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Original Article

Antioxidant and Antimicrobial Capacity of Phenolic Compounds of Mango (*Mangifera indica* L.) Seed depending upon the Extraction Process

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Abstract

The extraction method is critical for the recovery of phenolic compounds. The main goal was to evaluate the effect of an extraction process from mango seed on their phenolic profile, antioxidant and antimicrobial capacities. Phenolic extraction was performed in different steps: maceration, alkaline hydrolysis, acid/alkaline hydrolysis, polar and non-polar fraction of an ethyl acetate separation.The macerated extract showed a higher variety of polyphenols from mango seed:gallic (138.36 µg/g dry weight), coumaric (65.36 µg/g), ferulic (1376.67 µg/g) , chlorogenic (57.75 µg/g) anddicaffeoylquinic (219.29 µg/g) acids, catechin (16.78 µg/g) and rutin (6678.62µg/g). In alkaline hydrolyzed extract most of these compounds were lost, ferulic acid decreased 1356.77 µg/g dw and gallic acid increased 1383.89 µg/g dw. Gallic and chlorogenic acids increased 165 and 969. 45 µg/g dw respectively in acid/alkaline hydrolyzed, 109.57 and 841.38 µg/g dw respectively in non-polar and 277.15 and 77.88 µg/g dw respectively in polar extracts related to the macerated extract. Rutin was found only in acid/hydrolyzed and non-polar extract in lesser amount (87.62 and 78.51 µg/g dw) compared to macerated extract. The content of phenolic compounds was higher for the macerated extract (phenols=484.42 mg GAE/g and flavonoids=86.59 mg QE/g) than for the other steps. Acid/alkaline hydrolysis increased the antioxidant activity (1787.67 µmol TE/g for DPPH and 3692.86 µmol TE/g for TEAC); while the alkaline hydrolysis increased the antimicrobial effectivity (MIC=2.5 mg/mL for bacteria and 0.5 mg/mL for yeast). Results indicate that the acid or alkaline hydrolysis yields a stronger antioxidant and antimicrobial extract.

Keywords: Maceration, Alkaline hydrolysis, Acid hydrolysis, Phenolic compounds, Plant byproducts extracts

Introduction

Mango (*Mangifera indica* L.) is one of the most cultivated, marketed and consumed fruits in the world occupying the 2nd position as a tropical crop in terms of production and acreage used [1,2]. Its production is increasing due to consumer demand and it is cultivated in more than 103 countries [2]. Such fruit is popular due to its flavor, convenience, nutrients and antioxidants content such as ascorbic acid, carotenoids, and phenolic compounds [3]. Mango is generally consumed in fresh or as juice, pure, syrup, nectar, canned or slices and during its industrial processing the pulp is mainly used while the other parts of the fruit known as byproducts are discarded in huge amounts [2]. Only a fraction of by-products generated is reused and the rest is wasted causing an environmental pollution problem

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if it is not handled properly [4]. Depending of the mango variety, byproducts represent the 40-60% of the total weight of the fruit and specifically the seed represent 13-20%, and the peel the 1-20% [2,5]. Hence, it is important to contemplate different uses for mango byproducts. Nowadays, agricultural byproducts are gaining attention as a novel and economic sources of functional ingredients for containing bioactive compounds including phenolic compounds, flavonoids and carotenoids with antimicrobial and antioxidant properties that can be used as preservatives in foods [3, 6].

Mango seed has traditionally been used to treat some diseases around the world [7]. Several studies have showed that mango seed, as other byproducts, contains higher phenolic compounds and antioxidant capacity than its own pulp [8]. Major phenolic compounds found in mango seed extract are tannins, vanillin, coumaric acid, ferulic acid, caffeic acid, quercetin derivatives, gallic acid, ellagic acid and mangiferin [9]. Antioxidant properties of phenolic compounds is attributed to their ability to inhibit free radical or their propagation and chelate metals that can cause oxidation and damage to important biomolecules [10]. In addition, mango seed extract and its phenolic compounds have been associated to lower risk of major chronic health problems [10].

Phenolic compounds of mango seed have also been associated to antimicrobial properties against *Escherichia coli* O157:H7, *Salmonella C*holeraesuis, *Listeria monocytogenes*, *Staphylococcus aureus*, *Alternaria alternata*, *Bacillus subtilis*, *Bacillus cereus*, *Salmonella typhi* and *Pseudomonas aeruginosa* [3,6]. It has been suggested that phenolic compounds can affect cell membrane's permeability due to their interaction with hydrophilic amino acids of membrane proteins [11]. Therefore, causing alteration of pH and electric potential and subsequently the cellular release of protons, producing bacterial death [12].

During extraction of bioactive compounds from plant materials, yield, composition, and antioxidant-antimicrobial activity may be affected by the type and polarity of the used solvents, time and temperature of the extraction process [13]. Therefore, the extraction method is critical to optimize the recovery of these bioactive compounds [14]. Some phenolic compounds are conjugated to esters or glycosides that cannot be extracted by organic solvents; but, can be released using base, acid or both hydrolysis [15]. Mango seed extracts subjected to acid hydrolysis showed the highest antioxidant activity because of the produced gallic and ellagic acids [16]. In this regard, it is suggested that after hydrolysis, the free form of phenolic compounds may provide more antioxidant activity than the non-hydrolyzed linked phenolic compounds, being a major number of hydroxyl groups available to react as antioxidant of antimicrobial agents [17]. In this context, the present study was aimed to evaluate the effect of the extraction process of phenolic compounds (maceration, alkaline hydrolysis, acid/alkaline hydrolysis, polar and non-polar fraction) from 'Ataulfo' mango seed on their antioxidant and antimicrobial capacities.

