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Research Paper

Phytochemical screening of the antimicrobial fraction of *Solanum indicum* L. berries extract and evaluation of its effect against the survival of bacteria pathogens of plants

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Abstract. In this study, the effect of the antimicrobial fraction of *Solanum indicum* L. berries extract on growth and the survival of three species of bacteria pathogens of plants (*Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Pseudomonas syringae*) was evaluated. The inhibitory activity of thisantimicrobial fraction was observed on the growth of the three species tested. However, only a total inhibition of the growth of *Pseudomonas syringae* was observed. This antimicrobial fraction possessed also the capacity of causing the death of the cells of this species. Indeed, the percentage in reduction of Alamar blue indicating cell viability which was 100% in the medium without antimicrobial fraction decreased to reach the value of 5.08% in the medium at 2% of antimicrobial fraction for this species after five days of incubation. At this same concentration, only about 50 % of the cells were still alive after five days of incubation for *P. aeruginosa* and *P. fluorescens*. The phytochemical screening of the antimicrobial fraction revealed compounds including flavonoids, carotenoids and saponins. The Thin Layer Chromatography analysis on this antimicrobial fraction showed Rf values of 0.28, 0.38 and 0.95. This antimicrobial fraction could thus be used as natural substance to prevent infection of plants and crops by bacteria pathogens and also as preservative in food and feed as these berries have been ingested by humans for quite some time without any toxic effect noted.

Keywords: *Solanum indicum* L., berries, bacteria species, surviving cells, antimicrobial fraction.

INTRODUCTION

The nature has provided abundant plant health for all living creatures, which possess various properties (Bhatti et al., 1998). The important values of some plants have long been published but a large number of them remain are yet unexplored. In West Africa, about 350 species have been listed and described by Baumer (1995). Among these plants, there is *Solanum indicum* L. a wild plant widespread in tropical and temperate zones. It belongs to the family of *Solanaceae* and the genus *Solanum,* with more than 1,700 species. The fruits are berries used for culinary purposes in many parts of Africa where they are used as nutritious vegetables as they contain appreciable amounts of starch, calcium, vitamin A, ascorbic acid and phosphate (Bahgat et al., 2008). In addition to components mentioned earlier, these berries have been shown to contain polyphenols (N'dir et al., 2010) and steroidal glycosides (Ripperger and Himmelreich, 1994; Honbu et al., 2002). However, the use of this species has not limited to food. Indeed, *Solanum indicum* L. seeds, roots, leaves and berries are

used therapeutically for asthma, dry cough, chronic febrile afflictions and in dysuria. The berries have been suggested useful in leucoderma, pruritis and bronchistis and they have been claimed in folk medicine to have an antihypertensive effect (Rubaihayo, 1995). It has also been used in Chinese folk medicine as anti-inflammatory and wound-healing agents; as an analgesic, and for the treatment of rhinitis, cough and breast cancer (Syu et al., 2001). In West Africa, these berries are used in most of the cases as an additive for the treatment of some diseases. Indeed, in Nigeria, they are used as a laxative and digestive. In Ivory Coast, berries soup is used as an additive in the treatment of malaria. All these uses are not based on scientific studies but rather on empirical practices. Whether these berries are effective in treating any of these diseases, their use as food and medicine indicates that they have been ingested by humans for quite some time at many dosages. Furthermore, it was noted that in an area such as Ivory Coast, where climatic conditions are favorable to the infection of vegetables products by microorganisms and insects, these berries of *S. indicum* L. whatever their maturity stage, seem to resist to all infections, while for the other species belonging to the same genus, fruits are infected. Previous investigations have shown that the berries of this wild plant possess antimicrobial activities (Kouadio et al., 2011).

However, the effect of these berries extract on the survival of the bacteria is not known yet. The discovery of natural substances capable of inhibiting and killing bacteria species among which some are pathogens for plants is of a great importance (Davidson 2001). Thus, the present work was carried out in order to evaluate the action of the antimicrobial fraction of *S. indicum* L. berries extract against the survival of bacteria pathogens of plants for a contribution for the research in alternative in chemical substances for prevention of crops' rottenness. The phytochemical screening of this antimicrobial fraction was also done in order to identify its components.

