Harnessing Rosmarinus officinalis: A Dual Approach to Antioxidant and Antifungal Activity in Food Preservation

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Abstract

Food additives are natural or synthetic substances that can be added to foodstuff in small amounts to perform technological functions, namely color, sweetness or to extend shelf life. Preservatives, also known as antimicrobial agents, are food additives used to extend the shelf life of food by shielding them against deterioration caused by microorganisms [1]. In this work, the antimicrobial capacities of extracts from *Rosmarinus officinalis* are measured. They were chosen for their beneficial effects for human health and their notable properties and richness with phenolic compounds. The extract was obtained by the maceration method with a yield equal 14.34%, measuring the antifungal activity of Rosmarinus officinalis extract using three different concentration 250,200 and 150 mg/L, the extract showed activity against FOL *Fusarium oxysporum f. sp. lycopersici* varies depending on the concentration, it showed highest inhibition percentage at 250 mg/L with τ=30.5%, as for the antioxidant activity the total phenolic compounds was measured , and several test been made ,by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-Azinobis (3-ethylbenzothiazolin) -6-sulphonic acid (ABTS), Ferric Reducing Antioxidant Power (FRAP), and phenanthroline, the results showed that *Rosmarinus officinalis* extract exhibits an excellent antioxidant capacity.

Objective

The central objective is to undertake a comprehensive exploration of the extraction of phenolic compounds from natural sources this study seeks to evaluate their bioactive properties and investigate their potential applications as multifunctional food additives and natural preservatives in the food industry. By doing so, we aim to address key challenges in food production, with a focus on enhancing food safety, prolonging shelf life, and maintaining or improving sensory attributes.

Experimental

Extraction of *Rosmarinus officinalis*

The maceration technique was used for the extraction. Using 50g of powdered grained leaves, 500mL of nHexane was added in the first stage and stirred for 24 hours. The mixture was then filtered, and the powder was dried at 30°C for an additional 24 hours. The dried powder was then used in the second maceration. 500 mL of ethanol was added and stirred for 24 hours. The mixture was then filtered to obtain the ethanolic extract, and the excess solvent was removed using a Rotary evaporator. The mixture was then stored at 40°C until it was completely dry.

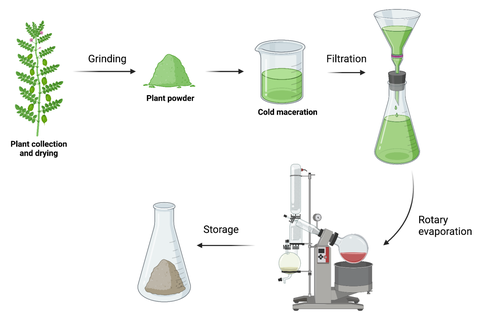
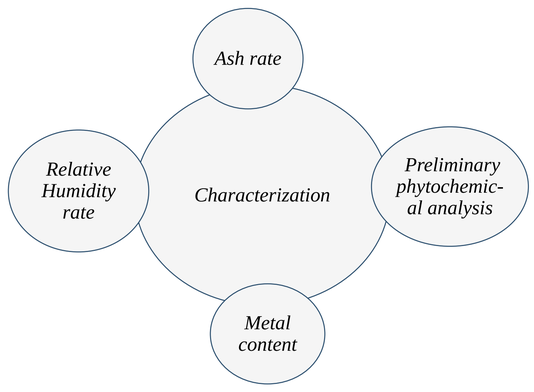


Figure1. The Extraction using maceration technique .

Hydrogel characterization

This scheme shows the key methods for characterizing the extract :

1. **Relative Humidity Rate:** Measures the moisture content of the sample.
2. **Ash Rate:** Indicates the inorganic residue remaining after combustion, useful for determining mineral content.
3. **Metal Content:** Quantifies the concentration of metals within the sample.
4. **Preliminary Phytochemical Analysis**: An initial examination of the bioactive compounds present.



Evaluation of anti-fungal and antioxidant activity

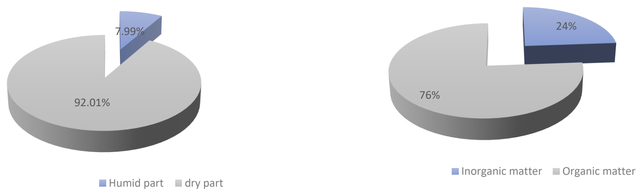
To determine the antioxidant capacities of the extract of *Rosmarinus* , four techniques were used including DPPH scavenging, ABTS , Phenanthroline and Ferric reducing antioxidant power (FRAP). Ascorbic acid BHA and BHT was used as reference material and the antioxidant capacities of the extracts were expressed with IC50. All experiments were made in triplicate.

