Antibacterial Activity of *Hyptis suaveolens* Leaves Extract on some Selected Clinical Isolates

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Abstract: *Hyptis suaveolens* is an important plant used traditionally as an ethnomedicine and widely distributed in Nigeria as well as African and Asian countries. In this study, antibacterial activity of ethanolic leaves extract of *H. suaveolens* was conducted. The plant metabolite was extracted using standard protocols and the antibacterial activity of the extracts was evaluated using the standard *in-vitro* methods against four (4) clinical isolates; *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes*. The result of the study revealed that the extraction process gave a yield of 6.2g extract (6.2%) of the original sample used. The plant extract exhibited a dose-dependent antibacterial activity against the test isolates with higher zone of inhibition of 18.67±0.33mm against *S. aureus,* followed by *E. coli* with 17.33±0.12mm both at 100mg/mL while lowest activity was recorded against *S. pyogenes* (6.1±0.66mm) at 12.5mg/mL. *S. aureus* showed a significant antibacterial activity, even at 12.5 mg/mL with the activity more pronounced at highest concentration of 100mg/mL. There was a dose-dependent increase in antibacterial activity across all test isolates. It was also the most susceptible of the isolates at all concentration while *P. aeruginosa* was the most resistant at all concentrations. *E. coli* and *S. aureus* had the lowest MIC of 25mg/mL and *S. pyogenes* had and 50mg/mL as its MIC, while *P. aeruginosa* on the other hand showed growth at all concentrations tested. Results of minimum bactericidal concentration showed that both the concentrations of 50 to 12.5mg/mL are unable to kill the gram negative isolates (*E. coli* and *P. aeruginosa*) while for the gram positive isolates (*S. aureus* and *S. pyogenes*) the concentration of 50mg/mL was found to totally kill the test isolates thus representing their Minimum Bactericidal Concentration (MBC). The study confirms that the leaves of *H. suaveolens* exhibited some antimicrobial activities owing to its significant zone of inhibition, lesser MIC and MBC as well as its broad spectrum of activity. Further phytochemical and pharmacological investigations are recommended.
Keywords: Antibacterial, Extract, *Hyptis suaveolens,* ethnomedicine, *in - vitro*

INTRODUCTION

Infectious diseases are caused by pathogenic bacteria, fungi and viruses and are of great burden to many societies and countries across the world which leads to the emergence of new infectious diseases every year, many of which have no treatment or cure (WHO, 2001). Bacteria are among the major culprits that cause devastating diseases that lead to losses of lives.

Medicinal plants are natural resources yielding valuable products used in the treatment of various ailments (Verma *et al.,* 2015), they provide a safe, cost-effective, ecological alternative to chemical antioxidants, which can be toxic on prolonged exposure (Hussein and El-Anssary, 2019). Since ancient time, people have been using medicinal plants to cater for their health needs and the enthusiasm for this kind of medicine has never been greater than it is today (Bashir *et al.,* 2015). Different parts of plants have been found to be rich in natural antioxidants that have the ability to counter the activity of free radicals that are highly reactive and thus can lead to severe physiological conditions commonly referred to as oxidative stress in the body (Mahdi-Pour *et al.,* 2012). Such free radicals can lead to damage of various cellular organelles and cause neurodegenerative diseases, liver diseases, cardiovascular diseases and respiratory diseases (Chiurchiù and MacCarrone, 2011).

Medicinal plants have been shown to posses various activities including but not limited to antibacterial, antifungal (Bashir *et al.,* 2013) antioxidant and anti-inflammatory activities (Sun *et al.,* 2012; Han *et al.,* 2016). This activity has been attributed to the plants' ability to produce secondary metabolite (Hussein and El-Anssary, 2019).

The use of medicinal plant in the treatment of diseases of microbial origin has been shown to be of great advantage due to its cheapness, biodegradability and availability (Mwitari *et al.,* 2013).

