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Full Length Research Paper

In vitro evaluation of *Bixa orellana* L. (Annatto) seeds as potential natural food preservative

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This paper evaluates the *in vitro* antimicrobial and antioxidant effect of the ethanolic extract of annatto seeds (EEBS). Minimum inhibitory concentration (MIC) of the EEBS was determinate through micro-dilution method in 7 bacteria and 5 fungi strains, all of which are ATCC. The EEBS shows a wide antimicrobial action spectrum for both Gram positive and Gram negative bacteria, as well as for fungi with MICs between 0.5 and 2048 µg/ml. The most sensitive bacteria to the EEBS effect were *Bacillus cereus*, *Staphylococcus aureus* and *Lactobacillus plantarum*; while, *Pseudomonas aeruginosa* and *Listeria monocytogenes* showed greater resistant against it. Yeast and moulds were more sensitive to EEBS than bacteria, with MICs ranging from 512 to 0.5 µg/ml, where *Saccharomyces cerevisiae* was the most resistant yeast against the extract and *Penicillium chrysogenum* the most sensitive. On the other hand, nisin (positive control) showed a strong inhibition of growth for Gram positive bacteria and lower capacity against Gram negative had no capacity against fungi. Besides the broad antimicrobial action, EEBS showed an anti-radical capacity at concentrations lower than MIC at the found values. These results demonstrate the antimicrobial and antioxidant potential of the EEBS, that can possibly make this extract a new alternative for natural food conservation.

Key words: *Bixa orellana,* antimicrobial activity, free radicals scavenging capacity, food preservatives.

INTRODUCTION

According to the World Health Organization (WHO), foodborne diseases are one of the most common health problems in the contemporary world and an important cause of productivity loss (FAO/WHO, 2006). The Disease Control and Prevention Centre of the United States estimates that 76 million people become ill each year; more than 300.000 are hospitalized and 5.000 die as a result of foodborne diseases (Satcher, 2000). The incidence of illnesses caused by microorganisms that are principally foodborne such as *Salmonella* species and *Campylobacter* species continued to increase considerably in many countries. Moreover, new and serious hazards have emerged within the food chain, such as infections from *Escherichia coli* (Pires et al., 2012).

Traditionally, some of the methods used to prevent food contamination are heating, reduced water activity, low temperatures, fermentation and the addition of antimicrobial agents (preservatives) (Leistner, 2000). The addition of preservatives has been an effective method to

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control microbial contamination, although in recent years, popular demand has shown an increased aversion towards synthetic chemical preservatives (Negowetti, 2013). This has resulted in a growing demand for natural products which are presumably safer, functional, and provide nutritional and health benefits. This demand has increased the importance of the research in alternative sources of natural preservatives (Cowan, 1999).

The medicinal plant*, Bixa orellana* (achiote, orlean, roucou, or annatto), native to Central and South America, is an ornamental shrub of 3 to 5 m tall with red flowers. Its fruit is an ovoid capsule containing the seeds, valued as a source of raw material for the extraction of a natural dye, the bixin and its water soluble derivative norbixin (Scotter et al., 1998) widely used in several industries such as food, pharmaceutics and cosmetic. Unlike its uses as a natural dye, annatto is also used in traditional folk medicine to treat diseases in the heart, stomach and intestine, as well as respiratory problems and burns, and also for sunscreen protection, insect repellent, and aphrodisiac (Cáceres et al., 1990). Some of its properties have been scientifically proven (Oboh et al., 2011; Haila et al., 1996; Kiokias and Gordon, 2003; Galindo-Cuspinera, 2003; Rojas et al., 2006; Braga et al., 2007). Several studies have shown that the annatto seed extract has antimicrobial capacity against *Bacillus subtilis, Staphylococcus aureus*, *Clostridium perfringens, Streptococcus thermophilus*, *Lactobacillus casei*, *Lactococcus lactis*, *Candida albicans, Candida famata, Rodotorula* species*, Aspergillus* species*,* and *Neurospora crassa* among others (Siva et al., 2011; Zarringhalami et al., 2009; Fleischer et al., 2003).