Material and Methods

Sample Preparation

Mangoes "Haden" were obtained from a local market in the city of Hermosillo, Sonora, Mexic oduring the period of June to July, 2012. Fruits were selected according to homogenous color and free of physical defects in a commercial maturity stage. Mangoes were sanitized in a chlorine solution (200 ppm), then were cut and seeds were removed from the fruit using a stainless steel knife previously sanitized. The seeds were dried at room temperatureand stored at -20 °C until its use for the extraction of phenolic compounds [3].

Extraction Process

Mango seeds were spliced and the kernel was removed manually. Kernels were lyophilized (E1). Then, 10 g of sample was left to macerate in 100 mL of ethanol 70% in darkness for 10 days at 25 °C. After that time, the extract was filtered to remove insoluble material in multiple layers of cheesecloth obtaining a crude macerated extract (E2). This was taken and the solvent was removed using a rotary evaporator (Büchi RE121, Brinkman, Flawil, Switzerland) and a water bath (Büchi 461, Brinkman, Flawil, Switzerland) at reduced pressure and temperature of 45 °C. Then, such concentrated was hydrolyzed using NaOH 4 M during 4 h in the absence of light (E3). Later, an acid hydrolysis was performed with HCl 4 M adjusting to pH 2.0 (E4). In the next step, the hydrolyzed extract was separated in two phases: aqueous ethanolic phase (E5) and ethyl acetate phase (E6), which were concentrated and dispersed within 10 mL of water [3]. Phenolic compounds and flavonoids, antioxidant and antimicrobial capacity were

evaluated from the obtained extracts: E1 (lyophilized seed), E2 (maceration), E3 (alkaline hydrolysis), E4 (acid/alkaline hydrolysis), E5 (polar phase) and E6 (non-polar phase).

Total Phenolic Content

Total phenolic content was measured by the method described by Singleton and Rossi [18], with some modifications. For this, 75 µL of Folin–Ciocalteu reagent (1:10) and 60 µL of 7.5% Na_2CO_3 were added to 15 µL of the sample in a 96-well microplate (Costar 96). After incubation in the dark for 30 min, absorbance was measured at 765 nm using a FLUOstar Omega spectrophotometer (BMGLabtech, Chicago, IL, USA). Total phenolic compounds were calculated using a standard curve of gallic acid and expressed as milligrams of gallic acid equivalents per gram of dry weight of extract (mg GAE/g dw). All the samples were analyzed in triplicate.

Total Flavonoid Content

Flavonoid content was determined based on the method described by Zhishen *et al.* [19], with some modifications. In darkness conditions, one mL of the sample was taken and added to 4 mL of deionized water, 300 µL of $NaNO_2$ (5%), 300 µL of $AlCl_3$ (10%), 2 mL of NaOH and 2.4 mL of distillated water. After 30 minutes of incubation, absorbance at 490 nm was measured using a FLUOstar Omega spectrophotometer (BMG Labtech, Chicago, IL, USA). Total flavonoid compounds were calculated using a standard curve of quercetin and expressed as milligrams of quercetin equivalents per gram of dry weight of extract (mg QE/g dw). All the samples were analyzed intriplicate.

Ultra-performance Liquid Chromatography for Phenolic Compounds

Ultra-performance liquid chromatography (UPLC) analyses of phenolic compounds were carried out by using an ACQUITY Ultra Performance LC^{TM} system (Waters, Milford, MA, USA) linked simultaneously to a PDA 2996 photodiode array detector (Waters) [20]. The ultraviolet-detection wavelength was set at 280 nm. Empower software (Waters) was used for controlling the instruments as well as for data acquisition and processing. The analysis was performed at 30 °C by using a reversed-phase column (BEH C18 1.7 µm, 2.1 x 100 mm; Waters). The mobile phase consisted of solvent A (7.5 mM acetic acid) and solvent B (acetonitrile) at a flow rate of 250 µL/ min. Gradient elution was used starting at 50/0 solvent B for 0.8 minutes, 5-20% solvent B for 5.2 minutes, isocratic 20% solvent B for 0.5 minute, 20-30% solvent B for 1 minute, isocratic 30% solvent B for 0.2 minute, 30-50% solvent B for 2.3 minutes, 50-100% solvent B for 1 minute, isocratic 100% solvent B for 1 minute, and finally 100-5% solvent B for 0.5 minute. At the end of this sequence, the column was equilibrated under the initial conditions for 2.5 minutes. The pressure ranged from 6,000 to 8,000 psi during the chromatographic rune. The effluent was introduced to a liquid chromatography detector (scanning range, 210-400 nm; resolution, 1.2 nm). The injection volume was 10 µL. The identification was made by comparison of UV spectra, using a database previously made with reference substances. Quantification was performed using standard curves of the corresponding compounds and reported as µg of the compound/g dry weight (dw).

