. Anpin Raja *et al.*
274 (2011) while trying to study the antibacterial properties of *Cecropia peltata* used
275 methanol as solvent for extraction and recorded strong antibacterial properties.

276 In this work, a variety of solvents were also employed in extraction and bioactivity of
277 *Hyptis suaveolens*. Zones of inhibition (Table 1) of the extract against different bacteria
278 were varied. This could be due to the active components present in the crude extract in
279 an unknown amount. It was not within the objectives of this study to include the isolation
280 and purification of the various active antimicrobial compounds from the plant leaves
281 therefore we could not determine the very active component of the plant leaves.

282 The disparity among the activities of the extracts (table 2) and between the standard
283 antimicrobial drug (Table 1) could be due to the mixtures of bioactive compounds
284 present in the extract thereby accounting for different mechanisms of action (presenting
285 an almost impossible means to escape the bactericidal or bacteriostatic effect for the
286 microorganisms), compared to the pure compounds contained in the standard antibiotic

(Olajuyigbe and Afolayan, 2012) which may have simple and directional mechanism of action.

The ethyl acetate fraction was the most active among all the fractions. When the concentration of ethyl acetate was further reduced to 10 mg/ml, it demonstrated inhibition against 8 out of the test bacteria. Thus, the ethyl acetate solvent could have possibly pooled most of the antibacterial components from the plant and therefore resulting in the high activity of the fraction. This result confirmed that of Malar *et al.* (2012) where they reported that ethyl acetate extracts of *Hyptis suaveolens* showed the widest spectrum activities with the maximum zone of inhibition 23 mm for a microorganism.

Ethyl acetate fraction, crude extract and standard antibiotics were selected for MIC and MBC (Table 3). *Bacillus cereus* and *Streptococcus pneumoniae* (009) were seen to have the least MIC and MBC across the three antimicrobials. Fabry *et al.* (1998) defined active crude extracts as those with MIC values < 8mg/ml and according to Simões *et al.* (2009), lower MIC and MBC values indicate higher efficacy and phytochemicals are routinely classified as antimicrobials when susceptibility tests have MICs in the range of 0.1–1mg/ml. Therefore, MICs and MBCs of less than 1 mg/ml in this study were considered to be of good activity.

Multiple antibacterial resistance by an organism is the ability of that organism to show resistance to two or more antimicrobial agents. The increasing prevalence of resistance by many pathogens has been reported over the years in different regions of the world including developing countries (Byarugaba, 2005). This increase results in the development and spread of multiple antimicrobial resistances among microorganisms in

the environment, treatment of illness has therefore becomes difficult to achieve with a single antimicrobial agent (Thompson, 1999; Martinez and Baquero, 2002; Wassmer *et al.*, 2006). In this study, there were observed multiple antibiotic resistances (Figure 1) recorded against all the standard antibiotics which are commonly used for treatment of various infections. This is worrisome and calls for the attention of concerned agencies to reduce the menace of rapid multi-drug resistance by bacteria.

Resistance of Gram positive cocci like *Enterococcus faecalis* and *Staphylococcus aureus* (70.0% each) to the commercially available antibiotics (Table 4 & 5) in this study is of serious concern just as Rice (2006) noted about their persistent resistance to antibiotics in the environment. The treatments and control of these pathogens are predominantly tough as most of them are multiple drug resistant pathogens that cause various forms of infectious diseases and transfer from person to person within different environments (Giwa, 2017). According to Rice (2006), control of infections and antibiotic selective pressure are important factors in its spread.

Staphylococcus aureus is one of the leading causes of nosocomia and wound infections usually resulting in purulent discharges (Nester *et al.*, 2004) with difficulties in treatments following their rapid development of resistance to different antimicrobial agents by the production of either plasmid encoded beta-lactamase, modification of penicillin binding proteins (Nester *et al.*, 2004).

Except for Pefloxacin and Streptomycin, *S. aureus* and *Enterococcus faecalis* were resistant to all the standard antibiotics used. Particularly, the high resistance recorded by these organisms to Erythromycin and Amoxicillin in this study was in agreement with the studies of Sani *et al.* (2012). Multiple resistance of *S. aureus* seen here is also in

agreement with Suller and Russell (2000); Martinez and Baquero (2002) who also documented similar reports.

Most enterococcal infections are caused by *Enterococcus faecalis* and the bacterium is the most important causes of nosocomial bacteremia, post-operative wound infection, and urinary tract infection. This bacterium has become resistant to many and sometimes all standard therapies (Huycke *et al.*, 2012). The observed antibiotic resistance pattern (Table 4 & 5) of *E. faecalis* in this research was also in concurrence with Adejuwon *et al.*, (2010) where similar drug resistance results were recorded for *Enterococcus faecalis*. *Bacillus cereus* was the most susceptible of all the test bacteria both to the crude extract, ethyl acetate and streptomycin. This result was in line with the previous work of Weber *et al.* (1988).

Conclusion

This study revealed that ethyl acetate, among other solvents was the most suitable for the extraction of antimicrobial compounds from the leaves of *Hyptis suaveolens* as the extract showed maximum zone of inhibition and increasing the concentrations of this extract could help eliminate most pathogens when they come in contact with the plant materials. Also, in this research all the test organisms used were resistant to more than two classes of antibiotics. A complete study of the various components of *Hyptis suaveolens* and the development of the plant into therapeutic agents could result in the production of a potent drug which could compete favorably with known standard antibiotics in the fight against the increasing antimicrobial resistance among microorganisms.

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