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Research Article

Extracts of Sweet Potato leaf (*Ipomoea Batatas*) and Taioba (*Xanthosoma Sagittifolium*): New Sources of Natural Bioactives for the Food Industry

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Abstract

The research was conducted to seek evidence of the potential of sweet potato leaf and taioba ear in the form of natural extracts to use as sources of bioactives in food. The leaf reduced to powder were subjected to a liquid-solid extraction with ethanol: water (80:20, v/v). In the extracts obtained, the identification and quantification of phenolic compounds was performed by High-performance liquid chromatography (HPLC). Their antimicrobial and cytotoxic activity was also analyzed. High-performance liquid chromatography (HPLC) analysis resulted in the identification of fourteen secondary metabolites for each extract. It was also found that the SPLE has antimicrobial activity with minimum inhibitory concentration (MIC) values in relation to *Bacillus cereus* from 500 to 1000 μg/mL and *Salmonella Typhimurium* from 250 to 1000 μg/mL. Both extracts showed moderate cytotoxic activity against *Artemia salina* with LD50 of 300.4 μg/mL for SPLE and 265.7 μg/mL for TLE. The results obtained indicate that the two extracts can be used for the development of bioactive ingredients with promising applications in food area.

Keywords: Natural Additives; Antimicrobial Activity; Food Preservation

Introduction

The consumption and enhancement of natural foods that carry bioactive compounds, which can contribute to the improvement of human health, have gained attention in recent years [1]. The effects of bioactive compounds on consumer health are linked to secondary metabolites of plants. These are natural antioxidants and antimicrobials and can be used by the food industry as natural additives [2].

The sweet potato (*Ipomoea batatas*) is a vegetable of great economic importance worldwide, with a medical ethnographic indication already confirmed in the literature [3]. However, due to the scarcity of information on the bioactive compounds found on their leaf and the beneficial effects on human health, there is still great fear in their use as food or as an additive for food preservation in the form of extracts.

Another plant source of worldwide relevance due to its consumption in countries in Asia, Africa, Central America, and the Pacific Islands Simsek [4] is taioba (*Xanthosoma sagittifolium*). This plant belongs to the *Araceae* family, has long leaf, reaching up to two meters in height [5]. Currently, despite the high consumption in eastern countries, there is one shortage of information about its phytochemical composition and biological activities [5-7].

In recent years, a major focus has been placed by the food industry on the development of healthier food products and this objective can be achieved using two possible strategies: a) the reduction of undesirable substances and b) increasing the levels of desired bioactive components [8]. In this way, sweet potato and taioba leaf can be new sources of promising raw materials for the investigation of natural bioactive components, especially the phenolic compounds, and thus be an option of natural preservatives for the food industry [9]. Plant extracts have several properties, including antimicrobial and cytotoxicity activity [10]. Thus, this work hypothesized that sweet potato leaf and taioba may contain evidence of valuable antimicrobial and cytotoxicity properties. Thus, aimed in this research investigate the potential of extracts from sweet potato (*Ipomoeas potatoes*) and taioba (*Xanthosoma sagittifolium*) leaf as new sources of bioactive compounds. The results of this study may open new horizons for the use of these extracts in the food area.

Material and Methods

Chemical materials

The standards of gallic acid, phenolic acids: Hydroxybenzoic, Vanillic, Syringic, p-cumaric, ellagic, Trã-cinnamic, Caffeine and Feluric, and flavonoids such as Rutin, Myricetin, Quercetin, Naringenin, Catechin, Hespertin, Kaempferol, and Crisina were obtained from Sigma-Aldrich (Sigma Co., USA). The methanol for HPLC and acetonitrile by J. T. Baker (Phillipsburg, USA). The Triphenyl tetrazolium chloride was purchased at DicaLab, products for laboratory Ltda (Londrina, Paraná). The Dimethylsulfoxide - DMSO was obtained from Loja Química LTDA (São Paulo-SP). The Mueller Hinton Agar and the Brain Heart Infusion (BHI) broth were purchased from Kasvi laboratory products Ltda (São Jose dos Pinhais, Paraná).