$DPPH^{\bullet}$ Radical Scavenging Activity

The total antioxidant activity was determined using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method which measures the ability of antioxidants to quench the $DPPH^{\bullet}$ stable radical [21]. A stock solution was prepared by mixing 2.5 mg of $DPPH^{\bullet}$ radical with 100 mL of pure methanol. The absorbance of the $DPPH^{\bullet}$ solution was adjusted to 0.70 measured at 515 nm using a FLUOstar Omega spectrophotometer (BMGLabtech, Chicago, IL, USA). Then, 140 µL of the radical solution followed by 10 µL of sample were added in triplicate to a 96-well microplate (Costar 96). After incubation for 30 minutes the absorbance was read at 515 nm in a microplate reader. Trolox was used as a standard and results were expressed as µmoles trolox equivalents per gram of extract at dry weight basis (µmol TE/g dw).

$ABTS^{\bullet+}$ Assay

$ABTS^{\bullet+}$ assay was carried out according to the method of Re *et al* [22]. This assay is based on the antioxidant ability to scavenge the $ABTS^{\bullet+}$ cation radical compared to the scavenging ability of the water-soluble vitamin E analog: trolox. The $ABTS^{\bullet+}$ radical cation was generated by mixing 5 mL of a solution of 7 mM ABTS (2,2-azino-bis(3 ethylbenzothiazoline-6-sulphonic acid) with 88 µL of a 0.139 mM solution of $K_2S_2O_8$. The reaction mixture was kept in the dark at room temperature for 16 h before use. Subsequently, the radical solution was adjusted with ethanol to an optical density of 0.7 measured at 754 nm. For the assay, 5

µL of sample and 245 µL of the ABTS$^{•+}$ solution were added to a 96-well microplate (Costar 96). Thereafter, the absorbance was measure after 6 minutes in a FLUOstar Omega spectrophotometer (BMGLabtech, Chicago, IL, USA). Results were expressed as µmoles Trolox equivalents per gram of extract dry weight (µmol TE/g dw). All samples were determined in triplicate.

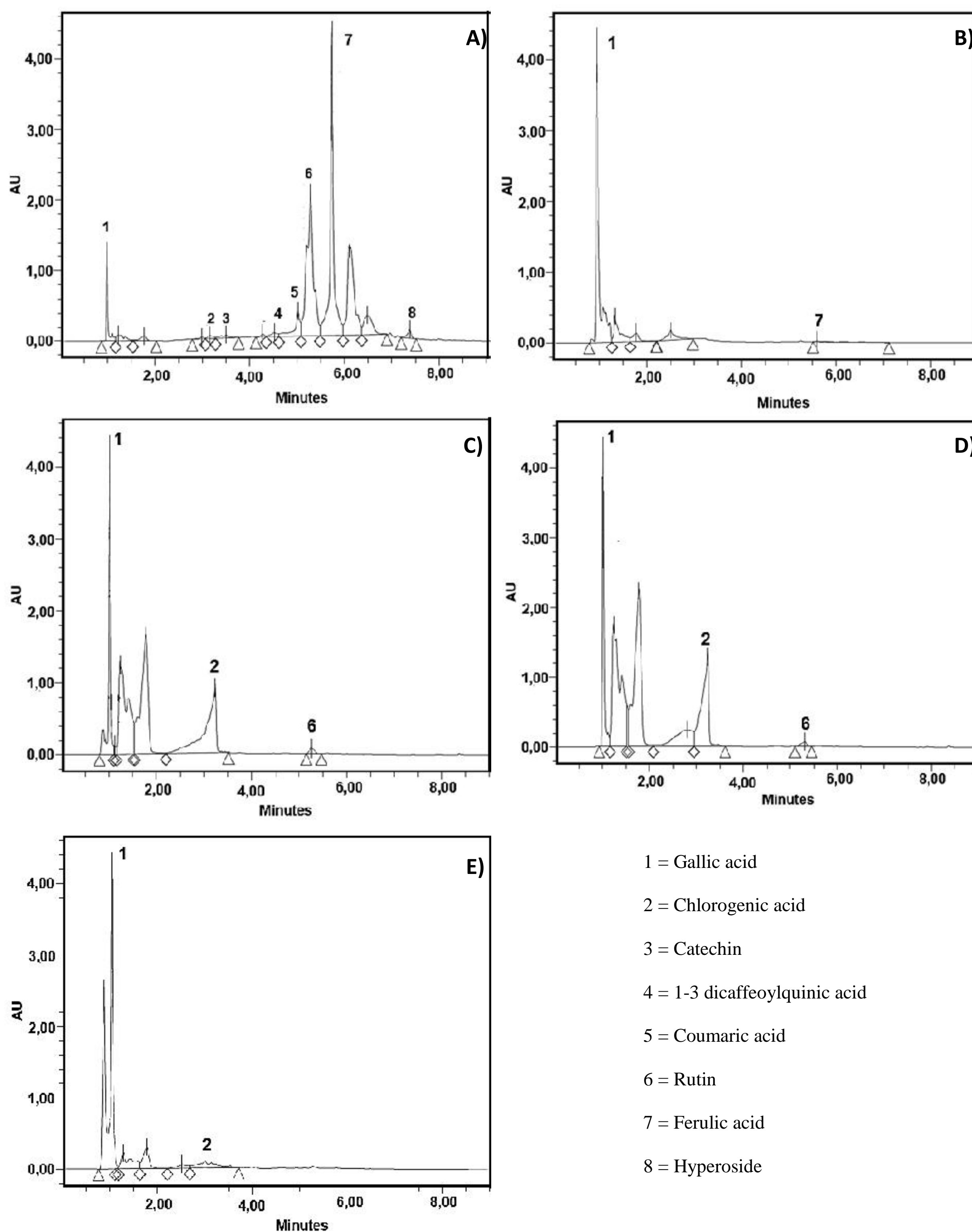

Fig. 1 UPLC-DAD chromatograms showing phenolic compounds identified in mango seed extracts A) Macerated B) Alkaline hydrolysis C) Alkaline/acid hydrolysis D) Polar phase E) Non polar phase.