MATERIALS AND METHODS

Biological material

In this study, red berries of *S. indicum* L. were used. These berries were collected from rural zones of the central part of Ivory Coast. Three species of bacteria pathogens of plants (*Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Pseudomonas syringae*) from the Pasteur Institute of Ivory Coast were also used. The bacterial culture medium used was the Brain Heart Infusion (BHI) broth.

Berries extract preparation

Red fresh berries of *S. indicum* L. were grinded and 30 g of the obtained homogenate were added to 150 ml of 100 % ethanol. The mixture was boiled in water bath at 80°C for 1 h under gentle stirring. The resulting mixture was centrifuged at 1500 rpm for 5 min. The supernatant was then filtered through Whatman paper of 11 cm of diameter and 1.2 µm of pore size. The resulting solution was evaporated to dryness under Fume Hood. The residue obtained of 1.5 g was dissolved into 15 ml of boiled distilled water and shaken until total dissolution. In order to purify the homogenate obtained and used the fraction containing the antimicrobial compounds, the method of purification by ethyl acetate was used. This purification of the extract was made by adding to the homogenate obtained, 15 ml of ethyl acetate. The resulting mixture was shaken during 1 min and centrifuged at 2000 rpm for 10 min. Aqueous and ethyl acetate phases were obtained. The ethyl acetate phase was recovered into a new tube. To the remaining aqueous phase, 15 ml of ethyl acetate were added again, shaken and centrifuged as described above. This purification was done three times.

The three ethyl acetate phases were put into the same tube and the aqueous phase into another tube and then, these two solutions obtained were dried under Fume Hood. The residues of the aqueous and ethyl acetate phases were dissolved respectively into 15 ml of distilled water and 15 ml of ethyl acetate and then filtrated separately onto 0.20 µm cutoff membranes to eliminate residues which were not dissolved and eventual contaminants. These aqueous and ethyl acetate fractions were evaluated for their inhibitory effect onthe three species of bacteria (*P. aeruginosa*, *P. fluorescens* and *P. syringae*).

Preparation of the tested species

A quantity of 1 ml of each bacterium species previously stored in glycerol 15 % at -20°C was thawed in 9 ml of Brain Heart Infusion (BHI). The obtained suspension was firstly incubated at 30°C for 8 h. In a second step, 1 ml of the microbial suspension obtained after 8 hours of incubation was put in 9 ml of BHI. The whole was incubated at 30°C overnight. The absorbance of this second culture was measured with a spectrophotometer at 630 nm. The optical density was adjusted at 0.6 by diluting and the microbial suspension was cultured. Afterwards, 1 ml of a second crop was put in 9 ml of Brain Heart Infusion (BHI). This microbial suspension obtained was used for different investigations.

Evaluation of the antimicrobial activity of the fractions obtained after purification of the berries extract by the ethyl acetate method

The method used was that of CLSI, 1999. Indeed, three solutions (Solution of BHI alone, solution of BHI at 1% of

ethyl acetate fraction and solution of Brain Heart Infusion (BHI) at 1% of aqueous fraction) of 300 µl each were placed in separate wells of the micro-plate without the bacteria strain. Each solution was placed in at least three wells of the micro-plate. In a second step, the microbial strain tested (30 µl) was cultured in other separate wells of the micro-plate contained the different solutions (270 µl each) mentioned above. These cultures were made in at least three wells of the micro-plate. The seeded plate was cultured in the Bioscreen apparatus at 30°C and at a wavelength of 600 nm for 24 h.

The measurement of the optical density expressing microbial growth was taken every 15 min. The BHI solution containing the fraction of the berries extract in which the inhibition of the bacterial growth was observed, was identified as the solution containing the antimicrobial fraction. On this antimicrobial fraction, various investigations were done. Indeed, this antimicrobial fraction was evaluated for its effect against the survival of the three species tested. The qualitative phytochemical screening was done in order to identify the families of compounds present in this antimicrobial fraction. The Thin Layer Chromatography (TLC) analysis of the antimicrobial fraction was also performed in order to determine the Rf values of these compounds.

