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Research Article

Phytochemical and Antimicrobial Activity of *Ocimum suave* Against Selected Human Pathogenic Bacteria

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Abstract

Worldwide, the use of antibiotics is losing effectiveness due to the substantial resistance to the antimicrobial developed by many pathogenic bacteria. African populations including Rwanda, use frequently traditional medicine for primary care. This study aimed to determine the phytochemicals and the antimicrobial activity of *Ocimum suave* (*O. suave*) against selected human pathogenic bacteria (two strains of *S. aureus*, *P. aeruginosa*, two strains of *E. coli*, *S. pneumonia*, *S. pyogene*, *K. oxytoca*, *H. influenza*, *S. sonei*). The leaf and stem extracts were prepared using maceration technique. The antimicrobial activities of extracts were evaluated using the impregnated disc and agar well diffusion methods. The tests revealed the presence of phytochemical substances such as tannins, flavonoids, saponins, steroids alkaloids and phenolics in leaves and only saponins in stem. *S. aureus* ATCC 43300 was the most sensitive while *H. influenza* was the most resistant among the microorganisms tested. Aqueous and methanolic crude extract of *O. suave* displayed maximum diameter of inhibition zone against bacteria (9-26.5 mm and 9-23.5 respectively) and Two-way ANOVA showed statistical significance difference between their means. T-test was used in comparison with standards antibiotics and showed that the distilled water extracts exhibited much higher activity against all tested organisms than activity of streptomycin. Combination of distilled water and methanol extracts with streptomycin revealed synergistic activity against *E. coli* ATCC 25922 and *S. pneumonia* ATCC 49618. The leaves of *O. suave* have a great potential antibacterial activity and should be fully explored for future treatment.

Keywords: phytochemical; antimicrobial activity; *Ocimum suave*, Rwanda

INTRODUCTION

Antibiotics play a significant role in reducing the burden of infectious diseases all over the world. However, the effectiveness of treatment of infections has become a global public health issue due to the Antimicrobial resistance (AMR) ^{1,2}. Low- and middle-income countries (LMICs) remain the most affected by both the infectious disease and the growing threat of antibiotic resistance where about 4M of deaths are estimated by 2050 due to the AMR ³⁻⁵. African traditional medicine has been used in the management of infectious diseases and is still the most widely as primary healthcare needs ⁶. According to the World Health Organization (WHO), 80% of the people in the developing countries including Rwanda rely on traditional medicinal plants for their health care ⁷. While African region continues to register an alarming rate of AMR, this region abounds in medicinal plants that have proven to be a source of new antibiotics or constitute an alternative to the current drugs ⁸. Therefore, traditional medicinal plants could be exploited as a source for drug development and might play a role in overcoming the growing problem of resistance and the toxicity of the currently commercial antibiotics ⁹. However, phytochemical and

antibacterial analysis of medicinal plants remain extensively unexplored.

Ocimum suave is abundant in different countries of the world including Rwanda (locally known as Umwenya) where is widely grown in gardens and forests ¹⁰. It is a flowering plant of Lamiaceae family with ascending erect, branched perennial herb and fine tomentose-pubescent ¹¹. It is used as a traditional herb with a wide array of phytochemical constituents such as flavonoids, polyphenols, saponins, alkaloid, phenols and tannins which have been described as pharmacologically active compounds ¹². In addition to anti-pests activity, the extracts of *O. suave* have been reported to have in vitro growth of bacterial and fungal isolates ¹³. The leave extracts have shown an antibacterial activity against *Escherichia coli* (*E. coli*), *Shigella*, *Salmonella*, *Proteus* *Bacillus subtilis* (*B. subtilis*), *Staphylococcus aureus* (*S. aureus*) and some fungi (*Trichophyton mentagrophytes* var. *interdigitale*) ^{14,15}.

In order to contribute to the growing need of developing new antibacterial agents for both Gram negative as well as Gram positive, it is necessary to investigate the chemical

composition and antimicrobial activity of *Ocimum suave* growing in Musanze district, a volcanic region of Northern Province of Rwanda. Keeping this in view, the chemistry of leaves and stem extracts was assessed and the in vitro biological activity was evaluated on selected human pathogenic bacteria.