Plant harvest

Five samples of fresh leaf of sweet potato (*Ipomoea batatas*) and taioba (*Xanthosoma sagittifolium*), without any physical, microbial, or visible insect damage, were collected in the municipality of Bananeiras, located in the Serra da Borborema, Paraíba, Brazil. Then, the collected vegetable materials were cleaned properly under running water, dried in an oven at 40 ºC for 24 hours. Subsequently, they were grounded using a knife mill (Willey, SL-31, Piracicaba, São Paulo) and stored in amber glass packaging for future use in the preparation of the extracts.

Extracts preparation

The leaf reduced to powder were subjected to a liquid-solid extraction with ethanol: water (80:20, v/v) as previously described by Cordeiro., *et al*. [11]. 1g of dry powder was suspended in 10 mL of the solvent followed by manual homogenization for 5 minutes and posteriorly the plant material was left in contact with the solvent for 15 days at room temperature. After this period, the mixtures were centrifuged at 4000 rpm for 15 minutes and the supernatants obtained were evaporated under vacuum in a rotary evaporator (Fisatam) and were placed in amber glass and stored refrigerated until their characterization.

Identification of phenolic compounds by High Performance Liquid Chromatography (HPLC)

For the identification of phenolic compounds, the samples were eluted with a gradient system consisting of solvent A (2% acetic acid, v/v) and solvent B (acetonitrile: methanol, 2: 1, v/v), used as a phase mobile, with a flow of 1 mL/min. The column temperature was maintained at 25 ºC and the injection volume was 20 μL. The gradient system started from 90% A 0 min, 88% A in 3 min, 85% A in 6 min, 82% A in 10 min, 80% A in 12 min, 70% A in 15 min, 65% A in 20 min, 60% A in 25 min, 50% A in 30-40 min, 75% A in 42 min and 90% A in 44 min. The total chromatographic run was 50 minutes. The peaks from phenolic compounds were monitored at 280 nm [12]. The columns used were a Shimadzu LC-18 column (25 cm x 4.6 mm, 5 μm particle size, from Supelco, Bellefonte, PA) and a C-18 ODS Shimadzu column. The LabSsolutions software (Shimadzu) was used to control the LC-UV and data processing system. The phenolic compounds were identified by comparing the retention times with the patterns of phenolic acids and flavonoids, being quantified in concentrations of mg/mL.

Antimicrobial activity

The extracts were tested against Gram-negative bacteria like *Salmonella Typhimurium* (ATCC 14028) and *Escherichia coli* (ATCC 101536) and Gram-positive bacteria like *Bacillus cereus* (ATCC 11778), *Listeria monocytogenes* (ATCC 19117), *Listeria Innocua* (ATCC 33090), *Staphylococcus aureus* (ATCC 15000) and *Clostridium perfringens* (ATCC 3624). The antimicrobial activity of the extracts was assessed directly by means of the minimum inhibitory concentration (MIC), using a method of microdilution in Brain Heart Infusion (BHI) broth based on the methodology described by the Clinical and Laboratory Standards Institute [13]. A 100μL aliquot of each extract was added to each well of a 96-well microplate containing 100μL of sterile BHI broth and bacterial suspension of 10^8 CFU/mL to reach final concentration ranges from 31.25 to 1000 μg/mL for the vegetable extracts. The microplates were incubated for 24 hours at 37 ºC. Subsequently, the interpretation of the results was based on the visual growth of the bacteria after incubation and confirmed by the color change of the culture medium with the addition of 20 μL of Triphenyl tetrazolium (0.5% v/v). MIC was defined as the minimum concentration capable of inhibiting the visible growth of bacterial cells.

