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Microwave assisted extraction of polyphenols of *Artocarpus heterophyllus* Lam. with antifungal activity against *Alternaria* sp.

Extraccion asistida por microondas de polifenoles de *Artocarpus heterophyllus* Lam. con actividad antifúngica contra *Alternaria* sp.

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Technological innovation: Microwave-assisted extraction was optimized as a function of obtaining *Artocarpus heterophyllus* leaf extracts with antifungal activity against *Alternaria sp.*

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Resumen

Introducción. La jaca (*Artocarpus heterophyllus* L.) constituye un cultivo estratégico, no sólo por su fruto, sino también porque es una fuente importante de proteínas y fitoquímicos con actividad antimicrobiana y otras propiedades. Teniendo en cuenta la incidencia de *Alternaria* spp., como microorganismo fitopatógeno y transmitido en alimentos, es interesante investigar las posibilidades de utilizar extractos de hoja de *A. heterophyllus* en procesos postcosecha de frutos dañados por este hongo. El objetivo del presente trabajo fue determinar las condiciones óptimas para el recobrado de polifenoles (PF) de hoja de jaca con actividad antimicrobiana contra una cepa de *Alternaria* sp. aislada del tomate, mediante la extracción asistida por microondas (EAM). Metodología. Los extractos se obtuvieron con etanol acuoso (4:1, v/v), en una relación 1:10 (hoja en polvo: disolvente). Se optimizó el efecto simultáneo de la potencia (600-1080 W) y el tiempo de extracción (1-3 min) en la concentración de fenoles solubles totales (FST) y flavonoides totales (FT) y la actividad, antifúngica (inhibición del crecimiento micelial), utilizando un diseño factorial 3^2. En todos los casos se registró la temperatura. El perfil polifenólico de los extractos de EAM obtenidos en condiciones óptimas se caracterizó utilizando el análisis de HPLC-MS. Se estudió el efecto de la extracción en 3 ciclos. Resultados. El aumento de potencia y tiempo hasta 840 W y 2 min favoreció el incremento de FST (148.8 mg GAE / g de ps) y FT (13.3 mg ER/ g ps). En estas condiciones se obtuvo 39.9% de inhibición del crecimiento micelial, con 1 mg. mL^{-1} de FST. Los

valores previstos de FST, FT y AA por el modelo, fueron satisfactorios en comparación con los valores experimentales. Además de ello, la EAM en 3 ciclos permitió obtener extractos con 277.3 mg EAG/ mg ps y 23 mg de ER/mg ps de FST y FT, respectivamente. Diferentes flavonoides y algunos ácidos fenólicos y orgánicos, reportados en extractos de jaca y de especies de *Ficus* fueron identificados tentativamente. Implicaciones. Los compuestos responsables de la actividad antifúngica deben ser aislados y encapsulados para mejorar su eficacia en futuras aplicaciones agroalimentarias. Originalidad. Por primera vez se notifica la actividad antifúngica de los extractos de hoja de *A. heterophyllus* contra *Alternaria* sp. Conclusiones. Se determinaron las condiciones óptimas de EAM para la extracción de polifenoles de hoja de jaca con actividad antimicrobiana significativa contra *Alternaria* sp (840 W y 2 min) y el efecto de increment del número de ciclos en la extracción de PF. Los resultados sugieren las potencialidades de este residuo como fuente de agentes antimicrobianos naturales para los procesos de postcosecha de tomate y otros cultivos.

Palabras clave: extracción asistida por microondas, polifenoles, *Artocarpus heterophyllus*, actividad antifúngica, *Alternaria sp.*

Abstract

Introduction. Jackfruit (*Artocarpus heterophyllus* L.) constitutes a strategic crop not only for its fruit, but also because it is a source of protein and different phytochemicals with antimicrobial activity and other properties. Taking into account the incidence of *Alternaria* spp., as a phytopathogenic and foodborne microorganism, it is interesting to investigate the possibilities of using *A. heterophyllus* leaf extracts in postharvest processes of fruit, damaged by this fungus. The objective of the present work was to determine the optimal conditions for the recovery of leaf jackfruit polyphenols with antimicrobial activity against an *Alternaria* sp. strain isolated from tomato, using microwave assisted extraction (MAE). Methodology. The extracts were obtained in ethanol: water solution (4:1, v/v), 1:10 leaf powder: solvent ratio. The simultaneous effect of power (600-1080 W) and extraction time (1-3 min) on the concentration of total soluble phenols (TSP) and total flavonoids (TF), and the antimicrobial activity (inhibition of mycelial growth) was optimized, using a factorial design 3^2. Temperature was registered in all cases. The polyphenolic profile of MAE extracts obtained at optimal conditions was characterized using HPLC-MS analysis. Besides that, 3 cycles MAE procedure was studied. Results. The increase of power and time until 840W and 2 min favored the increment of FST (148.8 mg GAE / g dry weight) and TF (13.3 mg RE / g dry weight) contents. At these conditions, 39.9% mycelial growth inhibition with 1 mg. mL^{-1} TSP was exhibited. Predicted TSP, TF and AA by the model values were satisfactory compared with the experimental values. Furthermore, the MAE in three cycles allowed to obtain extract with 277.3 mg GAE/ mg DW and 23 mg RE/ mg DW of TSP and TF respectively. Different flavonoids and some phenolic, and organic acids reported in jackfruit and other *Ficus* species extracts were tentatively identified. Implications. The compounds responsible by antifungal activity must be isolated and encapsulated to improve their effectiveness for future agrofood applications. Originality. The antifungal activity of *A. heterophyllus* leaf extracts against *Alternaria* sp. is being reported for the first time. Conclusions. The optimal MAE conditions for the polyphenolic jackfruit leaf extraction with significant antimicrobial activity against *Alternaria* sp were determined (840 W and 2 min). The results suggest the potentialities of this residue as a source of natural antimicrobial agents for postharvest processes of tomato and other crops.