Antibacterial Activity

The minimum inhibitory concentration (MIC) was determined for each extract against *Escherichia coli* O157:H7 ATCC 43890, *Salmonella* Choleraesuis ATCC 14028, *Listeria monocytogenes* ATCC 7644 and *Staphylococcus aureus* ATCC 65384. A loopful (~20 µL) of bacteria was transferred to a tube containing 10 mL of tryptic soy broth and incubated overnight at 37 °C. The initial inoculums of each bacteria were adjusted to 1 x 10^8 CFU/mL. Then, 5 µL of these inoculums were added to a sterile 96-well microplate (Costar 96), followed by 295 µL of each extract at different concentrations diluted in tryptic soy broth in order to achieve a final inoculum concentration of 1 x 10^6 CFU/mL . The microplate were incubated for 24 h at 37 °C. The MIC value was selected visually, according to the lowest concentration of the extract at which the tested bacteria did not show visible growth expressed as turbidity, and confirmed by agar counting[23]. In addition, growth curves were performed for each bacteria exposed to the extract MIC. The culture conditions were as previously indicated and as negative controls were used 300 µL of culture medium and the extract without bacteria. The plates were incubated at 37 °C during 24 h with intermittent shaking and the optical density at 600 nm was measured every 30 min in a FLUOstar Omega spectrophotometer (BMGLabtech, Chicago, IL, USA). All determinations were made in triplicate. The experimental growth data for each bacterial strain were fitted to the Baranyi function [24] using a complementary tool for Microsoft Excel (D-model, J. Baranyi, Institute of Food Research, Norwich, UK). Kinetic parameters, including lag time (h), maximum specific rate (µmax, UFC/h), and Ymax (UFC) for each growth curve, were calculated using the Baranyi function.

Antifungal Capacity

The MIC was determinate for each extract against *Saccharomyces cerevisiae* growth, similarly to the bacterial procedure previously described. Yeast was inoculated in potato dextrose broth for 24 h at 30 °C. The initial inoculum was adjusted to 1 x 10^8 CFU/mL. Then, 10 µL of this inoculum was added in triplicate to a sterile 96-well microplate (Costar 96), followed by 290 µL of different concentrations of each extract diluted in potato dextrose broth. The microplate was incubated at 25 °C 24 h and the MIC value was selected visually. Growth kinetic parameters were monitored at 25 °C for 24h with the same conditions as for bacteria [25].

Statistical Analysis

Experiments were analyzed using a completely randomized design. The evaluated factors were each step of extraction process (lyophilized seed, maceration, alkaline hydrolysis, acid/alkaline hydrolysis, polar phase and non-polar phase), while the response variables were the total phenol and flavonoid content, antioxidant capacity (DPPH and ABTS assays), MIC and microorganism growth parameters. Analysis of variance (ANOVA) was performed to estimate significant differences (p-value <0.05) between treatments and Tukey-Kramer test was applied for comparison of means (p-value <0.05) using the Number Cruncher Statistical Systems software

Results and Discussion

Phenolic and Flavonoid Content

The total phenol content from mango seed extracts are presented in Fig. 2. The macerated seeds had the highest values (p-value <0.5) for phenolic content (484.42 mg GAE/g), followed by acid/alkaline hydrolyzed (309.20 mg GAE/g) and alkaline hydrolyzed seeds (287.85mg GAE/g). The non-polar seed fraction (214.41mg GAE/g) and whole seed extracts (192.58 mg GAE/g) recorded the lower content in relation to other extracts. The lowest phenol content (192.58 mg GAE/g) was found in the polar phase seed extract. Similarly, the significant (p-value <0.05) differences were recorded for the flavonoid content (Fig. 2). The highest amount was observed for macerated seed (86.59mg QE/g) extracts followed by acid/alkaline hydrolyzed (64.40 mg QE/g), alkaline hydrolyzed (61.89 mg QE/g), no polar phase seed extract (34.20 mg QE/g), and the whole seed (28.01 mg QE/g) extracts. Also, the lowest value was found in the polar phase seed extract (22.54 mg QE/g). In this study, themaceration process produced the highest amount of phenolic content. The maceration is intended to soften and break the cell wall of plant in order to release the soluble phytochemicals [26].