Evaluation of cytotoxic activity

The toxicity of the extracts was evaluated using *Artemia salina* microcrustaceans based on the methodology described by Meyer., *et al*. [14]. The bioassay consists of inserting *Artemia salina* eggs to hatch in saline water with constant aeration for a period of 48 hours. Subsequently, 9 mL of each extract solution corresponding to the tested concentrations (15.62 to 1000 ppm) was added in microtubes containing 10 *Artemia salina* larvae totaling 10 repetitions for each concentration. After 24 hours of exposure of *Artemia salina* to plant extracts, live and dead microcrustaceans were accounted for. To establish the relationship between cytotoxicity and antimicrobial activity, the selectivity index (SI) was calculated according to the equation SI = log [LD_{50}]/[MIC]. Where positive values indicate high selectivity against microorganisms, while negative values point to the high toxicity of the extracts in *Artemia salina*.

Statistical analysis

Identification and quantification of phenolic compounds were performed in triplicate and the results were expressed as mean and standard deviation (mean ± SD). Data analysis was performed using the SAS® System (2012) software. The cytotoxic evaluation of the extracts was expressed as LD_{50}, a lethal dose capable of killing 50% of the microcrustaceans, with a 95% confidence limit, by means of linear regression, through the logarithm graph of the concentration used in the samples versus the percentage of mortality of the microcrustaceans, calculated using the Probity statistical method, using the Polo-plus 1.0 software.

Results and Discussion

The leaf of sweet potato and taioba are usually disregarded but are potential sources of phenolic compounds with promising properties for use in food [15,16]. However, little is known about the antimicrobial and cytotoxic potential of these species, especially of taioba. Thus, it was hypothesized that both vegetables in the form of extracts could effectively contain important bioactive compounds such as phenolic and flavonoid acids and be used as natural bioactives by the food industry.

Phytochemical composition of extracts

The determination of the phenolic profile revealed fourteen chemical compounds for each plant extract (Table 1). It was observed that the sweet potato leaf extract (SPLE) was the richest in terms of the concentration of the selected components. In this extract, large amounts of 3,4 dihydroxybenzoic acid, 4-hydroxybenzoic acid, 2,5 dihydroxybenzoic acid, vanillic acid, myricetin, and rutin were also observed. Meanwhile, in taioba leaf extract (TLE) the 2,5 dihydroxybenzoic acid, syringic acid, salicylic acid, and rutin were identified in greater quantities. Phytochemicals such as 4-hydroxybenzoic acid, sinapic acid, vanillic acid, myricetin, and Kaempferol were present only in SPLE and syringic, salicylic, feluric, Crisine and hespertin were only present in TLE.

Phenolic compounds	SPLE mg/100 g of extract	TLE mg/100 g of extract
Gallic acid	8.48 ± 0.00	17.62 ± 4.15
3,4-dihydroxybenzoic acid	1385.22 ± 71.96	110.13 ± 14.53
4-hydroxybenzoic acid	2458.06 ± 145.92	nd
2,5-dihydroxybenzoic acid	22979.18 ± 353.82	2189.54 ± 450.66
Synaptic acid	400.01 ± 5.99	nd
Vanillic acid	578.11 ± 25.98	nd
Syringic acid	nd	834.11 ± 24.92
Salicylic acid	nd	1139.55 ± 78.91
p-cumaric acid	39.57 ± 0.00	593.27 ± 16.61
Feluric acid	nd	154.19 ± 6.23
Trã-cinnamic acid	7.06 ± 1.99	8.81 ± 0.00
Rutin	661.51 ± 7.99	1215.92 ± 45.68
Myricetin	3142.19 ± 169.91	nd
Quercetin	117.32 ± 1.99	57.27 ± 26.99
Naringenin	360.44 ± 17.99	8.81 ± 4.15
Kaempferol	49.47 ± 9.99	nd
Crisine	nd	22.02 ± 2.07
Catechin	354.78 ± 13.99	182.09 ± 8.30
Hespertin	nd	4.40 ± 2.07

Table 1: Concentration (mg/100 g extract) of phenolic compounds present in sweet potato leaf and taioba extracts.

