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SHORT COMMUNICATION

In Vitro α-Amylase Inhibitory and Antimicrobial Potential of *Rauvolfia media* Extracts

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Abstract

Background: Medicinal plants have long been used to treat diabetes, microbial infections, and more. *Rauvolfia media* is traditionally known for enhancing male sexual and reproductive health. **Aim:** To investigate, for the first time, the antimicrobial potential, effects on glucose utilisation in the L6 myoblast cell line, and inhibitory action of *R. media* bark and leaf extracts on α-amylase activity through *in vitro* methods. **Methods:** The disc diffusion method was employed to assess the antimicrobial effects of acetonic, acetic acid, methanolic, and aqueous extracts of *R. media* bark and leaves on *Escherichia coli, Serratia marcescens,* and *Bacillus subtilis*. Among these, *B. subtilis* was most sensitive to all extracts. Methanol extracts exhibited maximum antimicrobial activity against *S. marcescens* at 500 µg/ml, with a Minimum Inhibitory Concentration (MIC) of 100 µg/ml. Acetic acid extracts, however, were ineffective against all tested microorganisms. Acetone, acetic acid, and aqueous extracts of the leaves and bark were particularly effective against *E. coli*, with MICs ranging from 25 to 50 µg/ml. **Results:** The study demonstrated that *R. media* bark and leaf extracts possess potential for treating diseases caused by human pathogenic bacteria. Leaf extracts showed superior α-amylase inhibition compared to bark extracts. **Conclusion:** The antimicrobial activity observed provides scientific validation for the traditional use of *R. media* against infectious gram-negative bacteria. These findings highlight its potential as a source for developing drugs to treat various diseases. Additionally, *in vitro* tests suggest that *R. media* extracts regulate and prevent postprandial hyperglycemia by inhibiting α-amylase and enhancing glucose uptake in the L6 cell line, showing superior efficacy compared to the standard drug, metformin.

Major Findings: *Rauvolfia media* bark and leaf extracts exhibited antimicrobial activity, particularly against *Escherichia coli* and *Bacillus subtilis*. The leaf extracts showed stronger α-amylase inhibition, indicating potential for managing postprandial hyperglycemia.

Keywords: Alpha-Amylase, Antidiabetic, Antimicrobial Activity, Glucose Uptake, Inhibition, *Rauvolfia media*

1. Introduction

The usage of medicinal plants to treat various human diseases dates back hundreds of years, making them a gift from nature to humankind[1]. Secondary plant metabolites (phytochemicals), formerly with unknown pharmacological activity, have undergone substantial research as a source of therapeutic medicines in recent years[2-4]. In light of this, it is envisaged that phytochemicals with sufficient antibacterial efficiency would be used for the treatment of bacterial infections[5,6] based on their mechanisms of action, such as chemotherapeutic, bacteriostatic, bactericidal, and antimicrobial[7].

The main advantages of using medications made from plants are that they are generally safer than synthetic alternatives, provide considerable therapeutic

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benefits, and are more cost-effective[8-10]. Antibiotic-resistant disease agents and illnesses, which were once assumed to be under the control of antibiotics[11], are now resurfacing[12-14]. Epidemics caused by these drug-resistant microorganisms are now a common occurrence around the world, posing serious public health concerns because multi-drug-resistant bacterial strains are significantly reducing the effectiveness of current medications and leading to the failure of infection treatment[15-17].

Similarly, diabetes mellitus, which is characterized by persistently increased blood sugar levels, or hyperglycemia, is a substantial cause of mortality. This problem results from either the pancreas producing insufficient insulin or the insulin produced not being able to effectively control blood glucose levels[18]. Targeting the carbohydrate-hydrolyzing enzymes α-amylase, α-glucosidase, and α-galactosidase, respectively, is an intriguing method to reduce postprandial hyperglycemia[19,20].

There are possibilities for the use of *R. media* (*Apocynaceae*) to treat hypertension, insomnia, diarrhoeas and dysenteries as a traditional medicinal plant; headaches, fevers and malaria, pneumonia and asthma, and immune deficiencies as well due to its broad pharmacological spectrum.[21]. Several doctors utilize this plant to calm down paranoid or schizophrenic patients[22-24]. The antioxidant[25], memory-improving[26,27], analgesic[28], anti-inflammatory[29], anti-tumor[30,31], anti-microbial and antiproliferative[32], hypoglycemic[33,34] and hypolipidemic[35] properties of *Rauvolfia sp.* are well documented. A systematic analysis was conducted to check the possible antimicrobial and anti-hyperglycemic activity of *R. media* in light of the enormous potential of plants as sources of novel drugs.