Phytochemical studies

The antimicrobial fraction obtained was subjected to various qualitative tests for the identification of constituents like flavonoids, alkaloids, saponins, glycosides and carotenoids.

Test for flavonoids

A few chop of 1 % NH_3 solution was added to 1 ml of the antimicrobial fraction in a test tube. The appearance of yellow coloration shows the presence of flavonoids compound (Andzouana and Mombouli, 2011).

Test for alkaloids

A quantity of 2 ml of Drangendroff's reagent was added to 1 ml of the antimicrobial fraction. The appearance of a turbid orange color shows the presence of alkaloids (Veerachari and Bopaiah, 2011).

Test for carotenoids

A quantity of 1 ml of the antimicrobial fraction was put in a test tube and dried under Fume Hood. A quantity of 10 ml of chloroform was added to the residue obtained and shaken vigorously. The resulting mixture was filtered and 85% sulphuric acid was added. The appearance of a blue color at the interface shows the presence of carotenoids (Ajayi et al., 2011).

Test for saponins

A quantity of 10 ml of the antimicrobial fraction was shaken vigorously, sat aside for 10 min. The appearance of a stable froth shows the presence of saponins (Veerachari and Bopaiah, 2011).

Test for glycosides

To 1 ml of the antimicrobial fraction, 1 ml of $FeCl_3$ reagent (mixture of 1 volume of 5% $FeCl_3$ solution + 99 volume of glacial acetic acid) and a few drops of concentrated H_2SO_4 were added. The appearance of a greenish blue color within few minutes shows the presence of glycosides (Trease and Evans, 1989).

Thin layer chromatography (TLC) analysis of the antimicrobial fraction

A quantity of 20 µl of the antimicrobial fraction obtained after purification, was loaded onto the Thin Layer Chromatography (TLC) silicate gel C18 plate (20 × 20 cm) containing a fluorescence indicator. The spotted TLC plate was run in a TLC chamber including mobile phase for 1 h. The mobile phase was butanol/acetic acid/water (60:20:20). The TLC plate was dried at room temperature for 5 min and then observed under UV fluorescence.

Bioassay analysis

A quantity of 2 ml of each bacterial suspension was put into different tubes aseptically. Into each tube, the antimicrobial fraction was added to obtain concentrations of 0.1, 0.5, 1 and 2%. Medium without antimicrobial fraction was used as control. For each concentration, 3 tubes were used. Then, all the tubes were incubated at 30°C under shaking at 250 rpm. The experiment was conducted over a span of 5 days. After each 24 h of incubation, 700 µl of the medium BHI and 300 µl of Alamar Blue reagent were added into each tube. The final concentration of the Alamar Blue reagent into each test-tube was 10%. Then, the microbial suspension with the Alamar Blue reagent was incubated at 37°C for 4 h. The BHI medium without the bacterial suspension but containing Alamar Blue reagent was also incubated. After the 4 h of incubation, the culture with pink color indicates the presence of surviving cells, while the culture with blue or purple color indicates the death of the bacteria cells. After this incubation time, 100 µl of each suspension was