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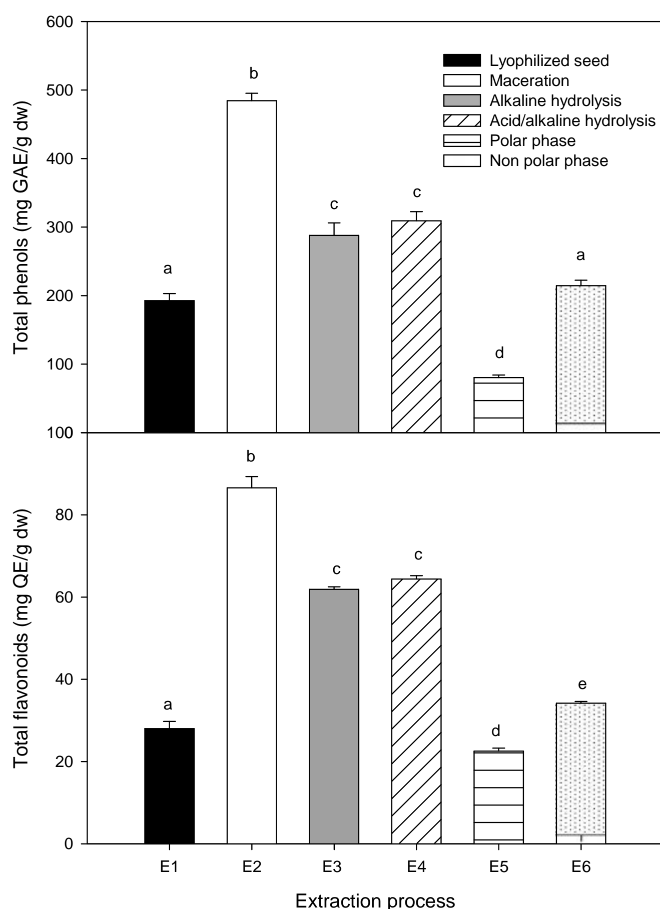

Fig. 2 Bioactive compounds content in mango seed extracts in different steps of the extraction process.

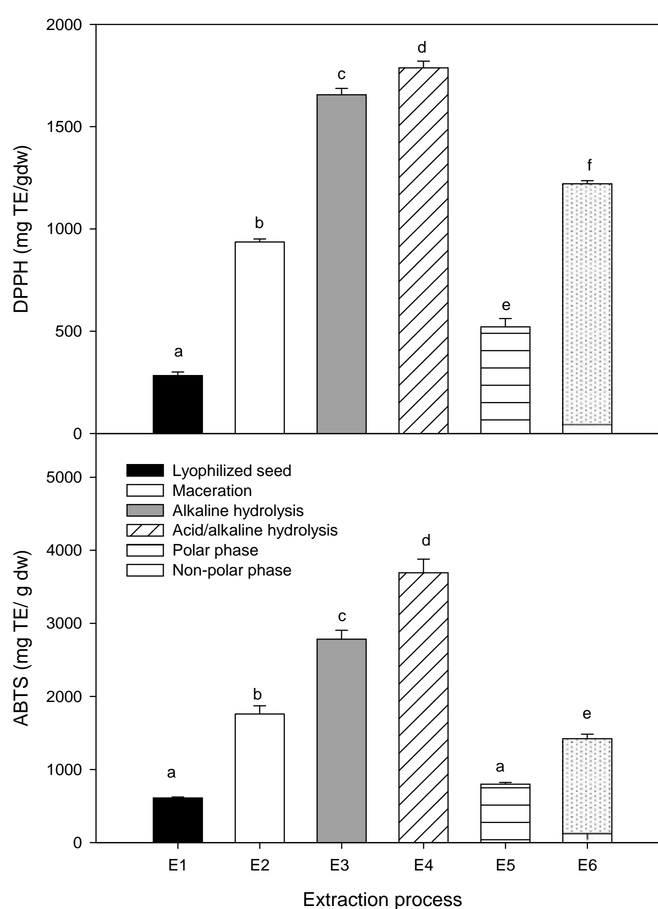

Fig. 3 Antioxidant activity of mango seed extracts in different steps of the extraction process.

Traditionally the solid-liquid extraction such as the maceration of plant material has been the main method to obtain phenolic compounds [27]. Several studies have been tested different solvents and ratios in maceration to recover phenolic compounds since the solvent and the chemical properties have the most important influence [28]. It can be noticed that the maceration in ethanol-water significantly increased the retrieval of phenolic compounds in response to their solubility and affinity. Previous studies have showed that extraction of phenolic compounds with hydro-alcoholic mixtures yields higher amounts [29]. The most widely used extracting solvents for mango byproducts are mixtures of methanol, ethanol, and acetone with water [4, 16, 30, 31]. According to these statements, this could be the reason why the polar phase contained the lowest amount of phenolic compounds.

Our results are in contrast with the study of Maisuthisakul and Gordon [32] who found that the extract of sun dried mango seed prepared with ethanol and acid hydrolysis showed a higher total phenolic content (286 mg tanin acid equivalents per gram (TAE/g)) than other conditions as shaking and refluxing (90 and 212 mg TAE/g respectively). They suggested that acid hydrolysis yielded higher amounts of phenolics, however, they did not contemplate the evaluation of other operations on this response.

We have expected that the acid and alkaline hydrolysis will release higher amount of phenolic compounds than the maceration step. Alkaline and acidic hydrolyses are the most common resources of releasing phenolic compounds. Alkaline hydrolysis breaks the ester bond linking phenolic acids to the cell wall and the acid hydrolysis breaks glycosidic bond and solubilizes sugars [33]. However, it could be possible that hydrolysis caused the loss of some phenolic compounds and that is the reason why the macerated yield the highest phenolic content.