Hyptis suaveolens (L.Poit) is a medicinal plant that is commonly used traditionally for the cure of many diseases including respiratory, gastrointestinal diseases, as an antipyretic and skin diseases (Chukwujekwu *et al.,* 2005). It is a fast growing perennial herb plant found in the tropical regions growing mainly as weed on road sides and over-grazed areas (Chukwujekwu *et al.,* 2005; Prince *et al.,* 2013). The plant is originally native to America but now can be found worldwide in French Guiana, Brazil, Venezuela, Ecuador in Southern America; United States in North America; Bangladesh, China and India in Asia; Benin, Kenya, Nigeria and thus known by many local names such as bush mint, bush tea, pignut, Hyptis a odeur (French), Betonica-brava (Brazil), Chao, Wilaiti tulsi (Hindi) (Prince *et al.,* 2013).

Several studies have been conducted before on the pharmacological profile of *Hyptis suaveolens* (Vera-Arzave *et al.,* 2012; Ghaffari *et al.,* 2014; Ogbonnia *et al.,* 2018). However most of the studies focused on the seeds of the plant, this research was conducted to determine the antibacterial activity of leaves extract of *Hyptis suaveolens* on some selected clinical isolates.

MATERIALS AND METHODS

Sample collection

Fresh leaves of *Hyptis suaveolens* were collected in May 2018 from Warwade dam in Jigawa states, Nigeria and transported to Herbarium of Bayero University Kano for authentication (Accession number BUKHAN 174). Authentication of the plant was carried out by expert botanists and voucher specimen kept in laboratory for future reference as per standard method earlier described (Bajpai *et al.,* 2016).

Sample processing

The plant sample was processed as per standard protocols described earlier (Harborne 1984; Aliyu *et al.,* 2008). The leaves were carefully separated from any stem part and thoroughly washed with sterile distilled water to remove any foreign particle and then dried indoor. The leaves were then weighed using electronic weighing balance and then crushed into powdered form with the aid of mechanical grinder. 100g of the plant sample was weighed and mixed with 1000mL ethanol (in a ratio of 1:10) and extracted using maceration technique, the extract was then concentrated using rotary evaporator and dried in oven at 40^{o}C to remove the remaining solvent according to Azwanida, (2015).

Media and Reagent preparation

All reagents used are of analytical grade (Loba Chemie PVT Ltd) and media (Mueller-Hinton agar, Mueller-Hinton broth and nutrient agar from Himedia laboratories PVT Ltd) are prepared as per the manufacturer's instructions. Reagents were stored in clean high density polypropylene bottles.

Bacterial Isolates

Staphylococcus aureus, Streptococcus pyogenes, *Escherichia coli* and *Pseudomonas aeruginosa* bacterial isolates were used for this work. The isolates were obtained from Microbiology unit of Dutse General Hospital, Jigawa State Nigeria.

Identification and confirmation of bacterial isolates

Bacterial isolates were identified by their morphological characteristics on selective media, gram staining and biochemical characteristics which include test for production of Catalase, Indole, Methyl red, Voges-proskauer, Oxidase test and citric utilization, as per standard method described (Cheesbrough, 2006; Aneja, 2012).

Preparation and standardization of inoculum

The standard turbidity solution (Barium sulphate standard turbidity solution) was prepared as per standard method by McFarland, (1907).

To 99mL of sterile distilled water, 1mL of concentrated tetraoxosulphate VI acid (H_2SO4) was added to arrive at 1% (v/v) solution of the acid. In another beaker, 0.5g of barium chloride ($BaCl_2.2H_2O$) was weighed and distilled water was added until the final volume of the solution reaches 50mL mark and stirred until it dissolved completely, this made 1% (w/v) barium chloride solution, 0.5mL of the barium chloride solution was then added to 99.5mL of the 1% H_2SO4 solution and shaken, this resulted in turbid solution (0.5 Mc Farland solution), a small volume of this turbid solution was taken in test tube which was used for comparing during standardizing inoculum of the test organisms.

For standardizing the inoculum, the test organisms were sub-cultured on nutrient agar plates and incubated overnight, colony material from this overnight culture of the test organisms was taken with the aid of a sterilized wire loop and transferred into a tube containing 5mL of normal saline and vortexed until the turbidity of the suspension matched with the turbidity of the prepared 0.5 McFarland standard, this was confirmed by comparing the turbidity of the diluted inoculum with that of the standard turbidity solution against the background of the of the printed white paper, where the inoculum is more turbid, a little more normal saline was added until its turbidity matched with 0.5 Macfarland standard to give a mean of 1.5 x 106 CFU/mL microbial population density (CLSI, 2016).