In addition to the antimicrobial properties, antioxidant capacity has been attributed to extracts from the annatto seeds, inhibiting *in vitro* oxidation of low density lipoprotein (LDL) related to heart diseases, at the same time protects DNA from oxidative damage that has serious consequences in some age-related cancers. The seed extract showed strong inhibition of triglycerides oxidation from rapeseed oil (Haila et al., 1996) and in the same way, the study of Kiokias and Gordon (2003) reported that norbixin was able to inhibit oxidative deterioration of olive oil.

Nonetheless, antimicrobial capacity studies have been limited to the evaluation of microorganisms that cause diseases not related to food, and the control substances have been commonly used antibiotics for the treatment of these diseases. This work presents potential applications of the *B. orellana* seeds ethanolic extract used as food preservative against important microorganisms in foodborne diseases, as well as its antioxidant activity.

MATERIALS AND METHODS

Preparation of the ethanolic extract from *B. orellana* L. seeds (EEBS), annatto seeds, was collected in the municipality of San Luis (Antioquia, Colombia). The seeds were identified as *B. orellana* L. red variety by the Herbarium of the Universidad of Antioquia (Colombia). The seeds were then dried in a conventional oven at 37.0±0.2°C for 48 h. The dry seeds were subjected to an extraction process with 95% ethanol (Merck®, Germany) for 48 h at 4.0±0.2°C in a single process. The resulting extract was concentrated in a rotary evaporator (Büchi R-124) followed by a freeze-dry process and stored at 4.0±0.2°C for 15 days.

Food-related microorganisms used for testing were *Bacillus cereus* ATCC 11778, *S. aureus* ATCC 6538, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella typhimurium* ATCC 14028, *Shigella sonnei* ATCC 29930, *C. albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 2601, *Aspergillus niger* ATCC 16404, *Penicillium chrysogenum* ATCC 10106 and *Byssochlamys fulvas* ATCC 9406. Growth curves for the bacteria and the yeasts were perfomed in order to establish the time for the exponential growth phase of each microorganism.

The moulds used were *A. niger, P. chrysogenum* and *B. fulvas,* and were cropped in slanted tubes (37.0±0.2°C) until the sporulation, spores were scraped with sterile water and this suspension at 0.1 absorbance, at 600 nm, was used in the broth micro dilution method.

Minimum inhibitory concentration (MIC) of EEBS

The method used for the evaluation of the MIC of the EEBS was a slightly modified colorimetric broth microdilution method was proposed by Abate et al. (1998). Diluted solutions of the extract were prepared. Concentrations of EEBS ranging from 1 to 1024 µg/ml were placed in 96-well microplates (Becton Dickinson Labware®, USA). Then, culture medium and microorganism, at its exponential growth phase (approximately 1.5×10^8 CFU/ml), were added to each of the wells. After incubation (37.0±0.2°C/5 h), 0.8 mg/ml of 3-{4.5-dimethylthiazol-2-yl}-2, 5-diphenyl tetrazolium bromide (MTT; Alfa Aesar®, Germany) was added to each well. In order to allow viable microorganisms to metabolize the yellow dye MTT into formazan (purple crystals), the mixture was incubated at 37.0±0.2°C for 1 h. The MIC value was considered as the concentration of the first well that did not undergo colour change (from yellow to purple). This methodology was successfully applied to all bacteria. For fungi, the incubation time was increased to 24 h. The procedure was repeated three times for each microorganism. The culture media used were Müeller-Hinton broth (Merck®, Germany) for bacteria and Sabouraud Dextrose broth (Merck®, Germany) was used for fungi. After determining the MIC, wells with none microorganism growth were reset to a solid medium. After 24 h of incubation, the concentration that showed no microorganism growth was considered to be the minimum bactericidal concentration against bacteria and the minimum fungicidal concentration against fungi.

