



Evaluation of Antibacterial Properties of Different Leaf Extracts of *Hyptis suaveolens* (L) Poit

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Authors' contributions

This work was carried out in collaboration among all authors. Author BAE designed the study performed the statistical analysis wrote the protocol. Authors AOA and OTO managed the analyses of study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: *Hyptis suaveolens* is well known for its immense medicinal properties and are beneficial as folk medicine. The objective of the study is to investigate the efficacy of the leaves for its antimicrobial activity.

Material and Methods: The leaves of *Hyptis suaveolens* was subjected to extraction process using n-hexane, ethanol and distilled water as solvents and the antimicrobial activity was analyzed against different bacterial strains viz.; *Escherichia coli*, *Proteus mirabilis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Enterococcus faecalis* by agar well diffusion method.

Results: Aqueous extracts showed inhibitory effect against the different tested bacteria organisms with variable zone of inhibitory range 6 -21 mm.

Conclusion: The aqueous extracts of *Hyptis suaveolens* exhibited the presence of highly effective bio-active compounds in these extracts. These can further be evaluated and characterize to improve upon what has been done to create a novel compound that can be useful for various medicinal purposes.

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Keywords: *Hyptis suaveolens*; leaf; antibacterial activity; extracts.

1. INTRODUCTION

Hyptis suaveolens is a fast growing perennial and aromatic herb belonging to family *Lamiaceae* [1]. It is one of the important traditional medicinal plant with about 0.4 – 2 m height; quadrangular stem having numerous trichomes. The leaves either ovate or obovate, generally measuring 3-5 cm long stalk while its petioles are up to 3cm long. The plant starts flowering early at an early of 2-3 months and produces copious blue flowers. The flowers are pollinated by numerous pollinators leading to enormous seed production [2,3]. Its use in Asian food recipes as an appetizer due to the presence of its essential oil has been reported by [4]. It is therefore serves as an edible aromatic flavoring for food.

Moreover, it has been reported to have metals like zinc, copper and iron in which the zinc plays a vital role in growth, aids the catalytic and regularity action of more than 300 enzymes and helps to maintain role in a wide range of physiological processes in the body which include iron utilization, elimination of free radicals, development of bone, and production of the skin and hair pigment called melanin [5].

2. MATERIALS AND METHODS

Hyptis suaveolens leaf alone were collected from the natural habitats and on road sides and farm steads in three different locations, with no altitudinal variation at Akungba Akoko, Akure in Ondo state. Owena in Osun state, South western part of Nigeria, The leaves was identified and confirmed by Mr A.A. Ibitoye Department of Crop and Pest management. Federal university of Technology Akure.

2.1 Preparation of the Crude Extract

Leaf samples of *Hyptis suaveolens* were air and shade dried for seven days and pulverized to powder. The powdered leaves samples (50g) were extracted with 200 ml of ethanol, n-hexane and aqueous using Soxhlet extractor one after the other at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whatman filter paper (No 1) and then concentrated in vacuum at 40°C using Rotary evaporator (RE52A China).

2.2 Collection of Test Organism

The test organism (*Enterococcus faecalis*, *Salmonella typhi*, *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*,

Streptococcus pneumoniae, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*) were collected from the Medical Microbiology Department of University College Hospital (UCH) Ibadan and the Federal Medical center Owo, Ondo state. Their identity was confirmed using biochemical and morphological characteristics before storing in slants and kept in the refrigerator.

2.3 Standardization of Test Organism and Antibacterial Assay

The test organism were individually grown in nutrient both at 37°C for isolation in separate conical flasks. The cell were then harvested and standardized from the stock culture using the method of [6,7]. The absorbance was measured using a spectrophotometer (Unico 1100RS series) 1ml of the harvested cell was pour plated. Two wells were bored using diameter 4mm of sterilized cork borer and 0.4ml of the liquor was introduced into one well while the same volume of sterile distilled water was added to the other well as control. The same process was used for the slurry. The plates were carefully incubated at 37°C for 24 hours in incubator (Uniscope) and the diameter of zones of inhibition measured. Standard antibiotic were used in the test organism for the control assay according to [8].

2.4 Statistical Analysis

The data gathered were processed using descriptive are way analysis variance SPSS version 23. The Duncan multiple range test was used as a follow up test.

3. RESULTS

Table 1 shows the standardized colony forming unit of each of the organism used for the antimicrobial assay of the extracts and the standard antibiotics discs in this research work.

Table 2 shows the mean and standard deviation of the diameter of zone of inhibition of the different extracts in the test organism. Comparison of the extracts with standard antibiotics showed that the extracts was highly effective in n-hexane and ethanol extract and less effective in distilled water extracts. The highest zone of inhibition was observed in *Escherichia coli* (A). *Staphylococcus aureus* (G) *Streptococcus pneumoniae* (H), *Proteus mirabilis* (D).