SPLE: Sweet Potato Leaf Extract; TLE: Taioba Leaf Extract; nd: Not Detected

These bioactive compounds are known for their effectiveness in controlling oxidative reactions, as well as in controlling inflammatory diseases, diabetes, cancer, and others. The hydroxybenzoic acids are phytochemicals that have antioxidant activity. However, their most interesting properties are associated with the ability to modify cell signaling processes. They induce a multiplying effect such as the activation of the Nrf2 pathway, which is one of the main mechanisms of cellular defense against oxidative stress, resulting in an improvement of endogenous antioxidant mechanisms [17].

The 2,5-Dihydroxybenzoic acid was the major phytochemical present in both extracts, being in a very expressive concentration in SPLE. This phenolic acid is responsible for inhibiting the formation of prostaglandins in response to lipopolysaccharides through Cox inhibition [18]. This suggests that foods rich in this acid may contribute to a decrease in the occurrence of heart attacks due to the formation of clots [17].

Another phenolic acid identified and quantified in SPLE was 4-hydroxybenzoic acid. It is an excellent antioxidant and due to its low toxicity. It is widely used by the cosmetics, pharmaceutical and food industry [9]. The 3,4-dihydroxybenzoic acid, also identified in greater quantity in SPLE, has several health benefits, such as antioxidant, antimicrobial, anti-inflammatory, anti-hyperglycemic, anti-apoptotic and antiproliferative activity [19].

In a previous study, Khan., *et al*. [20] reviewed several biological activities of 3,4-dihydroxybenzoic acid and reported excellent antibacterial, antiviral, neurological, anti-atherosclerotic, anti-fibrotic, anti-aging, anti-ulcer, and anti-cancer activities. Two distinct studies exhibited 3,4-dihydroxybenzoic acid antiatherogenic activity, where Wang et at., [21] attributed this effect to the combination of a decrease in miR-10b expression, together with an increase in the expression of ABCA1 and ABCG1, as well as in the accelerated transport of reverse cholesterol by macrophases. A study by Zheng., *et al*. [22] attributed this effect to the normalization of arterial inflammation by positive regulation of MERTK and inhibition of MAPK3/1 in lesional macrophases.

The Salicylic acid, on the other hand, was identified only in TLE, and the high concentration of this phenolic acid in taioba leaves extract also reveals the importance of this vegetable for health, since Peterson., *et al*. [23] reported that salicylic acid is an anti-inflammatory, antiatherogenic, anti-infectious and antifungal bioactive.

Flavonoids are a group of natural substances widely known for their biological activities responsible mainly for their strong antioxidant and antimicrobial action [24,25]. Among the flavonoids found in high concentrations in the two plant extracts, rutin stands out, a flavonoid with beneficial actions for health, such as protection of small blood vessels; inhibition of the free radical formation process, contributing with antioxidant and anti-inflammatory properties, in addition to having functions linked to the treatment of diabetic neuropathy, and considered a potent antithrombotic for cardiovascular diseases [26].

Another important flavonoid found only in SPLE was myricetin. It is one of the main compounds present in various foods and drinks. It is also a phenolic that has a wide range of activities, including strong antioxidant, anti-cancer, anti-diabetic and anti-inflammatory activities. These activities are associated with the central nervous system and their consumption can be beneficial against Parkinson's and Alzheimer's diseases [27].

In addition, catechin was present in the two extracts with higher concentration in SPLE. Its ability to scavenge free radicals have been reported as one of the main benefits of this flavonoid [28,29]. Furthermore, catechin has other medicinal properties, including anti-carcinogenic, anti-tumorigenic, and anti-mutagenesis properties, as well as preventing the growth of metastasis and tumors [30].