Keywords: microwave assisted extraction; polyphenols; *Artocarpus heterophyllus*; antifungal activity; *Alternaria* sp.

1. Introduction

According to the circular economy guidelines, the valorization of agro-food waste in México has showed growing attention due to the possibilities to recover high biological value compounds (HVBC) and biopolymers with potential applications in agrofood and other industries[1]. This approach has pushed the application of the emerging innovative solid-liquid extraction techniques such as pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE), and their optimization by response surface methodology (RSM) to produce high-added-value extracts [2-7].

Particularly, MAE is considered a green technology, less energy, solvent volume and time consumer that have been applied for recovering of polyphenols (PPhs) with antimicrobial and antioxidant properties from different vegetable matrixes [8-13]. The principle on which MAE is based is the dielectric heating, that is the process in which a microwave electromagnetic radiation heats a dielectric material by molecular dipole rotation of the polar components present in the matrix. The vegetal tissues will be modified, and extraction of analites enhanced [12, 13]. The successfully process will depend on multiple factors such as power, frequency, and time irradiation, composition, moisture content and particle size of sample, type and concentration of solvent, solid: liquid ratio, and number of extraction cycles[10-12].

Jackfruit (*Artocarpus heterophyllus* Lam.) from *Moraceae* family is commercially cultivated in some Asian countries, Australia, Florida (USA), the Caribbean, and Latin America, due to the high nutritional value and reported health benefits of this tropical fruit. In México the economic importance of this evergreen crop has significantly increased in the last years, reaching a production value around of USD 3.7 million[14].

At the same time, the attention has been dedicated to the study of jackfruit tree waste approach. Among different parts of the plant, great attention has been dedicated to leaf, due to its high protein and phytochemical contents (flavonoids, phenolic acids, terpenoids, etc.) in comparison to fruit, seed, stem and peel [15-20].

Particularly, in Nayarit State about 80 % of Mexican jackfruit production is covered, supported by 10 ha of leafy trees, which are pruned twice a year giving more than 10 thousand ton/ha of biomass. Really, its utilization would constitute an alternative source of protein and PPhs, and avoid environmental problems[5-7, 21].

Specifically, a broad antimicrobial activity has been evidenced by *A. heterophyllus* leaf extracts against foodborne bacteria and some phytopathogenic fungi[15, 16, 19]. Recently, polyphenolic extracts from *A. heterophyllus* leaf obtained by MAE, PLE and UAE methods[4], showed inhibitory action against mycelial growth and spore germination of *Coletotrichum gloesporioides* and *Penicillium italicum* strains. However, no reports have been found about the use of jackfruit extracts against *Alternaria* spp., which represent a cause of more than 50% of losses of tomato and other high commercial value fruit, and potential risk for consumers and animals due to its biosynthesis mycotoxin capacity [21, 22].

Considering this background, the present work was aimed to optimize the MAE process to maximize the recovery of polyphenolic extracts from *A. heterophyllus* leaf with antimicrobial activity against *Alternaria* sp.

2. Materials and equipments

2.1. Biological materials

The jackfruit tree leaves were collected in Zacualpan (Compostela, Nayarit, Mexico; latitude 21.248, longitude, 105.167, altitude 15 m), from July to September, 2019. The leaves were washed and disinfected with 2% sodium hypochlorite solution and distillated water, and dried for 24 h at room temperature (30 ± 2 °C). After that, were ground (grinder NB-201, Nutribullet, China), sieved through a standard 150 µm sieve and stored at 4°C. The *Alternaria* sp. strain was isolated from some infected tomatoes, collected in local markets of Tepic.

2.2. Chemicals

Folin-Ciocalteu 2N phenol reagent, gallic acid, and rutin were purchased from Sigma Aldrich (St. Lois, MO, USA), potato-dextrose-agar (PDA) from Becton Dickinson (Cuautitlán, Izcalli, México). Other chemicals were of reactive grade quality (sodium carbonate, aluminium chloride, sodium acetate, 98% ethanol, methanol, acetic acid, potassium persulphate, sodium phosphate and ferrum chloride), purchased from Thermo Fisher Scientific Inc.

3. Experimental methods

3.1. Extraction process

Previously, 20 g DW samples were mixture with the solvent for 10 min at room temperature and at 300 min^{-1}. The process was carried out using the ethanol: water mixture (4:1, v/v), at ratio of 1:10 (powder aqueous ethanol), in a domestic microwave oven system WMC30516 with power control (Whirpool, Benton Harbor, Michigan, USA) [5].

The MAE treatments were applied with 30 s pause intervals and no additional agitation was applied. The temperature was measured with a glass thermometer (± 2°C), each 30 s, and after the treatments the samples were maintained in an ice bath until 25°C was reached. The extracts were filtered (Whatman No. 41 paper) and centrifuged at 5000 g for 10 min. Additional, 3 cycles MAE procedure was carried out at selected conditions.