Table 1. Standardized colony forming unit per ml of each organism suspension used

Organism	Dilution Power	Cfu/ml	Spectrophotometric reading	Standard cfu/ml
<i>Escherichia coli</i>	10 ⁶	24	0.040	2.4 X 10 ⁷
<i>Enterococcus faecalis</i>	10 ⁶	12	0.050	1.2 X 10 ⁷
<i>Klebsiella aerogene</i>	10 ⁶	15	0.052	1.5 X 10 ⁷
<i>Proteus mirabilis</i>	10 ⁶	17	0.049	1.7 X 10 ⁷
<i>Pseudomonas aeruginosa</i>	10 ⁶	19	0.048	1.9 X 10 ⁷
<i>Salmonella typhi</i>	10 ⁶	20	0.047	2.0 X 10 ⁷
<i>Staphylococcus aureus</i>	10 ⁶	23	0.044	2.3 X 10 ⁷
<i>Streptococcus pneumoniae</i>	10 ⁶	21	0.042	2.1 X 10 ⁷

Table 2. Mean values and standard deviation of the diameter of zone of inhibition of the different extracts on the test organism

Solvent	Organisms							
	A	B	C	D	E	F	G	H
N-hexane	21.00±1.00 ^c	5.65±0.35 ^d	11.75±0.25 ^d	13.70±0.30 ^c	4.10±0.10 ^b	3.10±0.10 ^b	20.61±0.38 ^c	18.71±0.28 ^c
Ethanol	15.00±1.00 ^b	0.00±0.00 ^a	0.00±0.00 ^a	9.15±0.15 ^b	0.00±0.00 ^a	0.00±0.00 ^a	14.66±0.34 ^b	12.78±0.22 ^b
Diluted water	4.60±1.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	2.10±0.10 ^a

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)

Table 3. Antibiotic sensitivity of zone of inhibition on test organisms

Antibiotics	Organisms							
	E. coli	E. faecalis	K. aerogenes	P. mirabilis	P. aeruginosa	S.typhi	S. aureus	S. pneumoniae
CIP	24.26±0.74 ^e	11.61±0.38 ^d	19.77±0.23 ^d	21.43±0.57 ^d	9.76±0.24 ^b	12.62±0.37 ^b	22.60±0.39 ^e	0.00±0.00 ^a
TET	4.15±0.15 ^b	4.06±0.06 ^b	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	15.72±0.27 ^b
RTX	26.76±0.23 ^f	10.11±0.11 ^c	23.60±0.39 ^e	24.22±0.77 ^e	12.62±0.38 ^c	13.62±0.37 ^c	20.02±0.02 ^d	0.00±0.00 ^a
AM	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
GN	14.66±0.34 ^c	10.06±0.06 ^c	12.75±0.25 ^d	15.61±0.38 ^c	0.00±0.00 ^a	0.00±0.00 ^a	12.07±0.07 ^b	0.00±0.00 ^a
N	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
S	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
NA	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	18.01±0.01 ^c
AX	19.16±0.84 ^d	11.76±0.00 ^d	15.71±0.29 ^c	13.76±0.24 ^d	0.00±0.00 ^a	0.00±0.00 ^a	15.74±0.26 ^c	0.00±0.00 ^a

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)

Keywords: CIP – Ciprofloxacin TET-Tetracycline AX- Augmentin RTX -Rocephin AM-Ampicillin GN – Gentamicin N- Nitrofurantoin NA- Nalidixic Acid S – Streptomycin

Table 3 shows the value obtained for the standard antibiotics used. Most of the organisms were sensitive to Rocephin (RTX), Augmentin (AX), Ciprofloxacin, (CIP), and Gentamycin (GN). They are resistant to streptomycin (S), Ampicillin (AM), Tetracycline (TE). Nalidixic acid (NA) and Nitrofurantoin (N).

4. DISCUSSION

In this study, different extracts of *Hyptis suaveolens* used indicated variable inhibitory effect on some of the test organisms. *Pseudomonas aeruginosa*, *Salmonella typhi*, *Streptococcus faecalis* shows least inhibitory effect. This connotes that the leaves of *Hyptis suaveolens* contains bioactive components that had greater activity than that of the antibiotic, in inhibiting growth of test organism. This report collaborate with [1,9] that *Hyptis suaveolens* shows potent antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis* but not *Pseudomonas aeruginosa* and *Salmonella typhi* which was least inhibitory in this study. The highest inhibitory effect was exhibited by *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis* and *Streptococcus pneumoniae* with n-hexane. This is a proof that the extracts of these organism can be a substitute to the synthetic antibiotic in the treatment of infection caused by the bacteria. The use of ethanol as a solvents for the study collaborate with the report of [10,11]. The result of the present study coincided with previous observations of researchers. Many plants have been reported for antimicrobial properties across the world [12,13,14]. The report of study on the photochemistry of *Hyptis suaveolens* revealed that extracts of it different plants contain alkanoids, flavonoids, terpenoids and tannins [15,1]. These constituents of phytochemicals were responsible for inhibiting bacteria growth because they convey antimicrobial properties in their activities [16,17].

5. CONCLUSION

There are evidences of research being conducted on various parts of *Hyptis suaveolens* that it possess antimicrobial properties. However, more study should be harnessed to improve, identify and characterize it compounds that will establish novel drug which is available, affordable and potent useful as alternative to conventional drug in the treatment of infectious diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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