In this context, the main objective was firstly, to investigate the antibacterial efficacy of *R. media* leaf and bark extracts in various solvents, including acetone, methanol, acetic acid, and water, against human pathogenic bacteria such as *B. subtilis*, *S. marcescens*, and *E. coli* and secondly, to enhance the antidiabetic potential by inhibition of α-amylase.

2. Materials and Methods

2.1 Plant Material Collection and Extraction

The plant material was found in a wild population located on the *Mahajanga* hills of Madagascar. Bark and leaf samples were washed with care to remove any impurities, and put in the shade at 30°C till dry. Subsequently, they were powdered. The powdered plant material was stored in airtight jars at room temperature under vacuum before extraction. 25 g of the powder was loaded into the Soxhlet apparatus with 50 ml of each solvent (acetone, methanol and acetic acid), plus distilled water for extraction for a period not exceeding 72 h. The obtained extract was then concentrated using a rotary evaporator under reduced pressure. The samples obtained were stored in dark-coloured glass bottles at a cool place (around 4°C) and taken for analysis.

2.2 Determination of Antibacterial Activity by Disc Diffusion Method

The antibacterial activity of every extract was evaluated by the disc diffusion technique[36]. The bacterial strains were first incubated in a nutrient broth for 18 h. Then 200 µL of standardized bacterial suspensions were inoculated on MHA. About 20 µL of varied crude bark and leaf extracts were impregnated on sterile disc paper with a 6 mm diameter. The plates were then maintained in a sterile workstation for 30 min to allow the sample extract to be absorbed. Ciprofloxacin, 50 µg, was the positive control for both *S. marcescens* and *B. subtilis*[37], while that for *E. coli* was Amikacin, 50 µg, against which the activity of the test substance was to be benchmarked. The inhibition zones were measured in millimetres after overnight incubation of the plates at 37°C. The various extracts against test organisms were screened in triplicate. The inhibition zones, an indication of antibacterial activity, were measured and recorded using Vernier calipers.

2.3 Determination of MIC of the Effective Plant Extract

The MIC refers to the smallest amount of antimicrobial agent that suppresses microorganism growth after 24 h incubation. To determine the MIC by the disk diffusion method and assess the potency of plant extracts previously screened to exhibit very strong activity at 500 µg/ml, further testing of the most potent extracts was done[38]. Different concentrations of these effective extracts (0, 25, 50, 100, 250, and 500 µg/ml) were prepared by dissolving 10 mg in 25 ml of acetone, acetic acid, methanol, and distilled water. Sterilized solutions were impregnated onto sterilized Whatman filter paper

discs of 6 mm diameter. The bacterial strains grew in Mueller-Hinton agar, and the prepared filter paper discs had different concentrations of the plant extracts laid on the agar plates. The latter were kept at 5°C in the refrigerator for 2 h and then incubated at 35°C for 24 h. After incubation, the inhibition zones were measured in millimetres using Vernier calipers to determine the efficiency of the plant extracts at each concentration.

2.4 α-Amylase Inhibition Assay

The α-amylase inhibitory activity of *R. media* bark and leaf extracts, as well as Acarbose, was assessed with different extract concentrations of 1–4 mg/ml, using 5% DMSO. The reaction mixture was made up by the addition of 20 µl of α-amylase to 200 µl of 0.02 M sodium phosphate buffer and incubating it at room temperature for 10 min[39,40]. Thereafter, to this protein solution, 200 µl of 1% w/v starch solution followed by another incubation of 10 min. The reaction in the mixture was then stopped by the addition of 400 µl of the colour reagent 3,5-dinitrosalicylic acid, DNSA, and then heated for 5 min. The absorbance was read at 540 nm against a blank. The percentage of α-amylase inhibition against the extract concentration was plotted, and IC_{50} values were derived from the graph. The formula given was used to retrieve the inhibition percentage:

$$Percentage\ of\ inhibition = \frac{(Abs\ control\ -\ Abs\ extract)}{Abs\ control} \times 100$$