The solvent was removed by a vacuum rotary evaporation V10 basic (IKA, USA) at 50°C. All extracts were dried by FreeZone 4.5 Liter Freeze Dry System for 48 h (Labconco, Kansas City, USA) and stored at 4°C. For determination of total soluble phenols (TSP) and total flavonoids (TF) contents, and antimicrobial action (AA), extracts were dissolved in aqueous ethanol (2%) to obtain 1 mg. mL^{-1}. concentration.

3.2. Chemical analysis

3.2.1. Total soluble phenols

The TSP analysis was carried out with the Folin-Ciocalteu 2N phenol reagent[5] with modifications. After incubation at 25°C in darkness for 90 min, the absorbance was measured at 760 nm. Gallic acid was used as standard, and results were expressed as mg of gallic acid equivalents (GAE)/g of dry weight (DW) extract.

3.2.2. Total flavonoids

For TF determination reaction with flavonoid reagent, containing $AlCl_3$ was developed[5]. The absorbance was read at 530 nm, after incubation in darkness, for 30 min at 25°C. Rutin was used as standard, and results were expressed as mg of rutin equivalent (RE/g DW).

3.2.3. HPLC-MS analysis

The qualitative determination of compounds was carried out using an Agilent HPLC 1260 series/ MS spectroscopy 6100 system, with an Agilent Poroshell 120 EC-C18 column (5 µm and 4.6 mm x 250 mm) (Agilent Technologies, CA, USA). After injection of 10 µL of filtrated

extracts samples, a gradient elution was programmed using acidified water (0.6% acetic acid) and acetonitrile as mobile phases[4] at 0.3 mL. min^{-1}. Analysis was carried out using negative ion mode[5]. The spectra were acquired over a mass range from 50 to 1000, using capillary voltage- 4000 V, drying gas temperature- 210°C, drying gas flow- 8 mL. min^{-1}, and nebulizing gas pressure- 29 psi.

3.3. Antifungal activity

Antifungal activity was determined as a mycelial growth inhibition of *Alternaria* sp. strain. It was pre-cultured on PDA for 7 days at 28 ± 2°C. Then, PDA plugs (6 mm) were placed onto PDA medium containing the extracts [5]. In all cases, the growth diameter variation was measured after 7 days at 28 ± 2°C with a Trouper Vernier and the inhibition percentage was calculated according to Eq. 1.

$$Inhibition\ (\%) = \frac{dc-dt}{dc} x\ 100 \qquad \textbf{(Eq. 1)}$$

where: *dc* (mm) is the mean diameter of colony for the control sets and *dt* (mm) is the mean diameter of colony for the treatment sets.

2.6. Statistical analysis

All analytical assays were performed in triplicate. The confidence intervals for 95% of values (mean values ± 1.96 x standard deviation (S) were reported. An ANOVA was applied, using STATISTICA 10 software for Windows (StatSoft, Inc.). The dates with $p \leq 0.05$ were considered statistically significant.

A 3^2 factorial design was used. The experiences were performed three times (3 blocks and 27 randomized runs). Power (A- 600, 840 and 1080 W) and time (B- 1, 2 and 3 min) were considered independent variables. Results were fitted to a second-order polynomial model and multiple regression coefficients were determined for dependent variables (TSP, TF and AA) using the least squares method. The adequacy and statistical significance of terms were tested by ANOVA ($p \leq 0.05$). The coefficient of multiple regression (R^2) and model lack-of-fit (F) were used as indicators of the model adequacy. Determination of the optimal conditions was based on the desirability function value. The model validation was carried out comparing the experimental data with generated predicted values.

4. Discussion and results

The table 1 shows the characterization of extracts obtained by MAE at different power and time conditions.

4.1. Polyphenols content behavior

The experimental data of TSP and TF ranged from 95.6 ± 3.0 to 146.2 ± 2.0 mg GAE/g DW and from 6.2± 0.1 to 14.3± 1.2 mg RE/g DW, respectively (Table 1). These values are relatively inferior to those obtained for jackfruit leaf extracts obtained previously, with the same solvent at 45°C [5] (164.0 ± 6.3 mg GAE/g DW of TSP for extracts at 900 W after 3 min, and 16.3 ± 2.00 mg RE/g of TF, after 2 min). These differences must be caused by some factors related to the biological material origin and experimental procedures for management and extraction process[23]. The present work was developed using leaves collected in other zone of Nayarit and in different season period. Besides that, the used room-dried procedure could favor a partial PPhs decomposition caused by hydrolytic enzymes presence[24]. In addition, during extraction, the temperature increase was not limited.

Considering other studies about *A. heterophyllus* extraction carried out applying conventional methods, some authors [25] showed significant lower values for

methanolic jackfruit leaf extracts using maceration (TSP from 30.92 to 37.39 mg GAE/g DW and TF from 5.02 to 6.70 mg RE/g DW). Furthermore, hydroethanolic extracts with TSP and TF contents of 195.1 ± 1.2 mg (GAE/g DW and 11.0 ± 0.2 mg (AE)/g DW respectively, by continuous stirring at 200 min^{-1} for 3 days were obtained [24]. Meanwhile, leaf aqueous extracts with 101.1-183.3 mg GAE/g DW of TSP and 23.5-43.2 mg RE/g DW of TF by maceration were recovered, at 11-448 min, 5-100°C, and 13:1–47:1, ratios solvent: sample relation (mL: g) [20]. However, for these both last cases, the extraction efficiency was low, because long times were used. In fact, MAE superiority with respect to traditional methods is due to it less energy, solvent volume, and time consumption [11, 12].