Preparation of extract concentration

Different extract concentrations were prepared from the crude extract of *H. suaveolens* by twofold dilution according to standard protocols (Kumarasamy *et al.,* 2002; Malekinejad *et al.,* 2012). From the extract, 0.2g was taken and diluted with 2mL Dimethylsulfoxide (DMSO) to give a concentration of 100mg/mL which serves as stock solution, from this, 0.5mL was taken and diluted with fresh 0.5mL DMSO to give 50mg/mL concentration. This was continued in a similar fashion till 25mg/mL and 12.5mg/mL were obtained. The extract was then stored in a refrigerator at 4^{o}C before use (Deeni and Hussain, 1991).

Antibacterial activity test

Antibacterial activity test was carried out using well diffusion method as per standard protocol (Bauer *et al.,* 1966; CLSI, 2016). With the aid of sterile cotton swab, an inoculum was taken from the standardized bacterial cultures and inoculated onto freshly prepared solidified Mueller-Hinton agar and allowed to stand for about 15 minutes, this was then followed by making wells into the inoculated plates with the aid of 6mm cork borer and different extract concentrations (100µl) were dispensed into the well, DMSO was used as negative control while ciprofloxacine (10µg) was used as positive control. The plates were allowed for a pre-diffusion time of 15 minutes and then be incubated upright for 18-20 hours at 37^{o}C. Zones of inhibition were then measured at the end of incubation period.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was carried out by tube dilution assay as per the method reported earlier (Kumarasamy *et al.,* 2002; Malekinejad *et al.,* 2012) where a twofold dilution of the plant extracts was made by mixing the extract concentrations with Mueller-Hinton broth to give a concentration of 50, 25, and 12.5 mg/mL followed by the addition of the standardized test organism (100µl), a control tube was also set which contain the media and extracts only. The tubes were then incubated at 37^{o}C and observed after 18- 24 hours.

Determination of minimum bactericidal concentration (MBC)

A loop full from the positive MIC tubes were streak onto Mueller-Hinton Agar (MHA) plate and incubated at 37^{0}C for 24 hours. Absence of growth after incubation, determine positive result (bactericidal) (Olajuyigbo, 2012).

Statistical analysis

The experiment was performed in triplicates to minimise experimental error.

Data generated from the study were presented as mean and was subjected to statistical analysis using one-way analysis of variance (ANOVA) where results with a P value of $p \leq 0.05$ where considered significant.

RESULTS

The result of the study as indicated in Table 1 show that following the extraction protocol using ethanol as a solvent the percentage yield of 100g of dried leaves powder of *H. suaveolens* (L. Poit) was 6.2%. Additionally, the extract has been found to possess a greenish colour and sticky texture (Table 1).

Table 1: Physical characteristics of *H. suaveolens* (L. Poit) Leave Extract.

Sample weight (g)	Extract yield (g)	Percentage yield	Colour of extract	Texture
100	6.2	6.2%	Greenish brown	Sticky

Antibacterial activity

S. aureus showed a significant antibacterial activity, even at 12.5 mg/mL with the activity more pronounced at highest concentration of 100mg/mL.There was a dose-dependent increase in antibacterial activity across all test isolates. It was also the most susceptible of the isolates at all concentration while *P. aeruginosa* was the most resistant at all concentrations. The results are shown in table 2.

Table 2: Antibacterial activity of *H. suaveolens* against the clinical isolates

Test organisms	Mean zone of inhibition (mm)					
	100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	Positive control	Negative control
E. coli	17.33±0.12	11.67±0.39	9.0±.057	7.50±0.63	19.33±0.33	0.00
P. aeruginosa	12.00±0.58	10.67±0.88	7.67±0.33	6.22±0.33	20.33±0.33	0.00
S. aureus	18.67±0.33	13.67±0.33	9.67±0.33	8.10±0.58	21.67±0.33	0.00
S. pyogenes	14.67±0.43	10.33±0.88	8.53±0.67	6.10±0.66	20.33±0.26	0.00

NB: Positive control= Ciprofloxacine (10µg), Negative control= DMSO

Minimum inhibitory concentration

E. coli and *S. aureus* had the lowest MIC of 25mg/mL and *S. pyogenes* had and 50mg/mL as its MIC, while *P. aeruginosa* on the other hand showed growth at all concentrations tested as shown in table 3.