2.5 Glucose Utilization Assay

Determination of glucose uptake in L6 myoblast cells[41,42]. L6 cells were plated in 96-well culture plates at a density of 3000 cells per well and allowed to attach until 90% confluent. Two rows without cells were set up as a negative control for interference in the glucose uptake assay. When the cells had reached confluency, they were maintained in DMEM containing 2% FBS for an additional 5 days. The culture medium was replaced two days before conducting the glucose uptake experiment. The experiment was conducted by adding plant extract to multiple wells at different concentrations (1–4 mg/ml). In one column, metformin was added instead of the plant extract as a positive control at the concentrations: 1–4 mg/ml. In the cell cultures with the presence of either the plant extract or metformin, further incubation lowed for 48 h. Subsequent to the incubation period, 25 µL of the incubation buffer consisting of RPMI medium with dilution in PBS, 0.1% BSA, and different glucose amounts ranging from 1 to 4 mM were added to each well. Later on, the previous step also followed and again the cells were incubated at 37°C for three more hours. After which, the sample of the incubation medium from each well was further added to a new 96-well plate in duplicates using 5 µL. Following this, 200 µL of glucose oxidase reagent (SERA-PAK Plus, Bayer) was added to each well to assay their glucose content. Absorbance was then read on a Multiscan MS microplate reader from Lab Systems at 520 nm following incubation of the plate at 37°C for 15 min[43]. The glucose uptake was calculated by comparing the test samples to cell-free controls and expressed as a percentage of the untreated controls.

2.6 Statistical Analysis

A one-way ANOVA was conducted for data obtained from triplicates using R code software with a significance level of $p<0.05$.

3. Results and Discussion

Due to improper use, pathogens have gained mutations and antibiotic resistance. As a result, several researchers are looking for alternative medical treatments. By using the disc diffusion method, the antibacterial potential of crude *R. media* bark and leaf extract was examined in the current study against Gram-positive (*B. subtilis*) and Gram-negative (*S. marcescens* and *E. coli*) bacterial strains. The outcomes showed that the plant extracts had varying degrees of potential efficacy in inhibiting microbial development.

Methanol, acetone, and water were deemed to be the best extraction solvents for the active plant based on their antimicrobial activity. At 500 µg/ml, *R. media* bark and leaf extracts demonstrated significant antimicrobial activity against the bacterial strains.

Serratia marcescens showed high resistance to methanolic, acetic acid and aqueous extracts from bark (BMetOH, BAcAc, BH_2O) as well to acetonic and aqueous extracts from leaves (LAc and LH_2O), none of which displayed a zone of inhibition. The diameter of the inhibition zones was examined after the growth

was inhibited by the acetonic bark (7.0 ± 1.00 mm), methanolic (6.7 ± 0.59 mm), and acetic acid leaf extracts (7.5 ± 0.71 mm). The test bacteria *B. subtilis* showed decreased growth when exposed to methanolic leaf extracts (10.0 ± 0.45 mm), acetic acid leaf extracts (9.3 ± 1.16 mm), and acetonic extracts of the leaves (7.3 ± 0.84 mm), in that order. With a zone of inhibition of 35.0 ± 1.10 mm and 36.5 ± 0.72 mm, respectively, barks and leaves acetic acid (BAcAc, LAcAc) extracts were the most efficient extracts at slowing *E. coli* microbiological growth, whereas acetonic barks (BAC) extracts had the lower inhibitory effect with 14.8 ± 2.54 mm of the Zone Of Inhibition (ZOI) (Figure 1).

Moreover, the measured ZOI for the positive control ciprofloxacin (50 µg/ml) was 28.3 ± 0.71 mm against *B. subtilis* and 38.7 ± 1.41 mm against *S. marcescens*, while the measured ZOI for amikacin was 40.7 ± 0.20 mm against *E. coli*. Table 1 displays the antibacterial activity in terms of zone of inhibition.

