OBTAINING PHENOLIC COMPOUNDS USING HYDROALCOHOLIC SOLVENTS FROM CORN STALKS AND FTIR CHARACTERIZATION OF THE OBTAINED EXTRACT

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ABSTRACT

Phenolic compounds are important natural antioxidants present in various plant matrices. The extraction efficiency of these compounds is influenced by the type of solvent used, with alcohols being frequently used due to their polarity and solubilization capacity. This study investigates the extraction of phenolic compounds from corn stalks using different alcohols combined with microwaves. The dried stem underwent solid-liquid extraction (1:10 g/ml) using ethanol, methanol, and isopropyl alcohol plus 20% water in microwaves (100 W, 35 ºC, 5 min). FTIR analysis was employed to examine solvent-functional group interactions in the extracts. Ethanol yielded the highest flavonoid content (10.51 ± 1.16 mg/g). Methanol and ethanol showed no significant difference in phenolic extraction (23.9 ± 0.32 and 23.72 ± 1.05 mg/g, respectively), while isopropyl alcohol resulted in 16.72 ± 0.07 mg/g of total phenolics. FTIR analysis indicated solvent-organic compound interactions, particularly in regions with O-H bonds (3400-3200). This study demonstrates that alcohols are effective solvents for extracting bioactive compounds.

Keywords: Alcohool. Solvents. Extraction. Bark corn. Phenolic compounds.

1 INTRODUCTION

Solvents are one of the essential components in the solid-liquid extraction process, as they facilitate the recovery of bioactive compounds important for industries, such as pharmaceutical and food. Therefore, some considerations are important when choosing the solvent, such as selectivity and chosen plant matrix[1]. In this sense, alcohols are widely used due to their unique physicochemical properties, such as molecular interactions; the ease of forming hydrogen bonds with organic compounds that have polar functional groups[2]. Phenol hydroxyl groups (O-H) are bioactive molecules linked to a benzene ring. These bioactive molecules have been obtained using polar solvents such as alcohol, water, and its mixture. The phenolic compounds are interesting to the pharmaceutical, cosmetic, and food industries due to their antioxidant, antimicrobial, anticancer, and anti-inflammatory properties[3].

To obtain these compounds, it is necessary to use techniques that do not degrade them during the extraction process, therefore, modern extraction techniques are being studied and applied to expand the range of bioactive available for use at laboratory and, finally, industrial levels. Among them, microwaves are widely studied to obtain bioactive compounds such as phenolics. It is explained that it is environmentally friendly, as it consumes little solvent, energy, and time when compared to conventional methods. Furthermore, it has a versatility of materials that are used as a source of bioactives[4]. A rich source of phenolic compounds is lignocellulosic biomass, such as corn and its residues. It is estimated that 50% of the corn harvest stalks, therefore, a waste that can pose environmental concerns if not used. This material is rich in compounds and has been explored as a potential source of bioactives[5].

Fourier transform infrared spectroscopy (FTIR) is a widely used analytical technique to identify materials and characterize their chemical properties. This work applied the method to analyze the chemical structures and possible modifications in the absorption bands of the functional groups of the hydroalcoholic solvents and extracts obtained. In this sense, the objective of this work is the comparative analysis of three different alcohols, ethanol (C_2H_5OH), methanol (CH_3OH), and isopropyl (C_3H_8O), in terms of the potential for extracting phenolic compounds from corn stalks using microwaves as a technique extractive.

2 MATERIAL & METHODS

Biomass preparation: Sanitized by immersion in water/bleach solution, followed by washing in running water. After this, drying was carried out at 60 ºC in an oven until constant humidity. After that, corn stalks were milled to the range size (≤ 40 mesh) using the Tyler sieves series.

Microwave extraction: A microwave (Discover SP – CEM) was used. The biomass was mixed with three different hydroalcoholic solutions hydroalcoholic solutions prepared with ethanol, methanol, isopropanol, and 20% water (v/v), under the conditions of solid/liquid ratio 1:10 g/ml, 100 W power, 35 ° C for 5 min.