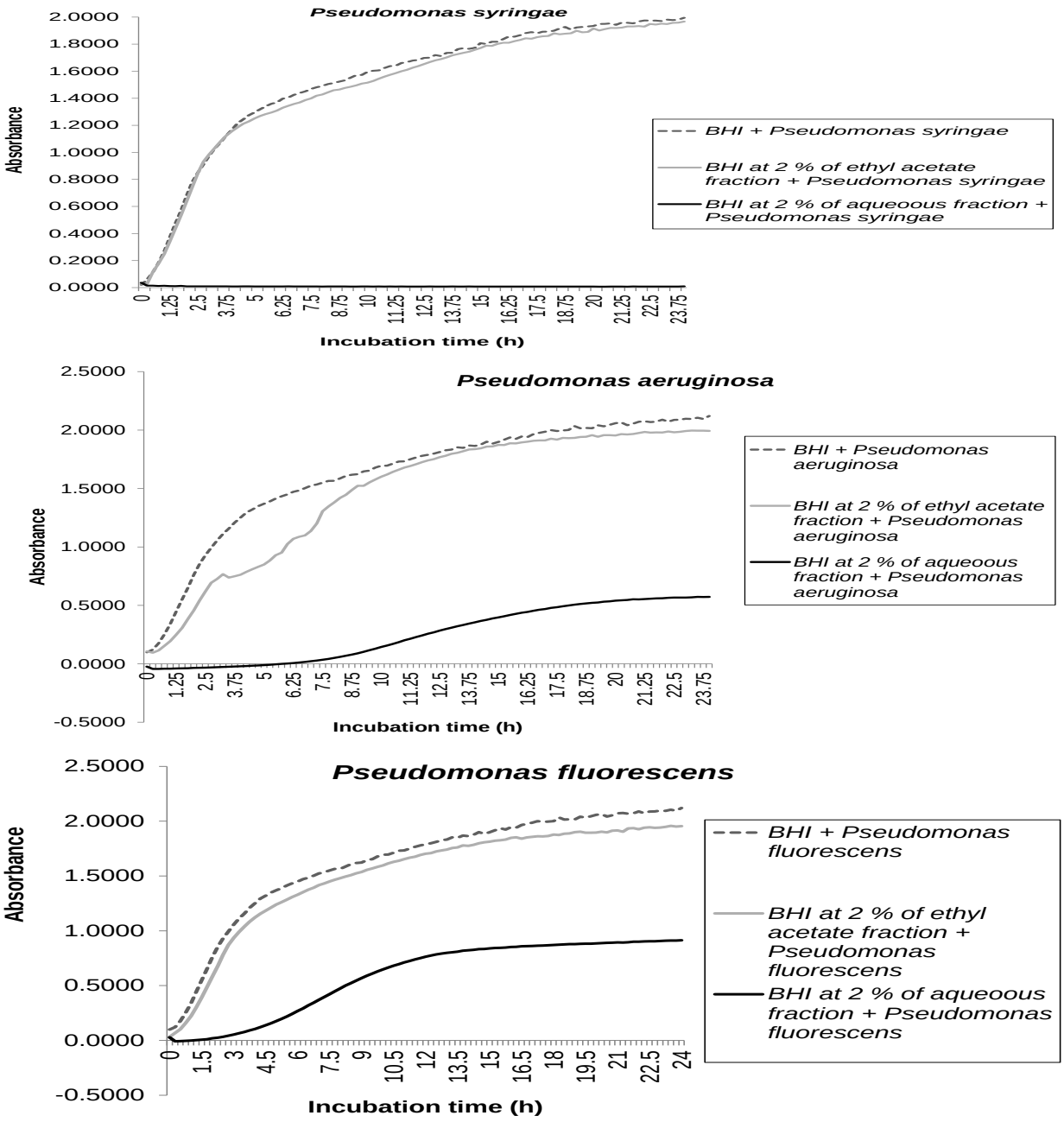

Figure 1. Inhibitory effect of ethyl acetate fraction and aqueous fractionof *Solanum indicum* L. berries extract on growth of *Pseudomonas syringae*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens.*

put into separate wells of a micro-plate and the absorbance was monitored at 570 nm using 600 nm as a reference wavelength in an apparatus Bio-Teck ELISA.

Statistical analysis

The statistical analysis of data was done by Analysis of Variance (ANOVA) using 5% level of significance. The statistical package used is IBM SPSS Statistics version 20. Tukey's Multiple Comparison test was used to identify these differences.

RESULTS

The results showed that the antimicrobial compounds of *S. indicum* berries extract are water-soluble. Indeed, the inhibition of the growth of the species (*P. aeruginosa*, *P. fluorescens* and *P. syringae*) tested was observed only in the medium containing the aqueous fraction (Figure 1). The families of compounds identified in this antimicrobial

Table 1. Phytochemical analysis of the antimicrobial fraction of *Solanum indicum* L. berries extract.

Phytochemical test	Antimicrobial fraction
Test for flavonoids	++
Test for alkanoids	--
Test for saponins	++
Test for glycosides	+
Test for carotenoids	++

++ = strongly present, + = slightly present, -- = absence

Figure 2. Thin Layer Chromatography of the antimicrobial fraction of *Solanum indicum* L. berries extract.

fraction were flavonoids, carotenoids and saponins. A lightly presence of glycosides was also observed (Table 1).