Antimicrobial capacity by direct bioautography

To perform this bioassay, EEBS and tannic acid as positive control were chromatographed in a silica gel 60 (Merck®, Germany). The mobile phase used was a 1:1 mixture of acetone:water (v/v). Also, microorganism *B. cereus* and *C. albicans* were grown in Müeller-Hinton and Sabouraud broth, respectively, and placed at incubation temperature (37.0±0.2°C) for 24 h. Then, 1 ml of the microbial suspension was resuspended on 9 ml of a sterile liquid culture media and put to incubation (37.0±0.2°C) in an orbital agitator to obtain a culture in logarithmic phase of growth. Subsequently, the microbial suspension was homogenized and added to the chromatogram run making sure it was completely covered by the solution. Each chromatogram was placed on sterile petri dishes covered with the lid and incubated at 37°C during 3 h for *B. cereus,* and 24 h for *C. albicans*. Finally, several bioautographies were

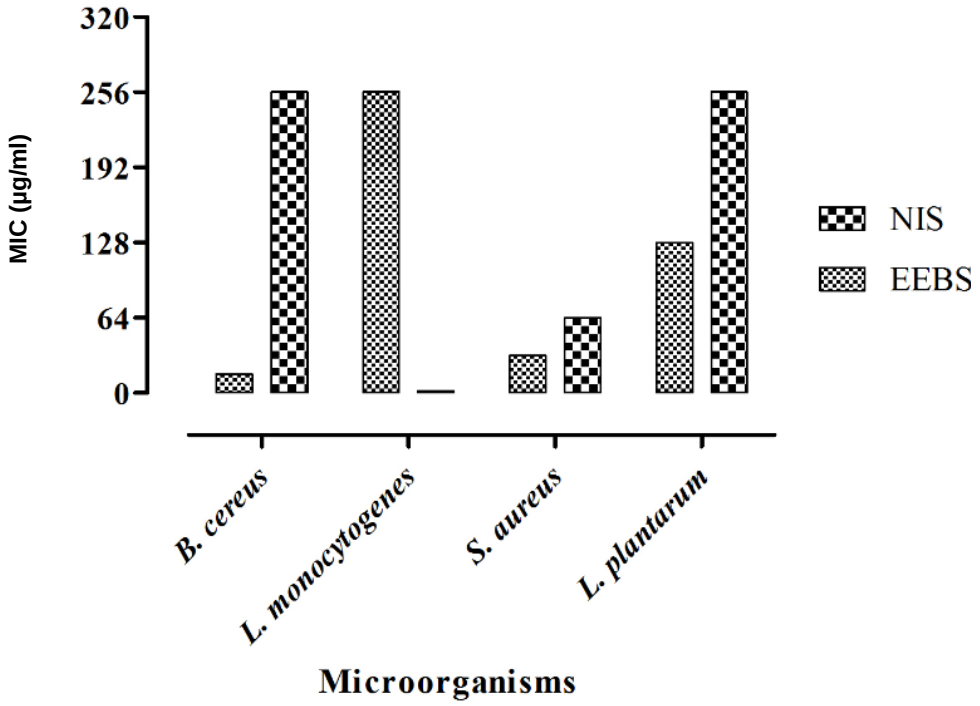

Figure 1. MIC values of EEBS and NIS against Gram positive bacteria: *B. cereus, S. aureus, L. monocytogenes* and *L. plantarum.*

developed with MTT and they were allowed to incubate for one more hour. When adding the developer reagent, the chromatograms turned to a deep violet colour, except for the inhibition areas of the microbial growth by the active metabolites present in the vegetable extracts. These spots were ivory coloured and their R_f was measured (Rahalison et al., 1991).

Total phenolic compounds in EEBS determination

To evaluate the concentration of total phenolic compounds in the EEBS extracts, the method developed by Singleton and Rossi (1965) was used that measures total phenolic compounds contained in a extract as a funtion of tannic acid. 100 µl of a EEBS solution (5 mg extract/ml methanol) was brought up to 500 µl using distilled water. The solution was then mixed with 250 µl of Folin-Ciocalteu reagent (Merck®, Germany) (1:1) and subjected to sonication for 5 min. The sonicated solution was mixed with 1250 µl Na_2CO_3 at 20% (Merck®, Germany) and was left to settle for 2 h in the dark. Then, the solution absorbance was measured at 725 nm and it was expressed as mg tannic acid/g extract. Each extract preparation was replicated three times using the same procedure.