During MAE, microwaves are absorbed and converted into thermal energy. The movement of dipolar molecules or ions will cause rubbing, and vibration, due to the variable electric field action. A pressure will build up inside the biological material, will modify its structure.

Table 1. MAE recovery of *A. heterophyllus* leaf phenolic extracts with antimicrobial activity against *Alternaria* sp. using an experimental design 3^2.

Power (W)	Time (min)	T (°C)	TSP (mg GAE/g DW)	TF (mg RE/ g DW)	AA* (%)
600 (-1)	1 (-1)	55	95.6 ±3.0	6.2 ± 0.1	28.8 ± 1.1
840 (0)	1 (-1)	75	114.3 ± 2.1	9.2 ± 0.7	41.7 ± 1.3
1080 (1)	1(-1)	80	109.0 ± 3.1	9.6 ± 0.4	37.9 ± 0.5
600 (-1)	2(0)	73	117.5 ±4.3	9. 2 ± 0.5	32.3 ± 0.4
840 (0)	2(0)	80	137.4 ± 2.4	12.6 ± 0.5	43.6 ± 0.4
1080 (1)	2(0)	80	137.3 ± 1.5	11.7 ± 1.4	36.2 ± 0.5
600 (-1)	3(1)	78	125.1± 2.9	11.6± 1.3	32.9 ± 0.8
840 (0)	3(1)	80	146.2 ± 2.0	14.3± 1.2	45.1 ± 0.5
1080 (1)	3(1)	82	129.1 ± 4.4	9.7 ± 0.1	35.8 ± 2.0

Each data represents the mean of randomized runs with three replicates ± 1.96 S,
*****AA- antifungal activity as mycelial growth inhibition of *Alternaria* sp. (%) at 1 mg. mL^{-1} of TSP.

and enhance metabolite extraction [9-12]. This causes a decrease of dielectric constant of the solvent, better mass transfer, and improved PPhs yield recovery [9, 13].

Besides the influence of the matrix/ solvent relation, their physicochemical properties and composition will define the PPhs selectivity and stability behavior during the extraction, and this depends strongly on the temperature [9, 12, 26]. The increment of the temperature was exhibited for all power conditions (600, 840 and 1080 W).

The temperature and polyphenolic content fallow-up evidenced that the temperature increment favored the polyphenolic extraction.

These events influence on the solubility, stability, antimicrobial and antioxidant properties of flavonoids and other PPhs[26-28].

Then, the determination of the temperature profile during MAE process must be carried out because it could significantly affect the PPhs recovery. It will depend on the sample volume and composition, and equipment design. In fact, it will define the extraction patterns of the PPhs [12]. But, the differences were evident at 600 W mainly. After 2 min

treatment very similar temperature values were registered.

Often, the satisfactory results of recovery from different vegetable sources have been obtained at temperatures even until 110°C, but after few minutes MAE treatments [9]. However, after two minutes the temperature increment affected the content of the compounds (Table 1). Depending on their structural properties heat generated by microwave energy diminishes their content due to the chemical modification or decomposition, respectively. In fact, under the effect of temperature, PPhs may suffer structural changes, related to the addition, or removal of OH^- groups, and their acylation, methylation, glycosylation, etc.[26, 29].

4.2. Antifungal activity behavior

The antifungal activity (AA) expressed as mycelial growth inhibition from 34.2 to 43.8% was observed, applying 1 mg. mL^{-1} of FST (Table 1). Previously, the antifungal satisfactory effect against *Alternaria alternata* has been reported with some polyphenolic extracts. The control of *A. alternata* strain infecting tomato by 80-100 µM of caffeic acid phenethyl ester, a propolis component, was reported (30-32.5%) [30]. Therefore, phenolic extracts of "chiltepin" (*Capsicum annum var. glabriusculum*) caused 38.5% mycelial growth inhibition of *A. alternata* at higher concentration (100 mg. mL-1) [31].

Regarding the antifungal properties of *A. heterophyllus* extracts, some results have been exhibited. The antifungal activity of silver nanoparticles with *A. heterophyllus* leaf extract against *Aspergillus niger* sp. was detected[32]. Besides that, the growth inhibitory activity has been evidenced for *Fusarium moniliforme* and *Saccharomyces cerevisiae* by the chitin binding lectin present in the jackfruit seeds (jackin)[19]. Recently, the inhibitory effect of 80% hydroalcoholic leaf extracts obtained by MAE (900 W at 2 min), at 0.5 and 2 mg/g DW, for *C. gloesporioides* L. (37.17 ± 0.25 and 36.21 ± 0.65%, respectively) and *P. digitatum* L. (34.21 ± 0.13% and 40.79 ± 1.3%, respectively) was reported [5].

In fact, our results gave evidences about the microbicide activity of jackfruit polyphenolic leaf extracts, against *Alternaria* sp., through its growth inhibition, which has not been reported previously. Its antifungal action could be related by suppression of mycotoxin production also.