Antimicrobial activity

It was found that the sweet potato leaf extract was more effective in relation to antimicrobial activity when compared to the taioba leaf extract, inhibiting two pathogenic bacteria, one Gram-positive, and one Gram-negative (Table 2). The concentrations that inhibited the strain of *Bacillus cereus* ATCC 11778, varied from 500 to 1000 μg/mL. While the concentrations that inhibited *Salmonella Typhimurium* ATCC 14028 ranged from 250 to 1000 μg/mL. The other bacteria tested were resistant to both extracts in all concentrations tested.

The antimicrobial activity of the sweet potato leaf extract demonstrated for the two pathogenic bacteria (*Bacillus cereus* and *Salmonella Typhimurium*) may be associated with the presence of phenolic acids such as gallic acid, 3,4-dihydroxybenzoic acid, and synapic acid, because the antimicrobial effectiveness of these bioactive compounds has been reported in previous studies [20,31,32].

Some flavonoids identified in high concentrations in the sweet potato leaf extract also have antimicrobial activity, which may have contributed to the effectiveness of this extract. Previous studies have shown that flavonoids such as naringenin, kaempferol, and quercetin can inhibit or reduce the formation of pathogenic microorganisms [33,34].

Sweet potato leaf extract (SPLE)						
Tested concentrations (µg/mL)						
Gram-positive bacteria	**1000**	**500**	**250**	**125**	**62.5**	**31.25**
Bacillus cereus	+	+	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-
Listeria monocytogenes	-	-	-	-	-	-
Listeria innocua	-	-	-	-	-	-
Clostridium perfringens	-	-	-	-	-	-
Gram-negative bacteria						
Escherichia coli	-	-	-	-	-	-
Salmonella Typhimurium	+	+	+	-	-	-
Taioba leaf extract (TLE)						
Tested concentrations (µg/mL)						
Gram-positive bacteria	1000	500	250	125	62.5	31.25
Bacillus cereus	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-
Listeria monocytogenes	-	-	-	-	-	-
Listeria innocua	-	-	-	-	-	-
Clostridium perfringens	-	-	-	-	-	-
Gram-negative bacteria						
Escherichia coli	-	-	-	-	-	-
Salmonella Typhimurium	-	-	-	-	-	-

Table 2: Antimicrobial activity of sweet potato leaf and taioba extracts.

SPLE: Sweet Potato Leaf Extract; TLE: Taioba Leaf Extract; (+) =There was Inhibitory Activity; (-) = There was no Inhibitory Activity

The bacteriostatic activity of sweet potato leaf extract against *Salmonella Tiphymurium* ATCC 14028 was extremely important, considering that mainly Gram-negative bacteria are well known for their greater resistance to antimicrobial drugs. This resistance is related to the lipopolysaccharides present in its outer membrane and by the presence of hydrophilic channels, known as porins, where these channels normally prevent the entry of hydrophobic substances [35,36].

However, it is possible to weaken this outer membrane by disintegrating the lipopolysaccharides, generically called permeabilizers, which is possibly what happened with phenolic molecules present in the sweet potato leaf extract in the concentrations that inhibited the bacteria. Thus, flavonoids present in sweet potato leaf extracts, such as quercetin, naringenin, myricetin, and Kaempferol, that have antimicrobial activity, in addition to the presence of a phenolic acid such as gallic acid may have acted in synergism and disintegrated the membrane of gram-negative bacteria [37,38].

Regarding the taioba leaf extract, no bacteriostatic activity was observed. This fact indicates the resistance of the bacteria to the phenolic compounds present in this plant. It is worth mentioning that the phenolic profile of the taioba leaves extract did not present important phenolic compounds for antimicrobial activity, such as myricetin and Kaempferol, which helps explain the result. These substances, when present, can act synergistically with other antimicrobial agents enhancing the antimicrobial activity [39].