Results of the antimicrobial activity of *R. media* bark and leaf extracts indicated that *S. marcescens* was the most resistant microorganism to the plant extracts, followed by *B. subtilis*, while *E. coli* was the most susceptible strain. From Table 2, it can be seen that the MIC results indicated all microorganisms were very sensitive to the *R. media* extracts at a concentration of 25 µg/ml, except for *B. subtilis*, which had the same with methanolic bark extracts at an MIC of 500 µg/ml and an MIC of 250 µg/ml with aqueous bark extracts. The MIC isolated for *E. coli* was 50 µg/ml with aqueous leaf extracts and a MIC of 100 µg/ml with methanolic extracts from both the bark and leaves. On the contrary, *B. subtilis* was resistant to the MIC of acetonic and acetic acid bark extracts alongside aqueous leaf extracts.

Using the disc diffusion method, *R. media* bark and leaf extracts were examined to determine their antibacterial activity against two Gram-negative bacteria (*E. coli* and *S. marcescens)*, and one Gram-positive bacterium (*B. subtilis)*. The bioactivities exhibited by plant extracts are mainly due to their secondary metabolites. Effective inhibition of these bacteria by the extracts is noteworthy, especially against the backdrop of increasing resistance by bacteria to conventional antibiotics[43,44].

Studies have shown that the extracts of *R. serpentina* were more efficient against Gram-positive bacteria than Gram-negative bacteria. Gram-positive bacteria possess an outer layer made of peptidoglycan, which is a poorly protective cell wall that makes them easily vulnerable to antibacterial agents[45,46].

Bacteria resistant to multiple antibiotics are on the rise. These often lead to inadequate response to treatment and an increase in expenditure incurred on health because of such treatment failures[47,48]. The

Table 1. Antibacterial activity (zone of inhibition) of *R. media* barks and leaves extracts

Zone of inhibition (mm) (mean ± SD)			
Solvents	***B. subtilis***	***S. marcescens***	***E. coli***
Barks			
Acetone	NZ	7.0 ± 1.00	14.8 ± 2.54*
Methanol	6.0 ± 0.45	NZ	24.3 ± 1.68*
Acetic acid	NZ	NZ	35.0 ± 1.10
Distilled water	7.0 ± 0.71	NZ	26.7 ± 1.40*
Leaves			
Acetone	7.3 ± 0.84	NZ	22.3 ± 3.42*
Methanol	10.0 ± 0.45	6.7 ± 0.59	17.3 ± 0.21*
Acetic acid	9.3 ± 1.16	7.5 ± 0.71	36.5 ± 0.72
Distilled water	NZ	NZ	25.3 ± 1.68*
Ciprofloxacin (50 µg)	28.3 ± 0.71	38.7 ± 1.41	-
Amikacin (30 µg)	-	-	40.7 ± 0.20

All values are expressed as mean ± standard deviation. A statistically significant difference in the means was considered at $p < 0.05$. "NZ" indicates no zone of inhibition.