In this sense, it is important to consider that the microbial inhibition effectiveness of the extracts will depend on the concentration and chemical composition, and synergism of different components [5, 23]. The PPhs can cause multiple disturbs at membrane level due to their interaction with proteins and lipids, inhibition of biomolecules synthesis such as enzymes, nucleic acids, etc., and inhibition of cellular processes where these molecules take part. Then, susceptibility of the fungal species to these phenomena is an important factor that must be considered. This can vary among strains even of the same species, and could depend on the composition and structural membrane characteristics, culture medium, environmental conditions and time of microbial growth also [23].

4.3. TSP, TF and AA optimization

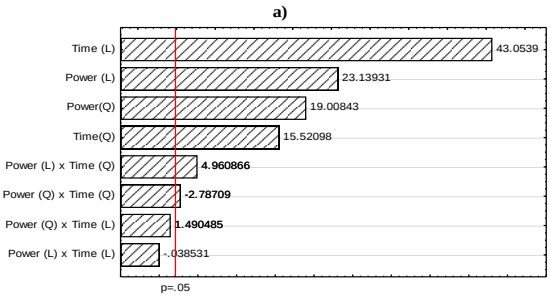
The response surface analysis was applied to optimize the PPhs recovery from jackfruit leaf with antifungal activity against *Alternaria* sp. strain.

Results were fitted to a second-order polynomial model and multiple regression coefficients for power and extraction time effects and their interactions for each dependent variable were determined. The

Pareto graphics of standardized effects (Fig. 1 a, b, c) show the significant independent variables and their interaction. Meanwhile, the Fig. 2 (a, b, c) exhibits the interaction among power and time, and their corresponding effect on the TSP, TF and AA by the response surface 3D plots analysis.

4.4. Effect of power and time on TSP

According to the Fig. 2a, the power (A) and the extraction time (B), and their quadratic terms A^2 and B^2 showed a negative significant effect on TSP ($p \leq 0.05$).



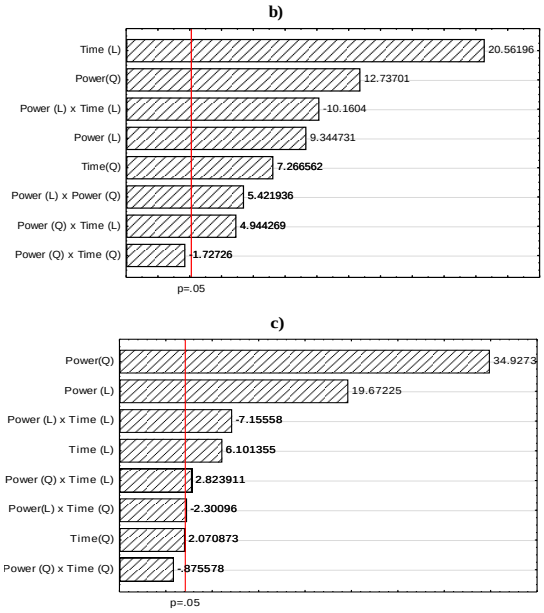


Figure 1. Pareto graphics significance of power and time and their interaction effects on, TSP (a), TF (b) and AA (c) of *A. heterophyllus* leaf extracts (significant terms, $p < 0.05$).

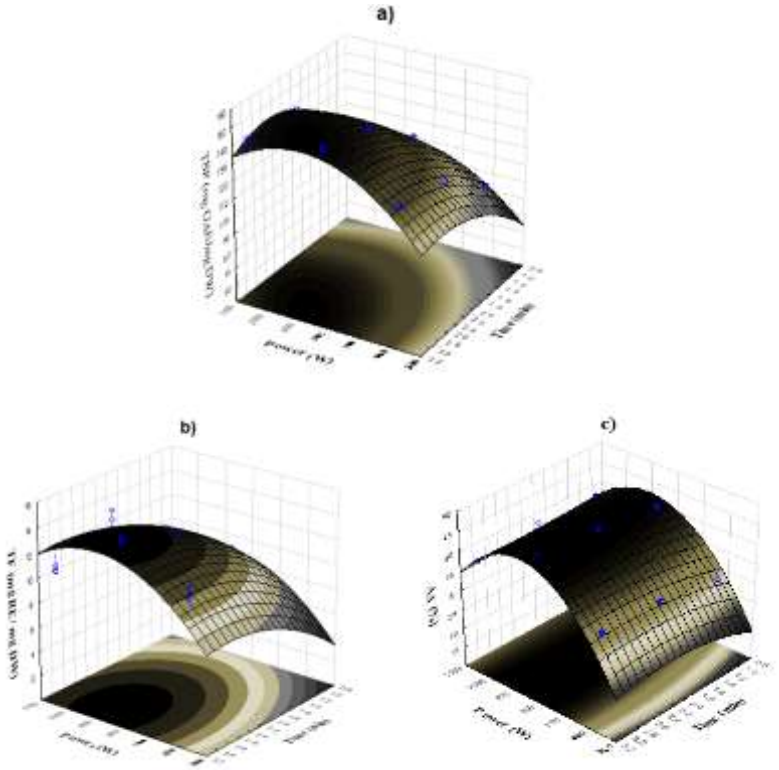


Figure 2. Response surface plots for TSP (a), TF (b) and AA (c) of the extracts of *A. heterophyllus* as function of power and time of MAE process.