The Thin Layer Chromatography analysis on this antimicrobial fraction showed Rf values of 0.28, 0.38 and 0.95 (Figure 2). The evaluation of the effect of this antimicrobial fraction on the survival of the bacteria species tested showed a decreasing of the surviving cells when the antimicrobial fraction content in the medium increased. Indeed, the evaluation of the percentage in reduction of Alamar blue indicating cell viability which was 100% in the medium without antimicrobial fraction, decreased to reach the values of 12.85% after one day of incubation in the medium at 2% of the antimicrobial fraction (Figure 3) for *P. syringae*. For *P. aeruginosa* and *P. fluorescens*. This decreasing of the surviving cells was influenced significantly by the antimicrobial fraction content in the medium (Table 2). It was also influenced significantly by the incubation time (Table 3). Indeed, from the first to the fifth day of incubation, the percentages in reduction of Alamar Blue mentioned above decreased to reach the values of 73.63, 61.41, 17.80 and 5.08% respectively in the medium at 0.1, 0.5, 1 and 2% of the antimicrobial fraction. However, no significant difference was observed between the percentages of reduced of Alamar Blue from the fourth to the fifth day of incubation except those observed with the culture in the medium at 0.5 % of the antimicrobial fraction (Table 3).

DISCUSSION

In this study, the aqueous fraction obtained after purification of *S. indicum* L. berries extract exhibited a significant inhibition of the growth of the three species tested. The phytochemical analysis of this aqueous fraction representing the antimicrobial fraction showed the presence of flavonoids, carotenoids, glycosides and saponins. The inhibition of the growth of the bacteria strain tested showed the antimicrobial activities of these phytochemical constituents. Previous studies have also reported the importance of the constituents such us saponins in various antibiotics used in treating common pathogenic strains (Kubmarawa, 2007; Mensah, 2008). As the saponins, it has been shown that the antimicrobial properties of propolis have been attributed to its high flavonoids content and in particular the presence of the flavonoids galangin and pinocembrin (Pepeljnjak et al., 1982; Grange and Davey, 1990; Bosio et al., 2000). It has also been shown that quercetin, one of the constituents of flavonoids caused an increase in permeability of the inner bacterial membrane and a dissipation of the membrane potential (Mirzoeva et al., 1997). These authors have also demonstrated that the flavonoids quercetin and naringenin significantly inhibited bacterial motility. Some investigations on carotenoids showed also that, these constituents have potential antimicrobial activities (Yan, 2003; Chen et al., 2008). The results have been confirmed by Zhang et al. (2008) and Tao et al. (2010). In addition to its inhibitory effect on