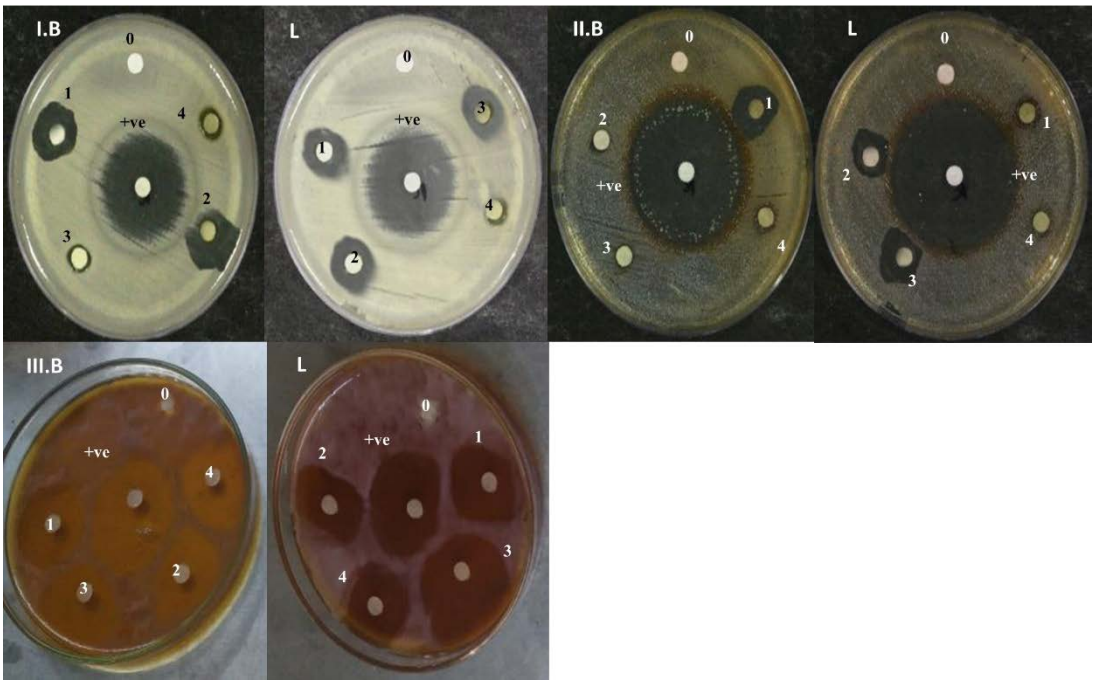


Figure 1. Disc diffusion assay of *R. media* **(B)** Barks and **(L)** Leaves extracts, **(0)** negative control; with 10% v/v DMSO, **(1)** Acetone (Ac), **(2)** Methanol (MetOH), **(3)** Acetic acid (Ac. Ac), **(4)** Distilled water (H_2O) compared to the standard **+ve (I)**, **+ve (II)** Ciprofloxacin, **+ve (III)** Amikacin, against **(I)** *B. subtilis* (BS), **(II)** *S. marcescens* (SM) and **(III)** *E. coli* (EC).

Table 2. Minimum Inhibitory Concentration (µg/ml) of *R. media* crude extracts against *B. subtilis* and *E. coli*

MIC		
Solvents	***B. subtilis***	***E. coli***
Barks		
Acetone	-	25 µg/ml
Methanol	500 µg/ml	100 µg/ml
Acetic acid	-	25 µg/ml
Distilled water	250 µg/ml	25 µg/ml
Leaves		
Acetone	25 µg/ml	25 µg/ml
Methanol	25 µg/ml	100 µg/ml
Acetic acid	25 µg/ml	25 µg/ml
Distilled water	-	50 µg/ml

rising threat has, therefore, underlined the need for new effective bioactive compounds derived from plants[49,50]. Herbs and their essential oils have been recognized for their varying levels of antibacterial activity since historic times[51,52].

The current investigation into the extracts' potential antibacterial activity against *S. marcescens*, *B. subtilis*, and *E. coli*, with very encouraging results. Broad-spectrum activity of the extracts against all the microorganisms was evidenced, except that *S. marcescens* was most resistant, as is usual for Gram-negative bacteria. Compared to this, *E. coli* was found to be more susceptible.

The tested acarbose is an oral hypoglycemic agent whose inhibitory activity is known for the action of the alpha-amylase and α-glucosidase of enzymes from this class. According to Table 3, at 4 mg/ml concentration, the acetonic extract of the bark has 87.83% inhibition and IC_{50} of 2.277 ± 0.01, and the acetic acid fraction of the leaf also has 87.55% inhibition and IC_{50} of 2.284 ± 0.001, *R. media* had good inhibitory activity, especially compared to the acetic acid extract of the bark, showing the same pattern. The inhibition was found to be 99% for standard acarbose, and its IC_{50} was established as 2.218 ± 0.25 mg/ml. Statistically, it was noted that there was no significant difference between Acarbose and the extracts. The leaf extracts generally performed better than the bark extract.

The worth of the research in medicinal plants with antidiabetics has progressively increased with