Fourier Transform Infrared Spectroscopy (FTIR): FTIR analyses were performed by direct reading on an Agilent Cary 630 FTIR, coupled to an ATR (Attenuated Total Reflectance). The scanning range used was 650 to 4000 cm^{-1} with 32 scans at a resolution of 4 cm^{-1}.

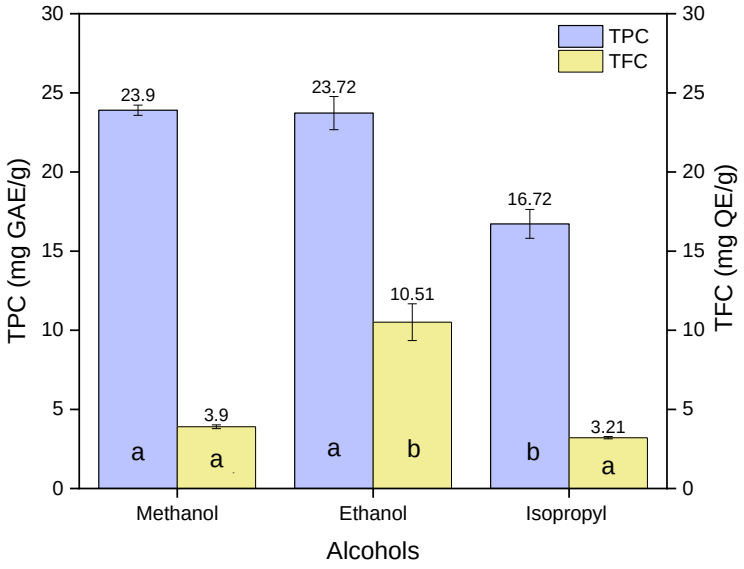
Total Phenolic and Total Flavonoid Content: The methodologies with adaptations described by Santos et al. (7). The Total Phenolic Content (TPC) was quantified in a calibration curve (y = 0.0093x + 0.0781; R2 = 1), being expressed in mg gallic acid equivalents per gram of extract (mg GAE/g). The Total Flavonoid Content (TFC) (was quantified by the curve y = 0.0054x + 0.059; R^2 = 0.9983 and expressed in mg equivalents of quercitin per gram of extract (mg EQ/g).

Statistical analysis: Excel® software was used for one way ANOVA analysis followed by the Tukey test. Values were considered significantly different using $p < 0.05$.

3 RESULTS & DISCUSSION

Overall, the results of the present study show that flavonoids and other phenolic compounds are present in the hydroalcoholic extract of corn stalks. Under the established conditions, it was possible to obtain 23.72 ± 1.05; 23.9 ± 0.32, and 16.72 ± 0.07 mg/g, respectively of TPC. As for flavonoids, ethanol had a better performance in relation to the other solvents, being 10.51 ± 1.16; 3.9 ± 0.12, and 3.21 ± 0.12 mg/g for ethanol, methanol and isopropanol, respectively. The TPC and TFC contents are shown in Figure 1.

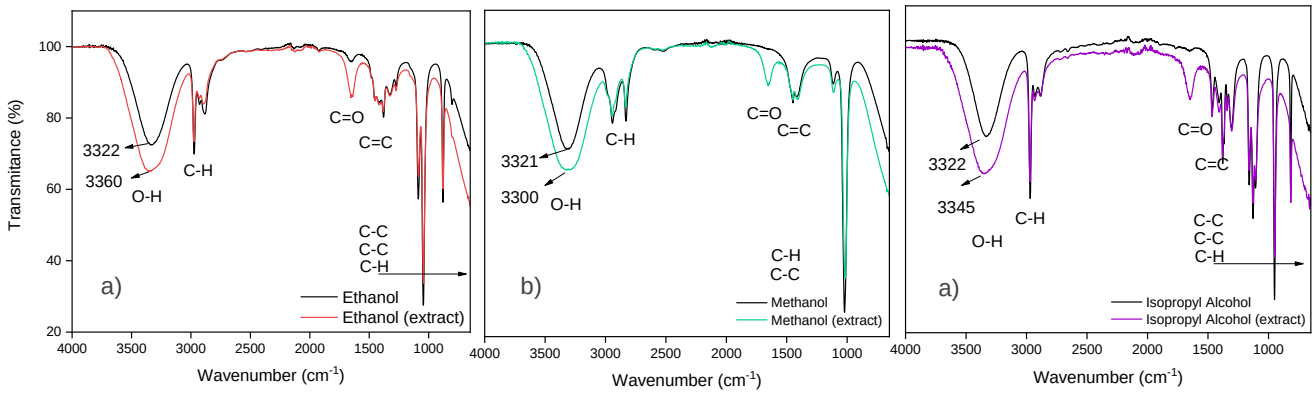
Figure 1 *Quantification of Total Phenolic Compounds and Total Flavonoid Content. Anova was applied to the TPCs to confirm the non-significant difference in extractions at a significance level of 5%. The same letters are significant differences.*