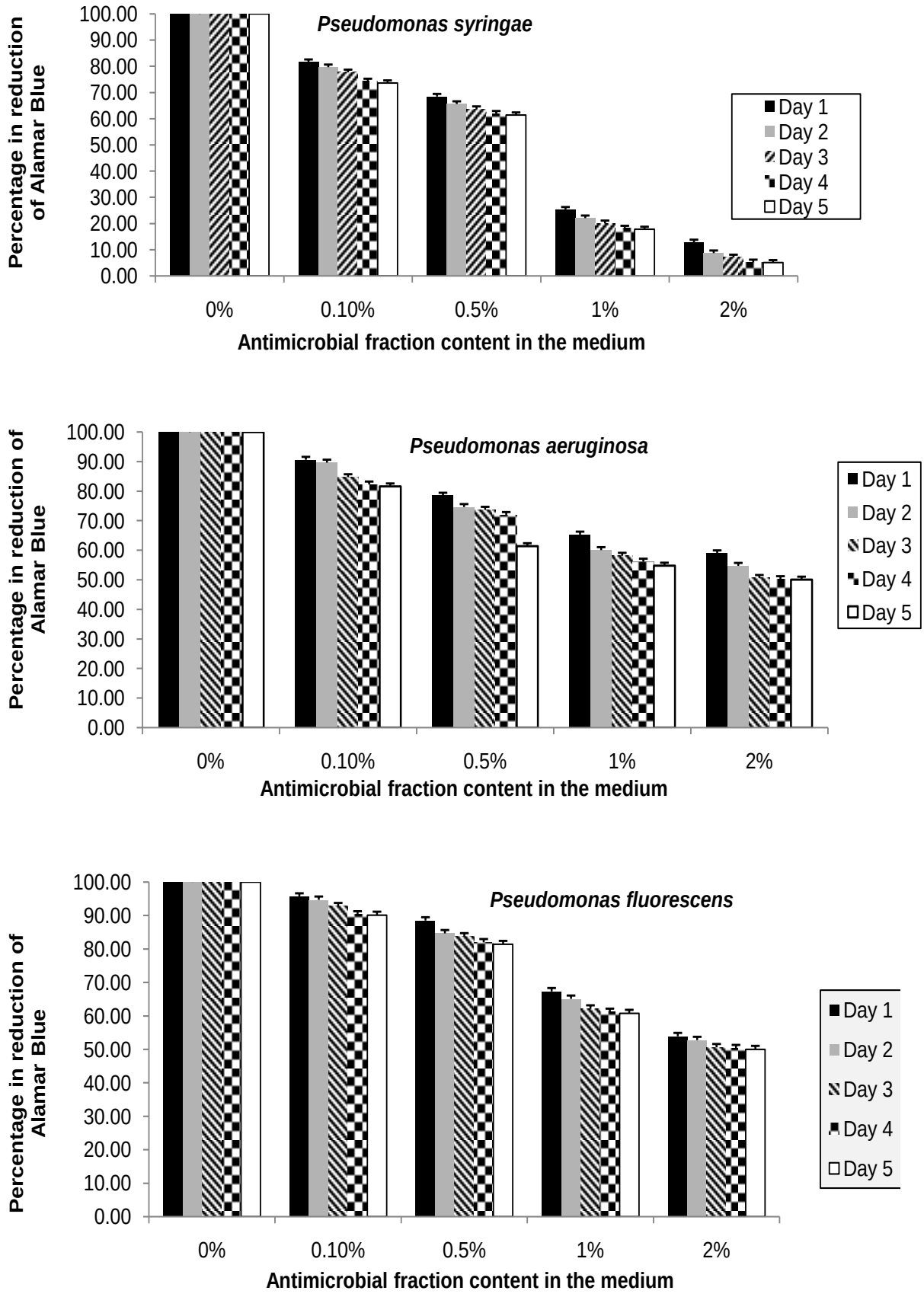

Figure 3. Effect of the antimicrobial fraction of *Solanum indicum* L. berries extract on percentage in reduction of Alamar Blue of *Pseudomonas syringae*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*.

Table 2. Dose-dependent effect of the antimicrobial fraction of *Solanum indicum* L. berries extract on cell viability of *Pseudomonas syringae*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* during 5 days of incubation.

Tukey HSD			Percentage in reduction of Alamar Blue				
Species tested	Antimicrobial fraction content in the medium	N	Subset for Alpha = 0.05				
			1	2	3	4	5
Psuedomonas syringae	Medium at 2% of antimicrobial fraction	3	12.85 – 5.08				
	Medium at 1% of antimicrobial fraction	3		25.31 – 17.80			
	Medium at 0.5% of antimicrobial fraction	3			68.47 – 61.41		
	Medium at 0.1% of antimicrobial fraction	3				81.62 – 73.63	
	Medium without antimicrobial fraction	3					100.00
	Significance		1.000	1.000	1.000	1.000	1.000
Psuedomonas aeruginosa	Medium at 2% of antimicrobial fraction	3	50.97 – 50.08				
	Medium at 1% of antimicrobial fraction	3		65.31 – 54.80			
	Medium at 0.5% of antimicrobial fraction	3			78.47 – 61.41		
	Medium at 0.1% of antimicrobial fraction	3				90.62 – 81.63	
	Medium without antimicrobial fraction	3					100.00
	Significance		1.000	1.000	1.000	1.000	1.000
Psuedomonas flourensces	Medium at 2% of antimicrobial fraction	3	50.90 – 50.03				
	Medium at 1% of antimicrobial fraction	3		67.30 – 60.80			
	Medium at 0.5% of antimicrobial fraction	3			88.45 – 81.40		
	Medium at 0.1% of antimicrobial fraction	3				95.61 – 90.63	
	Medium without antimicrobial fraction	3					100.00
	Significance		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. Uses Harmonic Mean Sample Size = 3.000.