a world that is searching for effective treatments against diabetes. On the other hand, the World Health Organization recommends regular physical activity and healthy eating as a means of effectively managing type II diabetes[53-55], but the opportunity also exists in the promotion of greens with antidiabetic and antioxidant properties as a way of encouraging healthier urban living through the daily diet. In the current work, a new approach is presented for the α-amylase activity inhibition mechanism. During the postprandial phase, inhibition of carbohydrate-metabolizing enzymes, such as α-glucosidase and α-amylase, is necessary to reduce postprandial hyperglycemia[56]. On the other hand, α-amylase metabolizes starch to maltose, which increases postprandial hyperglycemia. α-Glucosidases are enzymes that metabolize oligo- and disaccharides to an absorbable monosaccharide form of carbohydrate in the small intestine. Reducing blood glucose levels can be affected. Figure 2 shows the inhibitions of α-amylase activity and acarbose of the various tested extracts. All the substances tested showed dose-dependent inhibition. The acetonic and acetic acid extracts of the bark of *R. media*, with IC_{50} values of 2.277 ± 0.01 mg/ml and 2.284 ± 0.001 mg/ml, respectively, and acarbose with IC_{50} of 2.218 ± 0.25 mg/ml, were more potent than other substances tested. α-Amylase was inhibited by all crude extracts in a dose-dependent manner[57,58].

Acarbose is a member of the oral hypoglycemics' α-glucosidase inhibitor class and is well-known for inhibiting both alpha-amylase and α-glucosidase. Acarbose was utilized as a positive control as a result. From the data obtained (Table 3), at 4 mg/ml, it was found that acetonic extract of the bark (87.83% with an IC_{50} of 2.277 ± 0.01) and acetic acid fraction of the leaf (87.55%, IC_{50} of 2.284 ± 0.001) of *R. media* showed significant inhibitory activity when compared with acetic acid extract of the bark (Figure 3). The standard acarbose showed 99% inhibitory effects on the α-amylase activity with an IC_{50}= 2.218 ± 0.25 mg/ml.

L6 cell cultures, which were, in turn, used for the *in vitro* evaluation of glucose uptake were used; the outcomes are displayed in Figure 2 and Table 4. The study shows that *R. media* bark and leaf extracts caused a significant increase in glucose uptake at a glucose concentration of 20 mM. The acetonic bark extract, increased glucose uptake by 84%, and the aqueous leaf extract, which reached a 78% increase, both at the dose of 4 mg/ml. Metformin taken orally caused glucose uptake to jump by 96% at a concentration of 100 **µg**/ml. A comparison made by one-way ANOVA and post hoc tests pointed out no notable differences between the acetonic and aqueous bark extracts besides the aqueous leaf extract, that were additionally investigated in the study than the standard drug metformin.

This study of glucose uptake in L6 cells showed that *R. media* extracts increased glucose uptake significantly higher than metformin. This directly impinges that the crude extracts of *R. media* act like metformin in

Table 3. α - Amylase inhibition of barks and leaves extracts of *R. media*

	% inhibition				p-value	
	1 mg/ml	2 mg/ml	3 mg/ml	4 mg/ml	Within group	Between group
Barks						
Acetone	25.92%	47.85%	65.62%	87.83%	$2.18e^{-10}$*	<0.00001*
Methanol	48.58%	62.64%	72.72%	79.01%	$6.80e^{-18}$*	
Acetic acid	35.36 %	37.43%	39.52%	41.76%	$3.22e^{-23}$*	
Distilled water	7.97%	19.87%	29.91%	42.81%	$1.18e^{-22}$*	
Leaves						
Acetone	8.67%	38.54%	62.82%	83.53%	$1.40e^{-12}$*	<0.00001*
Methanol	54.53%	59.29%	68.14%	69.67%	$1.66e^{-17}$*	
Acetic acid	57.82%	68.21%	74.33%	87.55%	$6.92e^{-11}$*	
Distilled water	51.84%	61.14%	65.06%	66.14%	5.20e-^{16}*	
Acarbose	18.78%	45.86%	72.30%	90.17%		

All values are presented as mean ± standard deviation. A statistically significant difference in means was observed, with $p < 0.00001$.