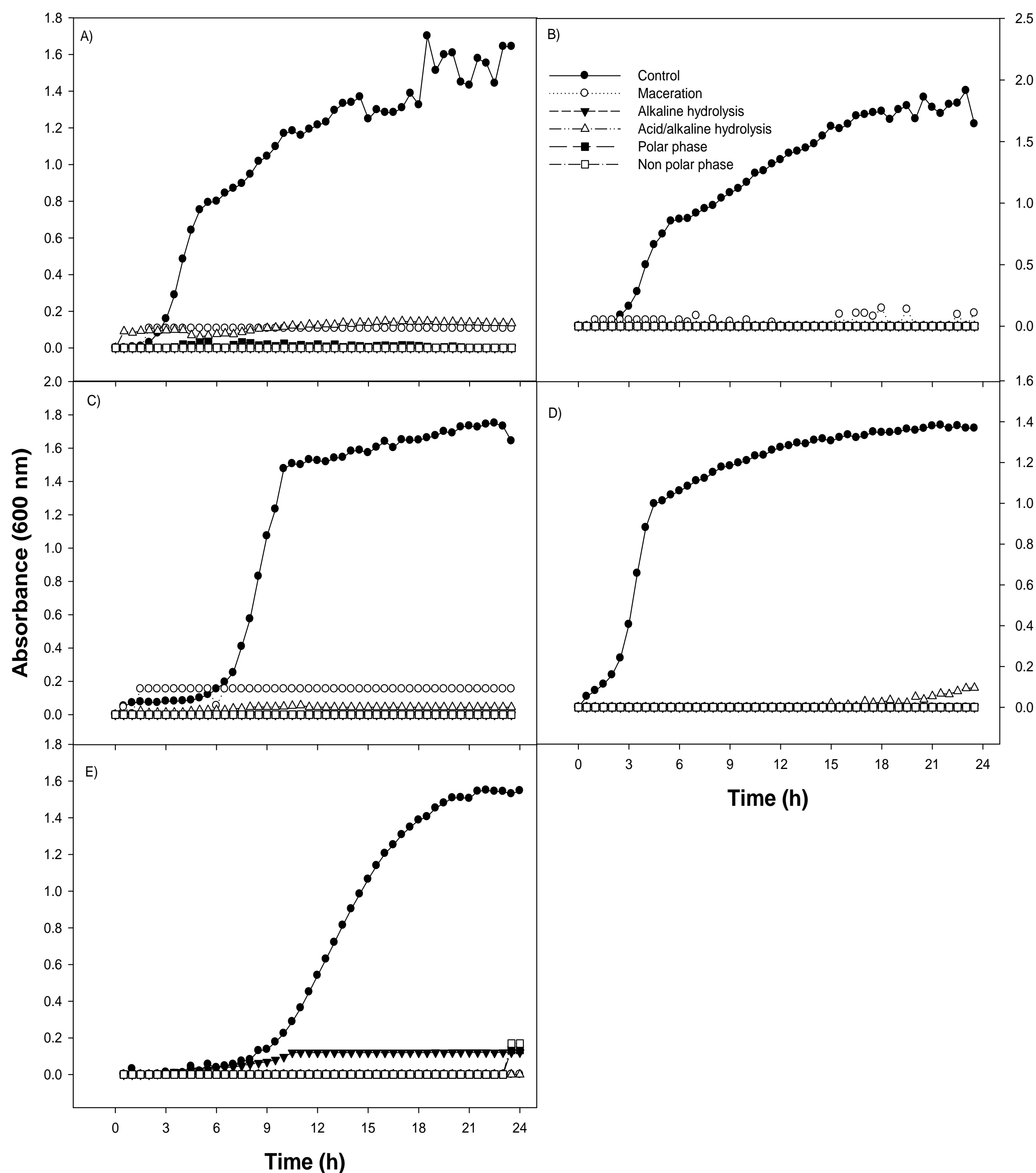

Fig. 4 Effect of the MIC of mango seed extracts in each step of the extraction process on the kinetic parameters of A) *E. coli*,B) *S. aureus*,C) *S.* cholerasuis, D) *L. monocytogenes* and E) *S. cerevisiae*.

Antioxidant Activity by DPPH and ABTS Radicals Assay

The antioxidant properties of mango seed extracts in each step of extraction were determined as free radical-scavenging ability by DPPH and ABTS methods (Fig. 3). A significant effect (p-value <0.05) on the antioxidant capacity was found among the extraction steps. The acid/alkaline hydrolyzed extract showed the highest antioxidant activity with 1787.67 μmol TE/g for DPPH inhibition and 3692.86 μmol TE/g for ABTS assay, followed by the alkaline hydrolyzed seed (1656.21 and 2784.03 μmol TE/g for DPPH and ABTS respectively), macerated seed (935.95 μmol TE/g for DPPH assay and 1759.93 μmol TE/g for ABTS), and the no polar phase with 1220.51 and 1421.00 μmol TE/g for DPPH and ABTS respectively.

Table 1 Ultra-performance liquid chromatography analysis for individual phenolic compounds changes during the extraction process.