growth, the antimicrobial fraction of *S. indicum* berries extract caused the death of the cells of the strain tested. Indeed, the more the antimicrobial fraction content in medium was high, the less the Alamar Blue reagent was reduced. This less reduction of the Alamar Blue reagent indicating a low rate of surviving cells was observed in the medium at 2% of the antimicrobial fraction. This reduction of surviving cells decreased during the incubation time. It was observed already in the medium at 0.1% of the antimicrobial fraction indicating that the minimum killing concentration could be at this value.

Conclusion

This work has revealed that the antimicrobial fraction of *S. indicum* L. berries extract was an appreciable source of flavonoids, saponins and carotenoids. The combination of these natural compounds could be proposed as effective and powerful antimicrobial agent against microorganism's growth and their survival. It highlights the discovery of natural substances for the research in alternative in chemical preservatives and additives in food and feed. The lowest percentage of reduced Alamar Blue reagent indicating the lowest rate of viable cells was observed in the medium at 2% of antimicrobial

Table 3A. Effect of the incubation time on the percentage in reduction of Alamar Blue of *Pseudomonas syringae* grown in the medium at 0.1, 0.5, 1 and 2% of the antimicrobial fraction of *Solanum indicum* L. berries extract.

Tukey HSD		Percentage in reduction of Alamar Blue				
Antimicrobial fraction content in the medium for each day	N	Subset for Alpha = 0.05				
		1	2	3	4	5
Medium at 0.1% of antimicrobial fraction for day 5	3	73.63				
Medium at 0.1% of antimicrobial fraction for day 4	3	74.27				
Medium at 0.1% of antimicrobial fraction for day 3	3		77.74			
Medium at 0.1% of antimicrobial fraction for day 2	3			79.67		
Medium at 0.1% of antimicrobial fraction for day 1	3				81.62	
Significance		0.392	1.000	1.000	1.000	
Medium at 0.5% of antimicrobial fraction for day 5	3	61.41				
Medium at 0.5% of antimicrobial fraction for day 4	3		61.95			
Medium at 0.5% of antimicrobial fraction for day 3	3			63.72		
Medium at 0.5% of antimicrobial fraction for day 2	3				65.64	
Medium at 0.5% of antimicrobial fraction for day 1	3					68.40
Significance		1.000	1.000	1.000	1.000	1.000
Medium at 1% of antimicrobial fraction for day 5	3	17.80				
Medium at 1% of antimicrobial fraction for day 4	3	18.15				
Medium at 1% of antimicrobial fraction for day 3	3		20.14			
Medium at 1% of antimicrobial fraction for day 2	3			22.07		
Medium at 1% of antimicrobial fraction for day 1	3				25.31	
Significance		0.52	1.000	1.000	1.000	
Medium at 2% of antimicrobial fraction for day 5	3	5.08				
Medium at 2% of antimicrobial fraction for day 4	3	5.24				
Medium at 2% of antimicrobial fraction for day 3	3		7.13			
Medium at 2% of antimicrobial fraction for day 2	3			8.74		
Medium at 2% of antimicrobial fraction for day 1	3				12.85	
Significance		0.975	1.000	1.000	1.000	

Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.000.

Table 3B. Effect of the incubation time on the percentage in reduction of Alamar Blue of *Pseudomonas aeruginosa* grown in the medium at 0.1, 0.5, 1 and 2% of the antimicrobial fraction of *Solanum indicum* L. berries extract.