Furthermore, the interactions of AB and AB^2 were found to be significant also ($p < 0.05$). Then, the model equation for TSP was obtained (Eq. 2).

$$TSP = -171.05 + 0.60\,A - 0.004\,A^2 + 155.85\,B - 38.38\,B^2 - 0.33\,AB + 0.09\,AB^2 \quad \textbf{(Eq. 2)}$$

Based on the regression coefficient values, the time of extraction (B) produced the highest effect on the TSP content. The non-significant value of lack of fit F = 0.99 and R^2 = 0.99 were obtained. This behavior shows that the model fitted well the experimental data and had a good prediction.

4.5 Effect of power and time on TF

For TF content, the power (A) and extraction time (B) showed a significant positive effect ($p \leq 0.05$), while their quadratic terms, A^2 and B^2, exhibited a negative effect, suggesting that TF content reached a maximum value and then decreased mainly with the increased time. The interactions AB, AB^2, A^2B were significant also (Fig. 1b). The coded equation for TF was defined (Eq. 3).

$$TF = -19.99 + 0.06\,A - 0.0004\,A^2 + 11.81\,B - 4.30\,B^2 - 0.03\,AB + 0.013\,AB^2 + 0.00002\,A^2B \quad \textbf{(Eq. 3)}$$

The values of F = 0.97 and R^2 = 0.98 showed that the model fitted well the experimental data and had a satisfactory prediction. The interaction effect between A and B on TSP and TF show that when power and time increased, both variables gradually increased until a maximal value, during the first two minutes mainly. The raising temperature could cause a significant effect on leaf cell wall integrity, allowing a major releasing of cellular components such as PPhs [3, 8-10]. Moreover, last years some reports have demonstrated that temperatures superior to 70°C ensure a maximal solubility of these compounds. Then, the solubility is recognized as a key parameter for designing and optimizing extraction processes[12, 26-28]. This behavior was demonstrated for hydroalcoholic chatechin solutions[27], and for naringin and its derivatives in water, ethyl acetate, and binary hydroalcoholic mixtures solutions[28]. On the other hand, due to the complex chemical structures and higher molecular mass of the condensed tannins, temperatures until 110°C may be necessary to get their liberation from vegetal biological matrices [27].

Elsewhere, when the treatment was extended until 3 min, a gradual decrease of TSP and TF contents was observed. It has considered that overheating at 80-82°C, may cause structural modifications or decomposition of some PPhs, as was mentioned previously [9, 26, 29, 33].

4.6. Effect of power and time on AA

The antimicrobial action was expressed as the percentage of inhibition (%) of mycelial growth of *Alternaria* sp. strain. In this case, significantly positive linear effects of both variables (A and B) was observed. Furthermore, negative quadratic effects of A^2 and B^2 were significant for this model also ($p \leq 0.05$). It indicated that AA reached a maximum and then starts to decrease rapidly with increasing of both power and time parameters. This suggests the relation of antifungal action with PPhs concentration in extracts. Among of variables interactions, A^2B and AB^2 were significant (Fig. 2c). Therefore, the model equation was derived as shown below (Eq. 4):

$$AA = -97.54 + 0.29\,A - 0.002\,A^2 + 29.92\,B - 8.7\,B^2 - 0.05\,AB + 0.00002\,A^2B + 0.02\,AB^2 \quad \textbf{(Eq. 4)}$$

The power was a major significant variable (Fig. 2 c), according to the regression

coefficients values for lineal and quadratic terms. The model predicts well the behavior of this response according to R^2= 0.98 and non-significant lack of fit, F = 0.98 values.

4.7. Validation of optimal extraction conditions

Considering the response surface graphs (Fig. 3 a, b, c) the optimal MAE parameters (power and time) were performed by maximizing the TSP, TF and AA, respectively. The model validation indicated that 840 W and 2 min were the optimal levels for the simultaneous maximization of dependent variables. At these conditions no significant differences were observed between the predicted and the experimental values (Table 2).

Desirability value of 0.83 was obtained, suggesting that theoretical model was in concordance with the experimental results. Concerning the optimization of MAE process to maximize content and properties of PPhs from

Table 2. Predicted extract TSP and TF contents, and AA by the model vs. experimental values.

Values	TSP (mg GAE/ mg DW)	TF (mg RE/ mg DW)	AA (%)
Predicted	137.3	12.5	43.5
Experimental	148.75 ± 6.5	13.3 ±1.0	39.9 ± 1.1

Experimental data represent the mean of three replicates ± 1.96 S, *****AA- antifungal activity as mycelial growth inhibition of *Alternaria* sp. at 1 mg. mL^{-1} of TSP.

different vegetal sources, it has been demonstrated that the RSM is a good way for multi-response optimization, adjusting quadratic polynomial models[8-11, 34-36]. For instance, the optimized MAE of coriander seeds PPhs at 19 min, 63% ethanol, and 570 W showed yields of TSP and TF (311.23 mg GAE/ 100 g DW, 213.66 mg catechin equivalent/100 g DW, respectively), and extracts antioxidant capacity (IC_{50}- 0.0315 mg. mL^{-1}) [34]. On the other hand, during MAE optimization of PPhs recovery from basil (*Ocimum basilicum* L.) by this same methodology, the maximum content of extracted TSP, TF and antioxidant capacity was obtained at 15 min, 50% of ethanol and 442 W) [35]. These results evidenced that the PPhs recovery will depend on the biological matrix characteristics, extraction conditions and PPhs physicochemical properties[13, 26, 30].