Table 3: Minimum inhibitory concentration of *H. suaveolens* against the clinical isolates

Test organism	Concentration (mg/mL)		
	50	25	12.5
E. coli	-	-*	+
P. aeruginosa	+	+	+
S. aureus	-	-*	+
S. pyogenes	-*	+	+

Key: += Growth, - = No Growth, *= MIC

Minimum bactericidal concentration

Result of minimum bactericidal concentration showed that both the concentrations of 50 to 12.5mg/mL are unable to kill the gram negative isolates (*E. coli* and *P. aeruginosa*) while for the gram positive isolates (*S. aureus* and *S. pyogenes*) the concentration of 50mg/mL was found to totally kill the test isolates thus representing their Minimum Bactericidal Concentration (MBC) as shown in Table 4.

Table 4: Minimum bactericidal concentration of *H. suaveolens* against the clinical isolates

Test organism	Concentration (mg/mL)		
	50	25	12.5
E. coli	+	+	+
P. aeruginosa	+	+	+
S. aureus	-*	+	+
S. pyogenes	-*	+	+

Key: + = Growth, - = No Growth, * = MBC

DISCUSSION

Medicinal plants have been known to have beneficial values across almost all civilizations, they are highly abundant, cheap and very valuable source of medicine used to cure for a variety of diseases (Kunle, 2012). A lot of work has been done in many countries such as Nigeria, India and china due to an increase of interest in a large number of natural products from medicinal plants, Chinese and Indian herbal medicine system has been widely accepted because many plant extract showed broad spectrum of activity (Pachkore *et al.,* 2011).

This work with ethanolic extract of *H. suaveolens* showed that all the four clinical bacterial isolates tested are susceptible to the plant extract at various concentrations (Table 2) with the activity more pronounced on *S. aureus* with 18.67±0.33mm zone of inhibition at 100mg/mL while the lowest zone inhibition was found on *S. pyogenes* with 6.1±0.66mm at 12.5mg/mL, which is in accordance with the work of Pachkore and colleagues (Pachkore *et al.,* 2011). Among all the isolate tested, *P. aeruginosa* showed the lowest susceptibility pattern toward all the extract concentrations which may be due to the fact that it is an encapsulated bacteria and have been known to resist many antibiotics as well, this finding is consistent with some earlier reported works (Mandal *et al.,* 2007; Bashir *et al.,* 2013).

Result of the minimum inhibitory concentration showed that *E. coli* and *S. aureus* have the lowest MIC (25mg/mL) as compared with the other isolates (*P. aeruginosa* and *S. pyogenes*) with MIC value of 50mg/mL. The result of the MIC is consistent with that of antibacterial assay as well as the other previously reported works (Iwu *et al.,* 1990; Egwari, 1999). On the other hand however, the result of minimum bactericidal concentration showed that both concentrations tested were unable to kill all the gram negative isolates but rather inhibit their growth only as opposed to other gram positive isolates which were totally killed at a concentration of 50mg/mL (Table 4).This is not surprising as gram negatives organisms were shown to resist more antibiotics than their gram positives counterparts due to differences in cellular morphology (Procop *et al.,* 2017). Bacterial infection has been a cause of mortality and morbidity in human population and many among the gram negatives such as *E. coli* were known to be associated with a variety of clinical diseases including the urinary tract infections, gastroenteritis and diarrhoeal diseases while *P. aeruginosa* is well known for its ability to resist many antibiotics (Bashir *et al.,* 2013).

As medicinal plants are shown to exert their effect on bacteria by various means such as stimulating autolysis, loss of electron and coagulation of cytoplasm (Cox *et al.,* 1998), activities of *H. suaveolens* may be attributed to the essential oils and compounds such as β- caryophyllene and β-pinene found (Iwu *et al.,* 1990; Mandal *et al.,* 2007). This study therefore supplements and confirmed the previously reported works.

CONCLUSION AND RECOMMENDATION

The study confirms that the leaves of *H. suaveolens* exhibited some antimicrobial activities owing to its significant zone of inhibition, lesser MIC and MBC as well as its broad spectrum of activity. Further phytochemical and pharmacological investigation are recommended.

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