In vitro antioxidant capacity of EEBS determination

The *in vitro* antioxidant capacity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method (Brand-Williams et al., 1995). The EEBS was diluted in methanol (Merck®, Germany) at concentrations ranging from 0.04 to 2.00 mg/ml, and then mixed in 10:990 volume proportion with a 25 µl solution of DPPH diluted in methanol. This mixture was incubated in the dark at room temperature for 30 min. The absorbance of the sample was measured using a spectrophotometer (Spectronic 20, Genesys TM) at 517 nm against a blank. The antiradical capacity was defined as the amount of antioxidant necessary to decrease the initial concentration of DPPH free radical down to 50%. For the study, ascorbic acid (Mol Labs®, Colombia) and tannic acid (Carlo Erba reagent®, Italy) were used as references.

Statistical analysis

The experimental values are reported as mean ± standard deviation (SD) of three samples. The Tukey post hoc test ($P<0.05$) was used for comparing the means.

RESULTS

MIC of EEBS

The MIC values of EEBS and nisin (NIS) are as shown in Figure 1. Against Gram positive bacteria, *B. cereus*, *S. aureus* and *L. plantarum* showed an increase of sensibility to EEBS (16, 32, and 128 µg/ml, respectively). These MIC values are lower than those found with NIS (256, 64 and 256 µg/ml, respectively). On the other hand, *L. monocytogenes* showed an increased sensibility to NIS (2 µg/ml) compared to EEBS (256 µg/ml).

EEBS was also active against Gram negative bacteria with MIC values of 256 µg/ml for *E. coli*, 1024 µg/ml for *P. aeruginosa* and 512 µg/ml for *S. sonnei* and *S. typhimurium* (Figure 2). Furthermore, NIS inhibited the growth of *E. coli*, *S. sonnei* and *S. typhimurium* at MIC

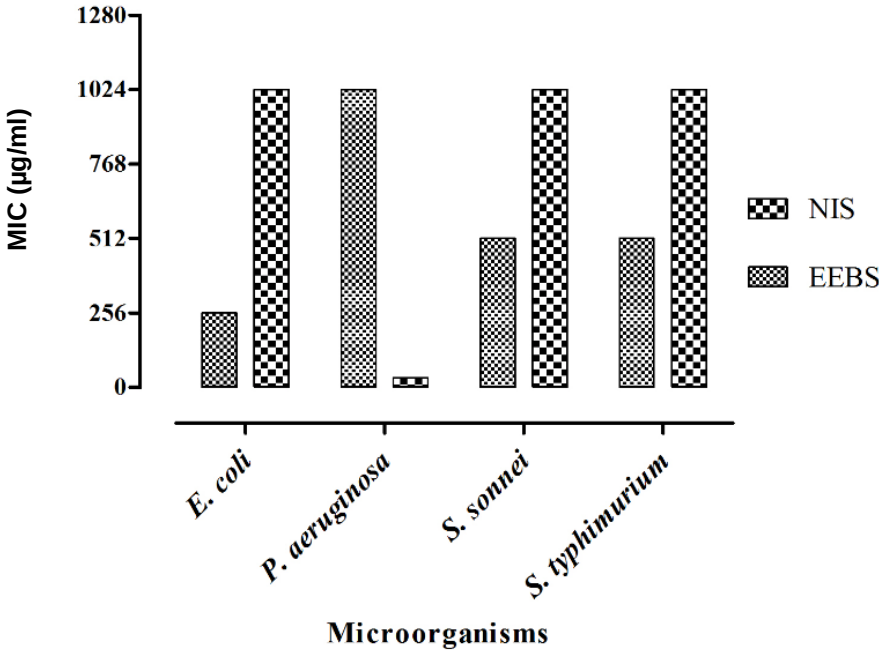

Figure 2. MIC values of EEBS and NIS against Gram negative bacteria: *E. coli, P. aeruginosa, S. sonnei* and *S. typhimurium*.