MATERIALS AND METHODS

Collection of plant samples and bacterial strains

The plant material was collected from the forests of Musanze district, Northern Province, of Rwanda. The leaves and stem with no dead parts were selectively collected and brought to the laboratory for processing. The plant parts were thoroughly washed in running water, rinsed using distilled water and finally dried at room temperature. The dried *O. suave* leaves and stems were then grounded to powder by cutter mill mixer (model/SM-400). The following ATCC bacterial strains obtained from Rwanda National Reference Laboratory were used for antimicrobial activity analysis: *S. aureus* ATCC 29213, *S. aureus* ATCC 43300, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *E. coli* ATCC 35218, *S. pneumonia* ATCC 49618, *S. pyogene* ATCC 2399, *K. oxytoca* ATCC 700524, *H. influenza* ATCC 9007, *S. sonei* ATCC 25931.

Preparation of plant extracts

20 grams of each powdered plant material was macerated in 200 ml of each solvent in a stoppered container. Ethanol (96%), methanol (98%), petroleum ether (95%) and distilled water were used as solvents. They were allowed to stand for different maceration period (4-7days) with continuous agitation (Orbital shaker / OS-340C), until soluble matter is dissolved. The extraction was carried out at room temperature with agitation at 200 rpm.

The soaked powder-solvent mixtures were filtered twice through a Whatman No. 1 filter paper. Solutions were concentrated to 1 ml with a rotor-evaporator (Heidolph rotary/Laborota 4010 digital) at 34-780°C. The concentrated leave and stem extracts were stored at 4 °C for further use.

To determine the sterility of the extracts, 2ml of the extract was put into solidified 20 ml of Muller Hinton agar, and incubated at 37°C for 24 hours. The absence of turbidity of the agar after the period of incubation confirmed a sterile extract.

Qualitative phytochemical screening of *O. suave*

The presence of resins, phenols, glycosides, saponins, tannins, flavonoids and alkaloids in the crude extract were determined according to the method defined by Harborne¹⁶.

Antimicrobial susceptibility testing

The bacterial suspensions were standardized to 10⁷ to 10⁸ cells/mL as with the barium sulfate standards, a 0.5 McFarland Standard is comparable solution to a bacterial suspension of 10⁸ CFU/ml. All of the tested bacteria were grown in Mueller Hinton broth at 37°C for 24 hours. The standard antibiotic discs (ciprofloxacin (CIP), levofloxacin (LE), streptomycin (S), ampicillin (AM)) were used as positive control and only the solvents alone were used as a negative control.

Impregnated disc methods

Plant extracts have been used to impregnate sterilized 6 mm blank discs for all solvents. After inoculation, the plates were incubated for 24 hours at 37°C. The diameter of inhibition zone was measured for the antibacterial activity assessment.

Agar well diffusion method

Six wells were prepared for each plate. Each hole was filled with the plant extracts and the Petri dishes were left at room temperature for 2 hours for diffusion and solidification. They were incubated at 37 °C for 24 hr. After this incubation period the diameter of the inhibition zones were measured and recorded.

Synergistic activity of *O. suave* and antibiotics

In this study, a disc-agar diffusion technique was performed to demonstrate the synergistic activity of *O. suave* with the respective antibiotics. Antibiotic discs were discretely impregnated with 50µL of different plant extracts and put on inoculated agar plates. The zones of inhibition produced by the plant extract in combination with standard antibiotics after overnight incubation was measured. The synergistic activity as described by Saquib *et al.*¹⁷.

Activity Index

The inhibitory effects of the methanolic, ethanolic and distilled water extracts were calculated and compared by measuring the activity index using the following formula¹⁸.

$$\text{Activity index} = \frac{\text{Zone of inhibition of extracts}}{\text{zone of inhibition of antibiotics}}$$

Statistical analysis

Each experiment was carried out in duplicate. Where applicable, the data were subjected to two-way analysis of variance (ANOVA) and the t-test were applied to compare the means of various extract parameters and Post-Hoc test was used to compare the differences between factors. P-values less than 0.05 was considered statistically significant.

RESULTS

Bioactive compounds

The biochemical assays were done to check the secondary plant metabolites present in the leaves and stem of *O. suave*. The bioactive compounds in leaves were tannins, phenols, saponins, alkaloids and steroids. Only saponins were observed in stem.