4.8. Influence of cycles number in MAE results

In order to achieve a maximum yield of the polyphenolic extracts of the jackfruit leaf, the process was carried out in three cycles. The table 3 shows that this procedure allows to significantly increase TSP (46.4%) and TF (42.2%) contents, obtaining an extract with 277.3 mg GAE/ mg DW and 23 mg RE/ mg DW of TSP and TF respectively. The additional cycles allow greater changes in matrix microstructure, and the use of fresh solvent volume improves the mass transfer favoring the PPhs recovery[13, 30]. However, according to yield values two cycles will adequate.

Table 3. Yield of MAE cycles of *A. heterophyllus* polyphenolic leaf extracts.

Cycles	TSP (mg AGE/ mg DW)	%	TF (mg RE/ mg DW)	%
I	148.8 ± 6.5^{a}	53.65^{a}	13.3 ± 1.0^{a}	57.83^{a}
II	94.8 ± 6.1^{b}	34.19^{b}	7.3 ± 1.^{b}	31.65^{b}
III	33.73± 4.6^{c}	12.17^{c}	2.4 ± 1.2^{c}	10.53^{c}
Total	277.27	100	23.0	100

Data represent the mean of three replicates ± 1.96S, values represented by different letter are significantly different at $p \leq 0.05$.

4.9. HPLC-MS identification

The chemical profile of leaf extracts at optimal MAE conditions (840W and 2 min) was characterized analyzing relative

abundance of deprotonated molecules and ionized products, and their mass/charge values (m/z) according the ion chromatograms. Reported accurate m/z values, fragmentation patterns, molecular mass of compounds previously identified in alcoholic extracts of *A. heterophyllus* leaf [5] and peel [37], and *Ficus* species leaf [38] were considered.

Thus, 17 signals corresponding mainly to flavonoids and some phenolic, and organic acids were tentatively identified. The flavonoids were the predominant compounds presented in extracts (12 compounds) (Table 3). Signals for two apigenin C-glycosides, apigenin-6,8-C-diglucoside (vicenin 2) and apigenin 8-C-xyloside-6-C-glucoside (vicenin 3), and isoschaftoside were tentatively identified (Table 3). The presence of vicenin 3 was also detected in ethanolic jackfruit leaf extract[5]. Elsewhere, among flavonols, quercetin glucoside- O-rutinoside and isorhamnetin glycoside were the major identified compounds, and benzyl-acetylpentosylhexoside glucoside were detected also.

Regarding the phenolic acids, three hydroxycinnamic acids derivatives were estimated. Signals corresponding to molecular ion and MS fragments of chlorogenic, 3,5-dicaffeoylquinic and *cis*-5-O-*p*-coumaroylquinic acids. Besides that, signals corresponded to oxylipin compound, (trihydroxy-octadecadienoic acid was registered, which was reported for jackfruit peel extracts also [38]. Finally, quinic and [5-glucopyranosyloxy-2-oxo-2, 3-dihydro-1H-indol-3-yl] acetic acid were the organic acids detected (Table 3). These both compounds were reported in methanolic jackfruit peel extracts also [40]. Meanwhile, quinic acid was found in hydroethanolic leaf extract [5] obtained by MAE.

Table 4. Characterization of polyphenolic profile of A. heterophyllus leaf extract obtained at 840W and 2 min.

No	Compound	Formula	$[M-H]^-$ (m/z)	Reported fragments	Detected fragments	Presence
1	Catechim (+)[a, b,c]	$C_{15}H_{14}O_6$	288.99	271.15, 245.20	282, 244.7	++
2	Epicatechin (+) [b,c]	$C_{15}H_{14}O_6$	289.16	245.19,	290, 244	++-
3	Epicatechin-O-rhamnoside	$C_{21}H_{24}O_{10}$	435.13	271.05, 150.03, 125.02	435, 271, 150,125	+
4.	5,7,8,4′-tetrahydro-xyflavanone (Carthamidin) [c]	$C_{15}H_{12}O_6$	286.56	**259.18**, 243.41, 219.31	259.5, 244, 243.2	++
5	Naringenin [c]	$C_{15}H_{12}O5$	271.24	**271.14**, 177.29	271, 176	+
6	Naringin [c]	$C_{27}H_{32}O_{14}$	579.16	**271.25**,253.37, 232.70, 225.43	271,250.6, 234, 224, 225.9	+
7	Apigenin 8-C-xyloside-6-C-glucoside (Vicenin 3) [b]	$C_{26}H_{28}O_{14}$	563.15	**473.2**, 443.17, 353.28	473.0, 551.4	+
8	Isoschaftoside [b]	$C_{26}H_{28}O_{14}$	563.20	545.22, **473.22**, 353.3	543, 473.1, 384, 358	++
9	Apigenin-6,8-C-diglucoside (Vicenin 2) [b]	$C_{27}H_{30}O_{15}$	593.30	503.19, **473.22**, 383.38, 353.39	595.0, 473.0, 353.1	+
10	Quercetin glucoside-O-rutinoside [c]	$C_{33}H_{40}O_{21}$	771.23	463.15, **301.18**, 255.27	771, 255.6, 466.6	++
11	Quercetin-3-O-glucoside (Isoquercitrin) [c]	$C_{21}H_{20}O_{12}$	463.49	343.10, **301.17**, 179.19	343.4, 178.3,301.3	+
12	Benzyl-acetylpentosylhexoside [c]	$C_{20}H_{28}O_{11}$	443.15	**401.14**, 131.03, 101.0	401.1, 131, 100.1	+
13	9,12,13 Trihydroxy-octadecadienoic acid [c]	$C_{18}H_{32}O_5$	327.21	**211.1,** 197.11,183.14, 171.10	326.8,209.6,196.7, 182.2,171.2	+
14	Chlorogenic acid [b]	$C_{16}H_{18}H_9$	352.75	**191.2**, 179.38	351.4,190.8, 178.3	+
15	*cis*-5-O-*p*-coumaroylquinic acid [b]	$C_{16}H_{18}O_8$	336.82	191.20, 173.17, **163.07**, 119.24	173.2, 163.3, 119.7	++
16	3,5-dicaffeoylquinic acid [b]	$C_{25}H_{24}O_{12}$	515.31	**353.25**,335.59, 267.66	351.4, 356.3, 265	+
17	Quinic acid [a,c]	$C_7H_{12}O_6$	191.05	129, 115, **111**, 103, 101	111.0,101.0102.8	++