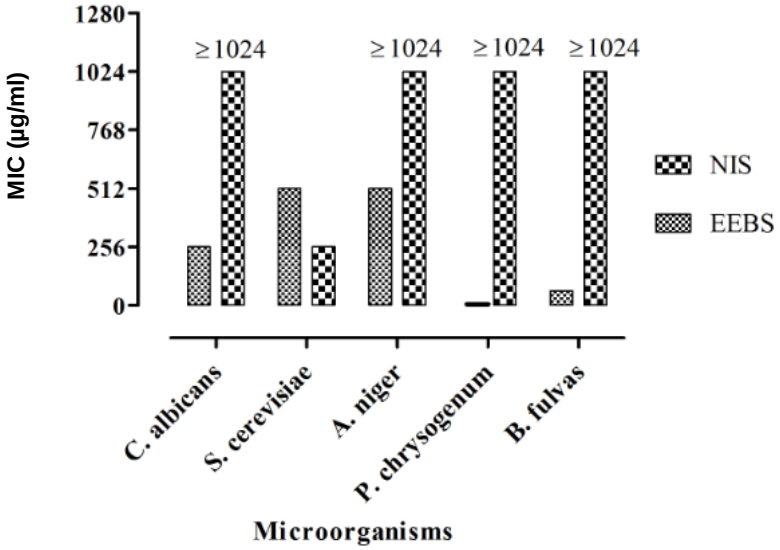

Figure 3. MICs of EEBS against fungi: *S. cerevisiae, C. albicans, P. chrysogenum, A. niger* and *B. fulvas.* **Nisin did not inhibit the growth of *C. albicans*, *P. chrysogenum*, *A. niger* and *B. fulvas* in the range of concentrations tested (1 to 1024 µg/ml).

values of 1024 and 32 µg/ml for *P. aeruginosa*. In addition, the antifungal efficacy of the EEBS against moulds and yeast of relevance for the food industry was proved.

Figure 3 shows the MIC values of EEBS against yeast: *S. cerevisiae* (512 µg/ml) as well as *C. albicans* (256 µg/ml), and against moulds: *A. niger, P. chrysogenum* and *B. fulvas* with MIC values of 512, 0.5 and 64 µg/ml, respectively. NIS was unable to inhibit the growth of fungal species at the tested concentrations (0 to 1024 µg/ml) except for the yeast *S. cerevisiae* which was the only microorganism in this group that showed susceptibility

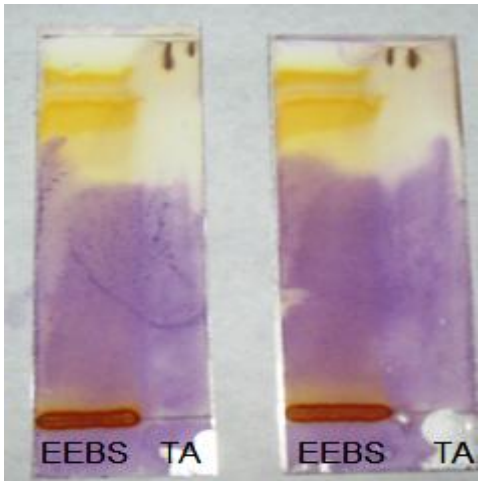

Figure 4. Direct bioautography of EEBS and tanic acid (TA)

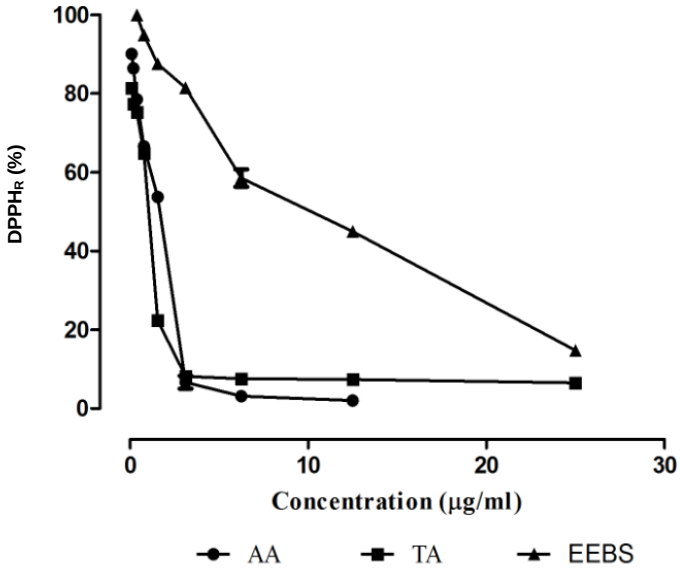

Figure 5. Antiradical capacity of ascorbic acid (AA), tannic acid (TA) and ethanolic extract from seeds of *B. orellana* (EEBS).

to the positive control (256 µg/ml).