It is observed that methanol and ethanol had no significant difference in obtaining TPC. However, ethanol stood out in obtaining flavonoids, one of the phenolic compounds most present in plant cells. Ethanol is used in a wide range of compounds and plant matrices, largely due to its low toxicity concerning the other two alcohols, mainly methanol, confirming ethanol as a suitable solvent for extraction. Furthermore, ethanol selectivity relationships may be more ideal for corn stalks than other solvents[3,8,9].

In Figure 2abc it is possible to observe the main functional groups observed in extracts and solvents. It is possible to verify that the samples presented similar peaks to each other and, therefore, bands in the same spectrum. As the analyzes were carried out between wavenumbers 4000 and 650 cm^{-1}, they present mid-infrared characteristics, as explained by Nandiyanto et. al. (10). Still according to Nandiyanto et. al. (10), the mid-infrared spectrum is divided into four regions: single bond (2500-4000 cm^{-1}); (ii) triple bond (2000-2500 cm^{-1}); (iii) double bond (1500-2000 cm^{-1}) and; digital printing (600-1500 cm^{-1}).

Figure 2 *FTIR characterization of hydroalcoholic solvents (ethanol, methanol and isopropanol) and corn stalk extracts.*



For the three residues, stretching of hydroxyl groups (O-H) and hydrogen bonds can be observed in the region 3400-3200 cm^{-1}, due to the polymeric association. For the extract obtained with isopropyl alcohol (2c), in this range, a greater amplitude of the curve is observed about the other extracts, which may be due to the greater quantity of O-H bonds present in isopropyl alcohol. Furthermore, in this range, there is the presence of phenols, which can justify the stretching of the bands in all extracts. The other peaks represent other compounds that are also obtained during the extraction process[11]. Between 2600-3100 cm^{-1}, unsaturated and saturated stretching of the C-H cluster is observed, with greater prominence also in isopropyl alcohol—functional groups with aldehydes, ketones, esters, and carboxylic acid (1600-1735 cm^{-1}). Between 1500 and 1600 cm^{-1} is characteristic of the aromatic functional group C=C. Below 1500 cm^{-1} is the fingerprint region where alcohols, esters, ethers, and aromatic rings can be found[12]. From 1500 cm^{-1}onwards, isopropyl alcohol has other prominent peaks compared to the others, this may be due to the bonds present in the solvent itself, facilitating the extraction of other compounds in addition to phenolics.

4 CONCLUSION

This study showed that alcohols are suitable solvents for the extraction of bioactive compounds. With emphasis on ethanol in obtaining flavonoids with levels around 10.51 ± 1.16 mg/g, the other solvents have no significant difference in the extraction of flavonoids, being 3.9 ± 0.12 and 3.21 ± 0.12 mg/g, for methanol and isopropyl, respectively. In phenolics, methanol, and ethanol had no significant difference in extraction (23.9 ± 0.32 and 23.72 ± 1.05 mg/g, respectively). For isopropyl alcohol, the TPC content was 16.72 ± 0.07mg/g. This lower value than the others does not necessarily represent that this alcohol is not as suitable as the others for the extraction of phenolics; the vegetable matrix may contribute to a reduction in this value. In the FTIR analysis, peak excisions were observed in regions with O-H bonds (3400-3200 cm^{-1}), bands characteristic of groups related to phenolics. Representing that there was an interaction between the solvent and the organic compounds.

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