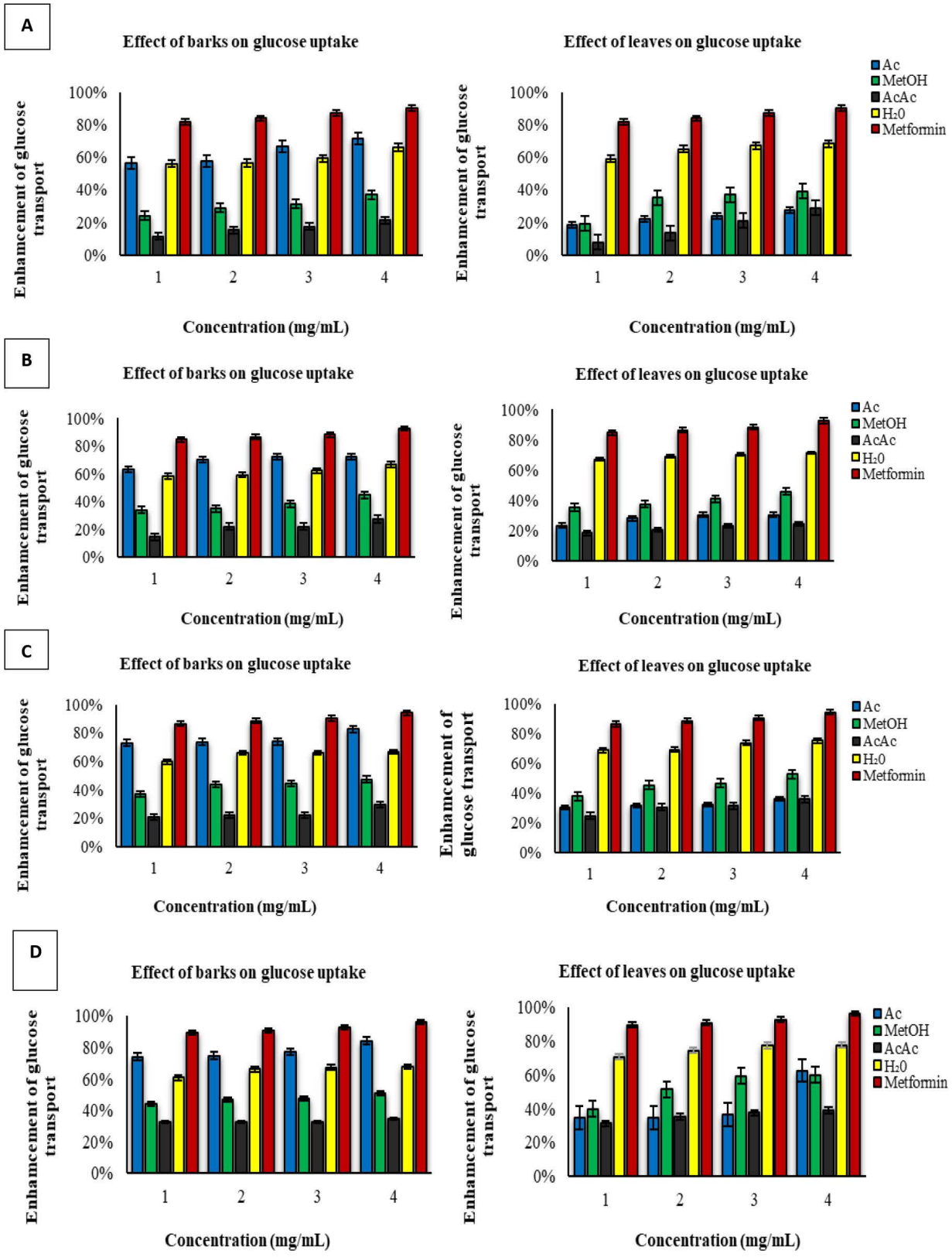


Figure 2. Effect of different concentrations of *R. media* barks and leaves extracts on glucose uptake at **(A)** 5mM, **(B)** 10Mm, **(C)** 15mM, **(D)** 20 mM, compared with Metformin (standard). Each assay was done in triplicate. Results are expressed in mean ± SD.