Antimicrobial activity analysis

Agar well diffusion methods and impregnated disc were used. Both methods showed almost the same results for tested bacteria strains but agar well diffusion method showed high effects compared to impregnated disc. Antimicrobial activity results of *O. suave* extracts showed that, there is no inhibition zone in stem extracts for both agar well diffusion and impregnated disc methods. All leave extracts inhibited the growth of six bacteria, with inhibition zones ranging from 8.5 to 26 mm. *S. aureus* 43300 and *K. oxytoca* 700524 were the most susceptible species to leave extracts. However, the remaining five bacteria resisted to leave extracts (Table 1).

Table 1: Antimicrobial susceptibility analysis of *O. suave* plant extracts against 11 human pathogenic bacterial.

Species	Inhibition zones in Millimeter (mm)							
	Distilled water		Methanol		Ethanol		Petroleum ether	
	AWD	ID	AWD	ID	AWD	ID	AWD	ID
<i>E. coli</i> 25922	14.75	14.5	11.75	13.5	11.5	11.75	10.5	9.5
<i>E. coli</i> 35218	0	0	0	0	0	0	0	0
<i>H. influenza</i> 9007	0	0	0	0	0	0	0	0
<i>K. oxytoca</i> 700524	23.5	21.5	20	20.5	19.25	19.5	14	13.75
<i>P. aeruginosa</i> 27853	20	19	19	18.5	18	18	12	13.5
<i>S. Pyogene</i> 12344	0	0	0	0	0	0	0	0
<i>S. aureus</i> 29213	0	0	0	0	0	0	0	0
<i>S. aureus</i> 43300	26	24	23.5	24.25	22.5	22.25	16.25	16.5
<i>S. pneumonia</i> 49618	9	9	9.5	9	9	9	8.5	8.75
<i>S. pyogene</i> 2399	12.5	12.5	11	11.5	12.5	12	11.5	12
<i>S. sonei</i> 25931	0	0	0	0	0	0	0	0

AWD: Agar Well Diffusion; ID: Impregnated Discs

Distilled water leave extracts were the most effective extracts and showed a strong antibacterial activity against human pathogenic bacteria. There was no inhibition zone observed in solvents used as negative control (figure 1).

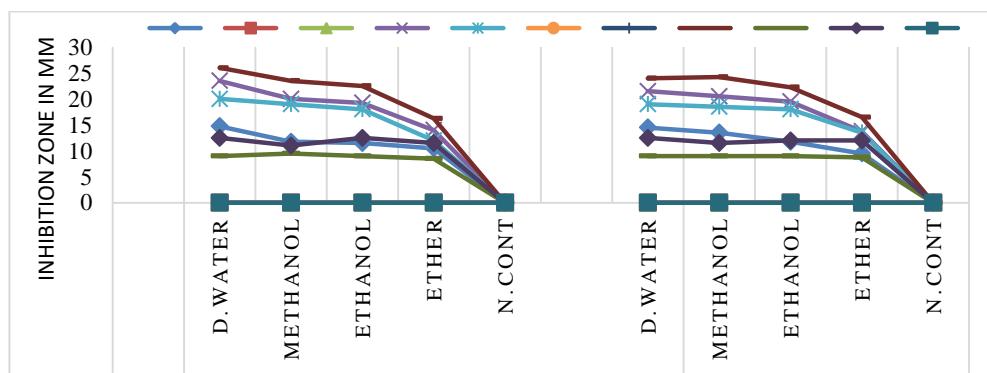


Figure 1: Inhibition zone of agar well diffusion and impregnated disc methods.

D. Water: Distilled water; N. Cont: negative control; AGAR. W.D: Agar well diffusion; I. DISC: Impregnated disc

Comparison of plants extract and standards antibiotics

All bacteria were susceptible to all standard antibiotics except *K. oxytoca* and *P. aeruginosa* which resisted to ampicillin. When the activities of *O. suave* was compared with these standard antibiotics, the distilled water crude extract showed

better antibacterial activity against almost all bacteria strain than streptomycin except *S. pneumonia*, but least activity than levofloxacin and ciprofloxacin. Methanol, ethanol and petroleum ether active crude extracts showed lower antibacterial activity against almost all bacteria strain than all standard antibiotics (figure 2).