Direct bioautography assay

In this study, a direct bioautography assay was implemented in order to detect the biological active compounds present in the EEBS (Figure 4). The yellow areas stated the inhibition of microorganism growth. The R_f values of the capacity areas of EEBS matching the Rf values of the standard phenolic compound (tannic acid) are between 0.73 and 1. The same behaviour was observed in the chromatograms for the yeast *C. albicans*, but in this case the zones of capacity were at a slightly lower R_f, with finding values between 0.73 and 0.93 for EEBS and tannic acid, respectively. These results suggest that the compounds involved in the antimicrobial capacity of EEBS are phenolic compounds. The quantification of total phenolic compounds of EEBS, found that the extract contains 104.86±0.69 mg of tannic acid per gram of dry extract.

In vitro EEBS antioxidant activity

The anti-radical capacity of EEBS was evaluated in this work using the technique of DPPH$^{\bullet}$ radical. The results were plotted as percentage of the remaining DPPH$^{\bullet}$ (DPPH$^{\bullet}_R$). For positive controls, tannic and ascorbic acids were used. Ascorbic acid was chosen due to its common use in the food industry as a natural antioxidant, as well as tannic acid since it has been reported as a metabolite in the plants extracts. Figure 5 clearly shows a dose-dependency of EEBS for the capability of capturing the DPPH$^{\bullet}$ radical, same as the positive controls in the range of concentrations used in this work (0 to 25 µg/ml). It is also evident that the slopes of the controls are steeper than the slope of the EEBS, indicating greater ability to capture the DPPH$^{\bullet}$ free radical. Furthermore, to decrease the amount of DPPH$^{\bullet}_R$ down to values below 10%, the controls required concentrations of approximately 3.12 µg/ml. To obtain the same percentage of DPPH$^{\bullet}_R$ for EEBS, concentrations greater than 25 µg/ml were needed.

The effective concentration (EC) decreases by 50% the DPPH$^{\bullet}$ initial concentrations (EC_{50}, µg/ml), which was obtained from the results recovered with the DPPH$^{\bullet}$ test (Table 1). The analysis of variance (ANOVA) shows no statistically significant differences ($p<0.05$) among the EC_{50} values for ascorbic and tannic acids (1.73±0.02 and 1.06±0.01 µg/ml, respectively). Statistically significant differences ($p<0.05$) between the controls and EEBS (8.28±1.63 µg/ml) were found.

DISCUSSION

MIC of EEBS

Annatto is known worldwide for its colouring properties, especially in the food industry where it is marketed as a natural dye. It is widely used as a condiment in traditional dishes and it is also used for the treatment of several diseases (burns, sore throat, headache, etc). Additionally, the commercial annatto extract has been shown to possess antimicrobial action (Galindo-Cuspinera and Rankin, 2005). This study, demonstrated that the ethanolic extract of annatto seeds (EEBS) showed a broad spectrum of antimicrobial action that was able to inhibit the growth of both Gram positive and Gram negative bacteria as well as fungi at very low MIC values(Figures 1, 2 and 3).

Table 1. EEBS and AA and TA control EC_{50} values.

Anti-radical	EC_{50} (µg/ml)
AA	1.73±0.02[a]
TA	1.06±0.01[a]
EEBS	8.28±1.63[b]

The results are shown as mean ±SD of three assays. Same letters mean no statistically significant differences ($p<0.05$) by Tukey test.

These results agree with those found by other authors, who have reported the antimicrobial action of the annatto extract against the Gram positive bacteria: *S. aureus*, *B. cereus* (Rojas et al., 2006; Zaouali et al., 2010), *C. perfringes* (Zaouali et al., 2010), *B. subtilis*, *L. plantarum* and *L. casei* (Galindo-Cuspinera, 2003). Similarly, Fleischer et al. (2003) shows that the ethanolic extracts of *B. orellana* L. seeds have antimicrobial capacity against Gram positive and Gram negative bacteria and also against yeast type *C. albicans*. The results in Figure 2 demonstrate the potent antimicrobial capacity of the EEBS against Gram negative bacteria, even better than those found with NIS, making the annatto seed extract (or its active components), a potential natural preservative substitute in the food industry. Moreover, Rojas et al. (2006) established the inhibitory power of EEBS against Gram negative bacteria, which was even more active than the antibiotic used as positive control.