Compounds (µg/g dw)	Retention time	Extraction process				
		Macerated	Alkaline hydrolyzed	Acid/alkaline hydrolyzed	Non polar	Polar
Gallic acid	0.986	138.36	1522.15	304.13	247.93	415.51
Chlorogenic acid	3.156	57.75	-	1027.20	899.13	135.63
Catechin	3.503	16.78	-	-	-	-
1,3 dicaffeoylquinic acid	4.522	219.29	-	-	-	-
Cumaric acid	5.022	65.36	-	-	-	-
Rutin	5.284	6678.62	-	87.62	78.51	-
Ferulic acid	5.734	1376.67	19.90	-	-	-
Hyperoside	7.376	298.88	-	-	-	-

Table 2 Minimum inhibitory concentration (MIC) of mango seed extract at different step of an extraction process.

Seed extract	MIC (mg/mL)				
	S. aureus	*E. coli*	*L. monocytogenes*	*S.* Cholerasuis	*S. cerevisiae*
Macerated	6	6	6	6	10
Alkaline hydrolyzed	2.5	2.5	2.5	2.5	0.5
Acid/alkaline hydrolyzed	5	5	5	5	0.2
Polar	24	24	24	24	12
Non polar	16	16	16	16	6

The lowest antioxidant activity was observed in the polar phase extract with 521.03 and 798.26 µmol TE/g for DPPH and ABTS, respectively, and in the whole seed (283.06 and 610.48 µmol TE/g for DPPH and ABTS respectively). Simple phenolic acids and flavonoids are the main phenolic compounds found in plant materials and they commonly occur as soluble conjugated to sugars or as insoluble or bound forms [33]. It has been demonstrated a significantly higher antioxidant capacity for the insoluble phenolics compared to free and soluble conjugated phenolics [33]. Therefore, in our study phenolics released by hydrolysis could have a higher antioxidant potential. There are few studies comparing the antioxidant properties of mango seed extract or other byproducts extracts in different steps of an extraction process. Maisuthisakul and Gordon [32] revealed that acid hydrolysis presented the highest values of antioxidant activity for DPPH (4.16 A_{AR}, 1/EC_{50}) and ABTS (1.41 mmol of trolox/g) assays compared to shaking (1.75 A_{AR}, 1/EC_{50} and 1.03 mmol of trolox/g, respectively) and refluxing (2.60 A_{AR}, 1/EC_{50} and 1.14 mmol of TE/g, respectively) conditions in the sun dried mango seed extract; however, this study did not contemplated the combination of hydrolysis. This result is attributed to the higher phenolic content due to that some phenolic conjugates are released by acid hydrolysis and the free form could provide a stronger antioxidant activity, same as in our study.

Vega-Vega *et al.* [3] assessed different methods for the extraction of phenolic compounds from mango seed. They compared two solvents (ethanol and methanol) for the step of maceration; then all macerated were hydrolyzed with NaOH and HCl, and each extract was subjected to a separation with ethyl acetate yielding two fractions: polar (aqueous phase) and non-polar (ethyl acetate phase). The results obtained were that the ethanol non-polar extract showed the major antioxidant activity with an EC_{50} of 0.04 mg/mL for DPPH assay and 272.41 mmol TE/g for ABTS assay than ethanolic polar, methanolic-polar and methanolic-non-polar extracts, after an acid/alkaline hydrolysis. However, this study did not evaluate the changes on the antioxidant and antimicrobial activities of the extract during the extraction process [3]. The effect of extraction solvent at 25 °C on the capacity of mango seed extracts to scavenge free radicals (TE and DPPH) was evaluated by Dorta *et al.* [28], showing that the acetone:water (1:1) was the most effective solvent to obtain a higher antioxidant extract followed by the ethanol:water and methanol:water at the same ratio possibly by the affinity of phenolic compounds profile to the polarity of those solvents.

The bound phenolic compounds in rice bran, the byproduct of rice milling process, were released by

an extraction with ethyl acetate and alkaline hydrolysis [34]. In addition, among phenolic compounds found in the extract, *trans*-ferulic acid was the major bound phenolic constituent in rice bran with strong antioxidant activity. In our study, the ferulic acid or gallic acid might be the responsible for the antioxidant potential of the extract obtained by alkaline/acid hydrolysis. In other study, pomegranate, peel was subject to several extraction methods to obtain an enriched polyphenolic extract [35]. The authors combined conventional extraction methods with others bases on the use of less polar solvents. The results revealed that a higher content of polyphenols was gained in the repartition in ethyl acetate due to ellagic acid, commonly found in pomegranate is poorly soluble in polar solvents [35]. However in our study, main phenolic compounds found in mango seed are soluble in polar solvents. Also, in our study the extract was separated in two phases after hydrolysis, in these fractions the content of phenolic compounds and antioxidant activity appears to decrease and this might be due to a possible distribution (or dissociation) of compounds hydrolyzed in accordance to their polarity in each of the phases (polar and non-polar). The less polar solvents such as ethyl acetate are used for the less polar compounds [28].