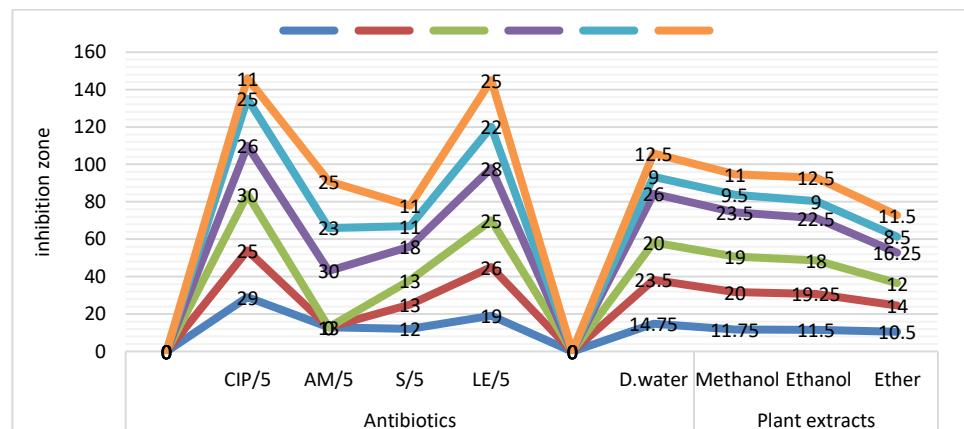


Figure 2: Inhibition zones of antibiotics vs plant extracts.

Methanol, distilled water, ethanol and petroleum ether revealed statistically significant difference when compared with ciprofloxacin and levofloxacin using Student's t-test with P- value of 0.041,0.035,0.002 respectively. Similarly, methanol, ethanol and petroleum ether exhibited non-statistically significant difference compared with ampicillin and streptomycin.

Synergistic activity of plant extracts with antimicrobial agents

The synergistic activity of individual plant in combination with antibiotics as well as activity index is presented below.

When distilled water extract was used in combination with streptomycin, it showed strong synergistic activity against *E. coli* and *S. pneumonia*. Combination of distilled water extract with levofloxacin, ciprofloxacin, amoxicillin revealed antagonism for all bacteria strains. Combination of methanol extract with amoxicillin and streptomycin showed a

synergistic outcome against *P. aeruginosa* and *S. pneumonia* respectively. The rest combination showed antagonism and there was no additive observed. Combination of ethanol and petroleum ether extracts with all antibiotics showed antagonism, no additive and synergistic was observed in both solvents.

Activity index

The activity index of extracts with the standard antibiotics is presented in table 2. Using ciprofloxacin as standard, high indices greater than unit (AI>1) were observed in *S. pyogene* for all the extracts and *S. aureus* for distilled water, *S. pyogene* showed great indices of 1.13 in distilled water. With amoxicillin as a standard all extracts had indices less than unity (AI<1) on almost all bacteria strain except *E. coli* 25922. Using streptomycin as a standard all bacteria showed high index greater than or equal to unity (AI>1) except *E. coli* 25922 and *S. pneumonia* 49618. Levofloxacin showed indices less than unit (AI<1) for all bacteria.

Table 2: Synergistic antimicrobial activity of *O. suave* with different antimicrobial agents

Bacteria strains	Antibiotics	Zone of Inhibition of Antibiotics	Mean Zone of Inhibition of <i>O. suave</i> extracts		Mean Zone of Inhibition of combination	Synergistic activity	Activity index	
			Water	Methanol			Water	Methanol
<i>E. coli</i> 25922	CIP	29	14.75	11.75	20	Antagonism	0.5	0.4
	AM	13			13	Antagonism	1.13	0.9
	S	12			27	Synergism	1.22	0.97
	LE	19			19	Antagonism	0.77	0.61
<i>K. oxytoca</i> 700524	CIP	25	23.5	20	27	Antagonism	0.94	0.8
	AM	0			22	Antagonism	N/A	N/A
	S	13			16	Antagonism	1.8	1.53
	LE	26			22	Antagonism	0.9	0.76
<i>P. aeruginosa</i> 27853	CIP	30	20	19	20	Antagonism	0.66	0.63
	AM	0			19	Antagonism	N/A	N/A
	S	13			18	Antagonism	1.53	1.46
	LE	25			24	Antagonism	0.8	0.76
<i>S. aureus</i> 43300	CIP	26	26	23.5	18	Antagonism	1	0.9
	AM	30			15	Antagonism	0.8	0.78
	S	18			14	Antagonism	1.44	1.3
	LE	28			19	Antagonism	0.92	0.83
<i>S. pneumonia</i> 49618	CIP	25	9	9.5	14	Antagonism	0.36	0.38
	AM	23			8	Antagonism	0.39	0.41
	S	11			24	Synergism	0.81	0.86
	LE	22			14	Antagonism	0.4	0.43
<i>S. pyogene</i> 2399	CIP	11	12.5	11	19	Antagonism	1.13	1
	AM	25			10	Antagonism	0.5	0.44
	S	11			11	Antagonism	1.13	1
	LE	25			9	Antagonism	0.5	0.44