The effectiveness of the EEBS against moulds and yeast (Figure 3), contrasts with the work of Braga et al. (2007), who showed that methanol extract of annatto seeds does not have anti-*C. albicans* action; whereas, Fleischer et al. (2003) reported that the ethanolic extract of these seeds showed antifungal action against this yeast.

The antimicrobial capacity results reported in the literature for this type of extracts vary greatly. This may be due to multiple factors such as the differences between concentrations of the active principle in the plant components and its respective extracts. These vary depending on environmental factors like the region where the plant is grown (climate, altitude, geographic location), time of the year when harvesting takes place, portion of the plant used and prior treatment before measurements (drying, grinding, storage) (Zaouali et al., 2010). Additionally, the method is affected by the amount of plant used, maceration time, and type of solvent used. Regarding the determinations of antimicrobial activity, variations may exist due to the half-life of the active compounds (photosensitive, thermo-labile, etc.), the use of different strains of microorganism (phenotype) and the quantification method used (Silva et al., 2010).

In this first phase of the paper, the *in vitro* antimicrobial efficacy of EEBS against important microorganism in the food industry could be corroborated, demonstrating the broad spectrum of antimicrobial action at low MIC values against both Gram positive and Gram negative bacteria as well as against fungi associated to food contamination. In addition, recent studies have demonstrated the antimicrobial potential of EEBS clinically. Such is the case of the study performed by Siva et al. (2011) who found strong antimicrobial capacity of the annatto dye against ten bacterial species: *B. subtilis, S. aureus, Vibrio fischeri, Yersinia enterocolitica, Proteus vulgaris, Lactobacillus* species, *Lactococcus* species*, Pediococcus pentosaceus, Staphylococcus* species, *Pseudomonas* species, and against five fungal organism: *C. albicans, C. famata, Rodotorula* species*, Aspergillus* spp.*,* and *N. crassa*. To date, there are not many records of the application of EEBS as natural antimicrobial supplements in food matrices with the exception of the study by Zarringhalami et al. (2009) who evaluated the antimicrobial effectiveness of annatto in sausages inoculated with 10^2 spores of *Clostridium perfringes* per 100 g of sample. After 7 days of storage, they found that the *C. perfringes* spores did not germinate the samples treated with annatto.

Direct bioautography assay

To date, several studies have been conducted to determine the compound or compounds responsible for the antimicrobial activities of some plants, and it has been found that the flavonoids and tannins possess antimicrobial properties (Coelho et al., 2003). Some authors have identified the chemical components of the ethanolic extract of *B. orellana* seeds, establishing the presence of several compounds such as bixin, isobixin and norbixin and others like β-carotene, criptoxanthin, lutein, orellin, etc (Chiste et al., 2011). In addition to these compounds, Tamil et al. (2011) have reported the existence of phenolic compounds, flavonoids, saponins, tannins, etc., in alcoholic extracts of *B. orellana* seeds, and its biological activities have been attributed primarily to such components.

Currently, there are numerous reports that analyze the potential antimicrobial effect of phenolic compounds present in plant extracts. The capacity is probably caused by the ability of phenol derivatives to form complexes with proteins, soluble and extracellular, and with the bacterial cell wall (Cowan, 1999). Once the phenolic compound crosses the cell membrane, it may interact with the enzymes or with proteins present in the membrane causing a counter flow of protons through it, thereby affecting the cellular capacity (Conner and Beuchat, 1984).

In vitro EEBS antioxidant activity

Antioxidants are compounds that by delaying or inhibiting the oxidative degradation of molecules help to prevent the formation of unpleasant colours and odours in food.

Phenolic compounds such as tocopherols, tocotrienols and flavonoids have a high ability to capture free radicals (Mustafa et al., 2010).