Tukey HSD		Percentage in reduction of Alamar Blue				
Antimicrobial fraction content in the medium for each day	N	Subset for Alpha = 0.05				
		1	2	3	4	5
Medium at 0.1% of antimicrobial fraction for day 5	3	81.63				
Medium at 0.1% of antimicrobial fraction for day 4	3	82.27				
Medium at 0.1% of antimicrobial fraction for day 3	3		84.74			
Medium at 0.1% of antimicrobial fraction for day 2	3			89.67		
Medium at 0.1% of antimicrobial fraction for day 1	3			90.62		
Significance		0.497	1.000	0.389		
Medium at 0.5% of antimicrobial fraction for day 5	3	61.40				
Medium at 0.5% of antimicrobial fraction for day 4	3		71.94			
Medium at 0.5% of antimicrobial fraction for day 3	3			73.72		
Medium at 0.5% of antimicrobial fraction for day 2	3			74.64		
Medium at 0.5% of antimicrobial fraction for day 1	3				78.47	

Table 3B. Contd.

Significance		1.000	1.000	0.349	1.000	
Medium at 1% of antimicrobial fraction for day 5	3	54.80				
Medium at 1% of antimicrobial fraction for day 4	3		56.15			
Medium at 1% of antimicrobial fraction for day 3	3			58.14		
Medium at 1% of antimicrobial fraction for day 2	3				60.07	
Medium at 1% of antimicrobial fraction for day 1	3					65.31
Significance		1.000	1.000	1.000	1.000	1.000
Medium at 2% of antimicrobial fraction for day 5	3	50.08				
Medium at 2% of antimicrobial fraction for day 4	3	50.24				
Medium at 2% of antimicrobial fraction for day 3	3	50.63				
Medium at 2% of antimicrobial fraction for day 2	3		54.74			
Medium at 2% of antimicrobial fraction for day 1	3			58.97		
Significance		0.975	1.000	1.000		

Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.000

Table 3C. Effect of the incubation time on the percentage in reduction of Alamar Blue of *Pseudomonas fluorescens* grown in the medium at 0.1, 0.5, 1 and 2% of the antimicrobial fraction of *Solanum indicum* L. berries extract.

Tukey HSD		**Percentage in reduction of Alamar Blue**		
Antimicrobial fraction content in the medium for each day	**N**	**Subset for Alpha = 0.05**		
		1	**2**	**3**
Medium at 0.1% of antimicrobial fraction for day 5	3	90.13		
Medium at 0.1% of antimicrobial fraction for day 4	3	90.27		
Medium at 0.1% of antimicrobial fraction for day 3	3		92.74	
Medium at 0.1% of antimicrobial fraction for day 2	3			94.67
Medium at 0.1% of antimicrobial fraction for day 1	3			95.65
Significance		0.392	1.000	0.525
Medium at 0.5% of antimicrobial fraction for day 5	3	81.41		
Medium at 0.5% of antimicrobial fraction for day 4	3	81.95		
Medium at 0.5% of antimicrobial fraction for day 3	3		83.73	
Medium at 0.5% of antimicrobial fraction for day 2	3		84.64	
Medium at 0.5% of antimicrobial fraction for day 1	3			88.47
Significance		0.692	0.512	1.000
Medium at 1% of antimicrobial fraction for day 5	3	60.80		
Medium at 1% of antimicrobial fraction for day 4	3	61.15		
Medium at 1% of antimicrobial fraction for day 3	3	62.14		
Medium at 1% of antimicrobial fraction for day 2	3		65.07	
Medium at 1% of antimicrobial fraction for day 1	3			67.31
Significance		0.272	1.000	1.000
Medium at 2% of antimicrobial fraction for day 5	3	50.03		
Medium at 2% of antimicrobial fraction for day 4	3	50.31		
Medium at 2% of antimicrobial fraction for day 3	3	50.58		
Medium at 2% of antimicrobial fraction for day 2	3		54.74	
Medium at 2% of antimicrobial fraction for day 1	3			58.97
Significance		0.975	1.000	1.000

fraction. Thus, this antimicrobial fraction could be used as preservative and additive in food and feed at this concentration or above as the berries of *S. indicum* L. have been ingested by humans for quite some time at many dosages without any toxic effect noted. It could also be used at this concentration or above for the prevention of plant infestation and crops' rottenness.

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