Vázquez-González et al. (2020) (a); Elhawary et al. (2018) (b); Zhang et al. (2017) (c).

The tentatively detection of the major compounds justifies the antifungal activity of *A. heterophyllus* leaf extracts obtained at 840 W and 2 min. (+)-Catechin is one of the most important proanthocyanidins, with antimicrobial, antioxidant, radical-scavenging and other properties[27, 37, 38]. Regarding to carthamidin (5,7,8,4′-tetrahydro-xyflavanone), it has been evidenced that most of the 5,7-dihydroxyflavonoids inhibit the *P. italicum* respiration rate, which is one of the most important antimicrobial mechanisms of flavonoids[5, 23]. Meanwhile, *Ficus pyriformis* extracts with significant isoschaftoside content showed a high antimicrobial activity against *Candida albicans*[38]. Furthermore, the inhibition of spore germination or reduction of mycelial growth by this compound has been evidenced against some phytophatogenic fungi such as *Sclerotinia sclerotiorum, Fusarium solani, Verticillium dahliae, Botrytis cinerea* and *Cercospora sojina*[39]. Even, a sub-minimum inhibitory concentration (μg.-L^{-1}) against *Aspergillus fumigatus* biofilm formation was detected for this phenolic acid[40].

Among flavonols, quercetin and its derivatives are the major polyphenolic flavonoids found in various vegetables and fruits. Moreover, these compounds revealed capacity to control the oxidant–antioxidant balance in biological systems[41]. Recently, antifungal activity of *Larrea tridentata* PPhs were registered against *B. cinerea, C. gloeosporioides, F. oxysporum* and *A. alternata*. Among compounds with antifungal and antioxidant properties quercetin was identified [42]. On the other hand, at minimum inhibitory concentrations of apigenin-7-O-glucoside and apigenin, reduction of intra- and extracellular reactive oxidative species was achieved, suggesting that reactive oxidative species inhibition could be a mechanism of antifungal action against *C. albicans* [43].

Regarding hydroxycinnamic acids derivatives, ethyl p-coumarate exhibited pronounced antifungal activity against *in vitro* mycelial growth of *A. alternata*, with half-inhibition concentration of 176.8 μg. mL^{-1}. Spore germination of the pathogen was inhibited in a dose-dependent manner. Moreover, *in vivo* test confirmed that both 100 and 800 μg. mL^{-1} of the compound, significantly reduced disease development of black spot rot in jujube fruit [44].

The results suggest that antifungal action of *A. heterophyllus* leaf extracts against *Alternaria* sp. depends on the polyphenols content and their chemical composition as recently was evidenced for *C. gloesporioides* and *P. italicum* strains also [5]. This behavior will depend on the multiple factors related with the PPhs recovery extraction (matrix composition, extraction conditions), and microbial sensibility properties, as was previously analized.

5. Conclusions

The response surface methodology was a good tool for simultaneous optimization of the microwave assisted extraction parameters that allows to maximize the extraction of polyphenolic compounds with antifungal activity against *Alternaria* sp. The evaluated model was successfully validated. The best extraction results, 148.75 mg GAE/g DW of TPS, 13.28 mg RE/g DW of TF, and 39.9 % of fungal mycelial growth inhibition were obtained at 840W and 2 min. The polyphenolic profile of jackfruit leaf extracts obtained at optimal conditions showed the tentatively presence of flavanols, flavanones, phenolic and quinic acids. This suggests that microwave assisted method could be used as a relative selective extraction method of PPhs with antimicrobial activity. It will depend on the PPhs content and chemical composition of *A. heterophyllus* leaf extracts.

Considering, the antimicrobial activity of *A. heterophyllus* leaf extracts against *Alternaria* sp., these results suggest the potentialities of their use as antimicrobial agents for the infection control in tomato and other crops during postharvest processes. Then, the efficient management of the jackfruit is an opportunity for Nayarit state, not only due to the commercial value of this fruit. Biorefinery approach for processing of this crop could be considered, including the use of the derived green biomass, to push sustainable alternatives of HBVC and protein recovery with potential use in own agrofood industry.

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