DISCUSSION

Antimicrobial resistance is becoming a serious public health problem and remain the major cause of death throughout the world. Alternative natural products of plants are known to have antimicrobial properties and could be of high interest in the therapy of microbial infections and reduction of the increasing incidence of antibiotic resistance ¹⁹. Various studies have been conducted in different countries, and have demonstrated the efficacy of this type of treatment ^{20,21}.

The phytochemical screening of *O. suave* leaves and stem revealed the presence of flavonoids, saponins, phenols, tannins and steroids for leaves and only saponins in stem and those results are in agreement with previous reports done by Tan *et al.*, ²². These classes of compounds are known to show curative activity against several pathogens and therefore could explain its wide usage traditionally for the treatment of wide array of illnesses ²³.

Extracts of leaves and stem of *O. suave* from different solvents were investigated to evaluate their antimicrobial activity against human pathogenic bacteria, including Gram-positive bacteria (*S. aureus* and *S. pyogene*) and Gram- negative bacteria (*E. coli*, *S. typhi*, *S. pneumonia*, *K. oxytoca*, *H. influenza* and *P. aeruginosa*) using agar well diffusion and impregnated disc methods.

The high inhibition zone observed in extract of distilled water could be due to the presence of high amount of polar bioactive compounds compared to than non-polar compounds ²⁴. Important to note, no inhibition zone appeared on wells with solvents only (controls). Thus, the inhibition observed in plant leaves extract was due to bio-active compounds present in *O. suave* leaves. However, stem extracts didn't show any activity against all bacteria strain. This could be due to the presence of only one compound (saponins) among seven tested bio-active compounds.

S. aureus ATCC 43300 was highly sensitive bacteria followed by *K. oxytoca* 700524 and the lowest sensitive was *S. pneumonia* 49618. *S. pyogene* ATCC 12344, *S. Sonei* ATCC 25931, *S. aureus* ATCC 29213, *H. influenza* ATCC 9007and *E. coli* ATCC 35218 resisted to all plant extracts (Table 1).

Impregnated disc method showed low activity against sensitive tested bacteria, while agar well diffusion showed high activity against sensitive tested bacteria, and this is due to paper disc retention properties ²⁵.

Meanwhile, the comparison with standards antibiotics showed that the distilled water extract exhibited much higher activity against all tested organisms than activity of streptomycin. Antibacterial activity of the standard antibiotics can be magnified by the combination of phytopharmaceuticals and modern medication ²⁶. In addition, the combination may be helpful in the preclusion of the emergence of resistant bacteria and reducing the drug toxicity ²⁷. Various in vitro experiments have established the fact that a combination of plant extracts and antibiotics possess a synergistic effect, which results in a significant decrease in levels of minimum inhibitory concentration for the antibiotics ^{28,29}.

The findings from the present study may help to understand the synergistic effect of combination therapy which could provide a new strategy for the adjunctive treatment for infections. Results from the current study revealed that a combination of distilled water and methanol extracts with streptomycin revealed synergistic activity against *E. coli* 25922 and *S. pneumonia* 49618. The above findings are in agreement with the previous study, in which the author had

reported synergism between plant extract and antibiotics against Gram positive and Gram negative bacteria ³⁰.

The plant extracts have shown higher activity index values against almost all tested bacteria strains in CIP and S which means that the extracts had good activity compared to those standard antibiotics. The high activity indices of the crude extracts is an indication of more promising therapeutic activity of the plants ³¹.

CONCLUSION

Leaves of *O. suave* showed a great potential activity in the inhibition of several bacterial strains and this should be fully explored in proper approach. Indeed, the aqueous crude of *O. suave* leaves had a great potential to inhibit Gram positives as well as Gram negatives bacteria. Further studies on other parts of the plant are recommended for better conclusive recommendations. In addition, antimicrobial analyses should be conducted on other species of bacteria and eventually on fungi strains.

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Declaration of interest statement

The authors have no conflicts of interest to declare.

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