Ultra-performance Liquid Chromatography for Phenolic Compounds

Table 1 and Fig. 1 show the phenolic compounds identified in each step of the extraction process. The macerated extract presented the most varied phenolic profil ethan the other extracts. The major compounds identified in this extract were simple phenols such as simple phenols such as gallic (138.36 µg/g dry weight), coumaric (65.36 µg/g), ferulic (1376.67 µg/g) and dicaffeoylquinic (219.29 µg/g dw) acids, flavonoids such as catechin (16.78 µg/g dw) and flavonoids glycosides such as rutin (6678.62) and chlorogenic (57.75 µg/g) acids. In the other side, the alkaline hydrolyzed extract lost most of the compounds observed in the macerated extract, showing as main compounds gallic and ferulic acid which increased 1383.89 µg/g dw and decreased 1356.77 µg/g dw respectively, compared to its content in the macerated extract. Gallic and chlorogenic acids were found in a larger amount in the rest of the extracts compared to the macerated extract. Gallic and chlorogenic acids increased 165 and 969. 45 µg/g dw, respectively in acid/alkaline hydrolyzed, 109.57 and 841.38 µg/g dw, respectively in non-polar and 277.15 and 77.88 µg/g dw respectively in polar extracts. Rutin was found only in acid/hydrolyzed and non-polar extract in lesser amount (87.62 and 78.51 µg/g dw) compared to the macerated extract.

Results show that hydrolysis has an effect over the diversity of phenolic compounds. Phenolic compounds with higher molecular weight and bounded to a sugar were found in macerated extracts such as rutin, hyperoside and 1,3-dicaffeoylquinic acid in comparison with the other extracts. This in accordance to total phenolics where the macerated extract showed the highest content. Lower molecular weight phenolics were found in hydrolyzed extracts; this could be explained due to the action of alkaline and acid hydrolysis that can free phenolic compounds from glycosidic or ester bound [36]. In addition, it occurred a loss of phenolic compounds during alkaline and acid hydrolysis as previously reported, for example some hidroxicinamic acids such as caffeic, *o*-coumaric, *p*-coumaric, isoferulic, ferulic acids. This could be the reason why a lower diversity and quantity in phenolic compounds were found in hydrolyzed extracts. In the case of polar and non-polar extracts, composition was similar to hydrolyzed extracts, differing in content between phases.

Antimicrobial Activity

Table 2 shows the minimum inhibitory concentrations (MIC) of each extract against different microorganisms used for this test. The alkaline hydrolyzed seed extract was the most potent antimicrobial agent against bacteria and yeast with a MIC of 2.5 mg/mL and 0.2 mg/mL respectively, followed by the acid/alkaline hydrolysis extract with MICs of 5 mg/mL for bacteria and 0.5 mg/mL for yeast. Next, the maceration extract presented MICs value of 6 mg/mL and 10 mg/mL for bacteria and yeast, respectively. Then, the non-polar phase achieved concentrations of 16 mg/mL and 6 mg/mL for bacteria and yeast inhibition, respectively. And finally, the polar phase was the less potent extract to inhibit these microorganisms with MICs of 24 mg/mL and 12 mg/mL for bacteria and yeast, respectively. Fig. 4 ratified the inhibitory effect of mango seed extracts on the growth of *S. cerevisiae*, *E. coli, S.* Cholerasuis, *S. aureus* and *L. monocytogenes*.

In a previous study, the antimicrobial effect of mango seed kernel ethanol extract against 18

species of bacteria was evaluated [30]. The extract presented a broad antimicrobial spectrum and was more effective for Gram (+) than Gram (-). In our study, it could not be observed differences between the Gram status. The range of MICs reported for enteropathogenic *E. coli* was 1-2.5 mg/mL and for *Salmonella* was 2.5 mg/mL. MICs for Gram (+) were 50 and 100 µg/mL for *S. aureus* and *L. monocytogenes*, respectively. This MICs were lower that the obtained in our work for each bacteria. A mango seed kernel methanolic extract at concentration of 100 mg/mL showed an antimicrobial effect on methicillin resistant *S. aureus* and *E. coli*. The extract was able to inhibit a major zone (mm) than a commercial antibiotic chloramphenicol as a positive control [37].

Furthermore, mango seed extract has been reported as effective antimicrobial against fungus. Vega-Vega *et al*. [3] reported that the ethanolic non-polar extract from mango Haden seed showed 89.78% of inhibition in *Alternaria* growth applying 6.25 mg/mL of the extract. Also, this extract caused 100% inhibition of bacteria like *S.* Cholerasuis, *S. aureus* and *L. monocytogenes* and 84.5% for *E. coli* at a concentration of 25 mg/mL.

Comparing the antioxidant activity to the antimicrobial potential of each extract, it can be noticed that a greater ability to inactivate free radicals increases the lethality against microorganisms, since the extracts with highest antioxidant capacity such as alkaline and acid/alkaline hydrolyzed extracts, are also the best antimicrobials. This could be attributed to the phenolic monomers in these extracts, which are smaller molecules and can more easily pass through the membranes of microorganisms to perform its inhibitory effect [38].

Conclusion

In the present work the content of phenolic compounds,antioxidant and antimicrobial activities from mango seed extract at different steps of an extraction process have been determined. The study suggested that the macerated extract increased the profile variety of phenolic compounds in mango seed extract. Also, the results demonstrated a significantly higher antioxidant capacity and antimicrobial activity against pathogenic and deteriorative microorganism with an acid/alkaline hydrolysis of mango seed. These findings contribute to generate a stronger antioxidant and antimicrobial mango seed extract and to consider the potential of such extract as alternative use for the discarded byproducts. These could reduce environmental problems, create new sources of bioactive compounds and provide a greater economic returns to agroindustries.

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