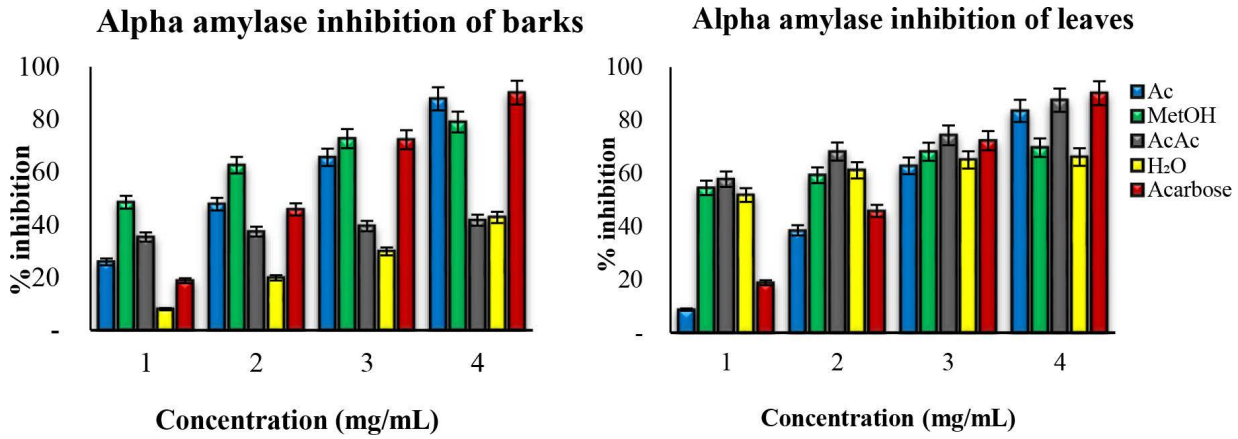


Figure 3. α-amylase inhibitory activity of *R. media* barks and leaves extracts in acetone (Ac), methanol (MetOH), acetic acid (AcAc) and distilled water (H_2O), compared with Acarbose used as standard.

Table 4. ANOVA analysis of different extracts of the barks and leaves on glucose uptake:

Extracts Within group		p-value	
		Between-group	
Barks	Acetone	0.4892	<0.05*
	Methanol	0.0049*	
	Acetic acid	0.0039*	
	Distilled water	0.7211	
Leaves	Acetone	0.0015*	
	Methanol	0.0053*	
	Acetic acid	0.0016*	
	Distilled water	0.7587	
Standard	Metformin		

All values are presented as the mean ± standard deviation. A p-value of $p < 0.05$ was considered statistically significant, indicating a meaningful difference between group means.

their ability to enhance glucose uptake in the liver. Metformin is an oral hypoglycemic agent and falls under Biguanides. Its major blood sugar-lowering effect is attributed to the activation of AMPK in the liver, which may give rise to various pharmacological effects, including inhibition of the production of glucose and lipids and improved hepatic insulin sensitivity[59-62].

4. Conclusion

This study appears to be the first attempt to determine the antibacterial activities of *R. media* under *in vitro* conditions. The results indicated the highest concentration of antibacterial compounds in the methanolic and acetic acid extracts of the bark and leaves among the four extracting solvents tested, viz., acetone, acetic acid, methanol, and water. This plant possesses the marked antibacterial capacity to be crucial in the formulation of new medicinal drugs in the near future. While this discovery opens up the possibility of developing new drugs, it would also highlight the role of native plants in the management of bacterial infections.

Studies from the laboratory indicated the application of the plants in combating bacterial infections. *In vitro* techniques that can be used to screen plants for antidiabetic activity include measurement of glucose uptake, inhibition of enzymes such as α-glucosidase and α-amylase, and measurement of an organism's ability to absorb glucose. The inhibitions of the α-amylase enzyme by the leaf extracts were found to be different.

The results thus suggest further exploration with the view to developing the extracts from the bark and leaves of *R. media* as potential sources of new, effective antibacterial and antidiabetic use agent products. The study posits that the presence of phytochemicals, flavonoids, stilbenes, triterpenoids, phenols, alkaloids, coumarins, and other classes of compounds in these plants could be responsible for these inhibitory actions, which are very useful as herbal bio-active compounds. Further studies are necessary to become familiar with their physiological, pharmacological, and clinical activities against various diseases. Their structure must be characterized at the molecular level;

chromatographic techniques are very helpful in the isolation and partial purification of active principles. The antimicrobial molecules must be elaborated upon in detail not only for their forthcoming application but also for their chemical constitution. Further *in vitro*, *ex vivo*, and *in vivo* studies will need to be conducted to further confirm these plant components for antidiabetic activity.

5. Author Contribution

Conceptualization, E.R. and R.C; methodology, E.R. and R.C; writing—original draft preparation, E.R. and R.C; writing—review and editing, E.R. and I.S.; supervision, E.R and I.S. All authors have read and agreed to the published version of the manuscript.

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