There is scientific evidence for the responsible compounds for the EEBS antioxidant properties. Adding a mixture of annatto and corn flour (0.4 g/100 g) to ground chicken meat is an alternative to enhance its colour and also to minimize lipid oxidation during storage at -18°C for 120 days (Castro et al., 2011). In another study, the effect of norbixin on DNA damage of *E. coli* cells induced by UV radiation as well as for hydrogen peroxide (H_2O_2) and the superoxide anion were evaluated. This found that norbixin protects cells against these agents increasing their survival at least for ten times (Júnior et al., 2005). Similarly, Kiokias and Gordon (2003) evaluated the antioxidants effects of β-carotene, bixin and norbixin of olive oil and oil/water emulsions at 60°C finding that norbixin was the only carotenoid that is able to inhibit the oxidative deterioration of lipids in both systems with a similar outcome as δ-tocopherol. In results reported by Haila et al. (1996), the antioxidant effect is attributed to bixin since the natural colorant of annatto (which contains a 3.8% of bixin) strongly inhibits the autooxidation of rapeseed oil. Furthermore, it has been reported that the greater anti-radical capacity of the annatto seed was observed in extracts obtained from the most polar solvents (ethanol and methanol) showing the highest content of phenolic compounds (Cardarelli et al., 2007).

Given that the EC_{50} value (8.28±1.63 µg/ml) of the extracts is below 1024 µg/ml, that is, the highest MIC value obtained in the tests of antimicrobial capacity in this study, the results of anti-radical capacity is EEBS promising. This means that a possible application of EEBS in a food product as a natural preservative would take advantage of the antimicrobial as well as the anti-radical activities. In addition to this, several studies have shown that the dye extracted from annatto seeds has no mutagenic, genotoxic or carcinogenic effects, and therefore is safe for human consumption (Paumgartten et al., 2002; Hagiwara et al., 2003; Alves de Lima et al., 2003; Agner et al., 2004). The annatto extract is recognized by FAO/WHO (2006) as a product of no toxicity. The acceptable daily intake for bixin and norbixin is 0.065 mg/kg.

For all the aforementioned, EEBS emerges as a new natural alternative for food conservation that reduces the chemical hazard often implicit in the use of synthetic additives, and thereby safeguarding food safety. The next stage in of our investigation will consist of applying this extract in a specific food matrix and as a consequence evaluating its efficacy *in situ*.

Conclusions

EEBS presented a wide spectrum of inhibition against Gram positive bacteria (*B. cereus*, *S. aureus*, *L. plantarum*, and *L. monocytogenes*) and Gram negative bacteria (*E. coli*, *S. sonnei*, *P. aeruginosa* and *S. typhimurium*). The antimicrobial capacity is higher on Gram positive bacteria: *B. cereus*, *S. aureus* and *L. plantarum*, and inhibits bacterial growth at very low concentrations. The Gram negative more sensitive bacteria to the ethanolic extract were *E. coli* followed by *S. sonnei* and *S. typhimurium*. Furthermore, the MIC values for EEBS are lower for most bacteria found with nisin. Due to its broad spectrum of antimicrobial action against bacteria associated with contamination in meat, dairy and vegetables, this extract is an alternative to use as a natural preservative in any food matrix. Yeasts and molds tested also showed high sensitivity to EEBS with MIC values ranging from 512 to 0.5 µg/ml *S. cerevisiae* being the most resistant fungus to the extract and *P. chrysogenum* the most sensitive.

The EEBS anti-radical capacity results are promising because the EC_{50} value (8.28±1.63 µg/ml) of the extracts is below 1024 µg/ml, which is the highest MIC obtained in antimicrobial test results encountered in this study, that is, the possible application of EEBS in a food product as a natural preservative, its antimicrobial and anti-radical activities could be exploited.

With the goal of replacing chemical nature preservatives in the Food Industry, EEBS becomes a natural alternative of preservation that minimizes the chemical risk with the use of synthetic preservatives, safeguarding food safety. Therefore, studies are required on specific food matrices for evaluating EEBS *in situ* behavior testing the sensorial effect that can be generated upon the product to be preserved, as well as the effect of manufacturing processes and other additives on the antimicrobial and antioxidant properties demonstrated in this study.

The understanding of the mechanisms of antimicrobial action could take researches towards obtaining preservatives from plants and studying its potential application in food matrices.

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