Cytotoxic activity

For the first time, with in-vitro tests, the cytotoxic activity of various concentrations of leaf extract sweet potato and taioba in *Artemia salina* was evaluated. Table 3 expresses the lethal doses (LD) that were able to kill 50%, 90%, and 95% of *Artemia salina* larvae for the two plant extracts. The selectivity index (SI) was made only for the sweet potato leaf extract, as it was the only one that demonstrated to have some antimicrobial activity. The sweet potato leaf extract (SPLE) showed a high selectivity index for Bacillus cereus and *Salmonella Typhimurium*. Therefore, the SPLE is a promising antimicrobial agent, as it exhibited good antimicrobial activity against the two pathogenic bacteria (*Bacillus cereus* and *Salmonella Typhimurium*), while the cytotoxicity corresponding to LD_{50} was considered medium. The lethal dose capable of killing 50% of *Artemia saline* was 300.40 μg/mL whereas the other lethal doses (LD_{90} and LD_{95}) ranged from 504356.50 to 588203.40 μg/mL.

Sweet potato leaf extract (SPLE)			Bacterial selectivity index (SI)		
Lethal dose	Dose in (μg/mL)	Confidence limit		*B. cereus*	*S. Typhimurium*
LD_{50}	300.4	164.8 < DL < 783.6		0.60	1.20
LD_{90}	504356. 5	36776.8 < DL < 267697.4		-	-
LD_{95}	588203.4	154204.5 < DL < 0.0		-	-
Taioba leaf extract (TLE)					
Lethal dose	Dose in (μg/mL)	Confidence limit			
LD_{50}	265.7	129.1 < CL < 827.6		-	-
LD_{90}	81909.6	7877.2 < CL < 625405.8		-	-
LD_{95}	416105.7	22169.9 < CL < 0.0		-	-

Table 3: Cytotoxic activity and selectivity index of sweet potato leaf and taioba extracts.

SPLE: Sweet potato Leaf Extract; TLE: Taioba Leaf Extract

In a recent study, Nascimento., *et al*. [40] verified the antimicrobial and cytotoxic activity of extracts from *Senna* and *Cassia* species and reported a high antifungal selectivity index against the yeasts *Candida albicans*, *Candida tropicalis* and *Candida glabrata* and low toxicity of their extracts, corroborating with the results found in our study.

The taioba leaf extract (TLE) did not show selectivity in relation to the studied bacteria, however, it was more effective in decreasing the survival viability of *Artemia salina* with lower values compared to the sweet potato leaf extract, corresponding to the lethal doses (LD_{50}, LD_{90}, and LD_{95}). Even so, the result for LD_{50} also indicated a medium cytotoxic activity of this extract. The lethal dose capable of killing 50% of *Artemia salina* was 265.7 μg/mL, while the other lethal doses (LD_{90} and LD_{95}) varied from 81909.6 to 416105.7 μg/mL.

According to Bussmann., *et al*. [41] doses below 249 μg/mL are considered highly toxic; doses between 250-499 μg/mL are considered to have medium toxicity; doses between 500 to 1000 μg/mL are considered low and doses above 1000 μg/mL have no toxicity.

Conclusions

In general, the phenolic profile showed that the two extracts are rich in bioactive compounds. The antimicrobial activity of these extracts was also evaluated against strains of Gram-positive and Gram-negative pathogenic bacteria, however, only the sweet potato leaf extract was highlighted, inhibiting the strains of *Bacillus cereus* ATCC 11778 and *Salmonella Typhimurium* ATCC 14028 which supports the use of this species in traditional medicine to treat infections caused by these bacteria, as well as its application in food contaminated by these bacteria as well. In addition, these extracts had medium cytotoxic activity. In general, the data obtained show that sweet potato leaf and taioba extracts can be used for the development of bioactive ingredients with promising applications in food and nutraceutical. However, comprehensive studies are needed to clarify the antimicrobial bioactivity, mainly of the taioba leaf extract against pathogenic bacteria using higher concentrations, as well as to study the synergy between both, seeking to enhance the action.

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