The antifungal activity of the ethanolic extracts was assessed against Fusarium oxysporum f. sp. lycopersici (FOL), a primary pathogen causing tomato vascular wilt and a significant threat to tomato crops. The evaluation was performed using the broth dilution method, where Potato Dextrose Agar (PDA) was used as the growth medium to support fungal proliferation. Each ethanolic extract was tested at three distinct concentrations: 250 mg/L, 200 mg/L, and 150 mg/L. For each concentration, measurements were taken to observe the extent of fungal inhibition and to assess any dose-dependent effects on FOL.

Results

Ash and humidity rate

The charts represent the relative humidity rate (T=105°C, t=24h) , and the ash rate (T= 600°C, t= 6h)



Refrences

[1] M. Silva and F. Lidon, 'Food preservatives - An overview on applications and side effects', *Emir. J. Food Agric*, vol. 28, no. 6, p. 366, 2016, doi: 10.9755/ejfa.2016-04-351.

[2] Z. Ö. Erdohan and K. N. Turhan, 'Olive leaf extract and usage for development of antimicrobial food packaging', 2011.

Metal content

(Acid mineralisation of Ash)

Elements	Concentration (ppm)
Cu	0.1
Co	0.1685
Fe	173.87
Cd	0
Zn	2.99
Pb	6.3055
Hg	0

Preliminary phytochemical analysis

Compounds	Result	Color in test tube
Tannins	+	Blue-black
Catechin tannins	+	red ring
Gallic tannins	+	Blue-black
Alkaloids	+	Precipitates
Sterols and triterpenes	+	violet ring
Free anthraquinones	+	Red + precipitate
Flavonoids	+	yellow brown
Flavonols	+	Red
Saponosides	+	visible foam
Leucoanthocyanes	+	brick red
Mucilage	+	Flocculent precipitate
O-heteroside	+	Red + precipitate
C-heteroside	+	Red

+ : Present, - : Absent.

Total phenolic content , total flavonoids content TPC TFC

The Folin–Ciocalteu reagent determines the total phenol content (including other readily oxidized substances), The total phenolic content in the R.E was 513,6 ± 13,11 µg gallic acid equivalents/ml of extract. The AlCl3 colorimetric method has been widely used to estimate flavonoids. The level of our R.E was 47,3±2.63 µg quercetin equivalents/ml of extract.

Total phenolic content	513,6 ± 13,11(µg GAE/ml)
Total flavonoids content	47,3±2.63 (µg QE/ml)

*Values were expressed as means ± SD
*Total phenolic compounds were expressed as µg gallic acid equivalent/ml (µg GAE/ml)
*Flavonoids contents were expressed as µg quercetin equivalent /ml (µg QE/ml

Antifungal activity

The antifungal activity of the ethanolic extracts was evaluated against *Fusarium oxysporum f. sp. lycopersici* (FOL), a primary pathogen responsible for tomato vascular wilt. The broth dilution method was used for testing, with Potato Dextrose Agar (PDA) serving as the growth medium. Each extract was tested at three different concentrations: 250 mg/L, 200 mg/L, and 150 mg/L, to assess the concentration-dependent effectiveness of the extracts.

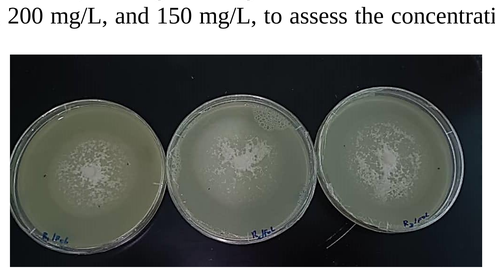


Figure2. The FOL growth in different concentration .

The results demonstrated that the ethanolic extracts exhibited varying levels of antifungal activity against FOL, with inhibition increasing as concentration increased. Among the tested extracts, *Rosmarinus officinalis* showed the most significant antifungal effect, achieving the highest inhibition rate at the maximum tested concentration of 250 mg/L, with an inhibition percentage of τ = 31.9%. This suggests that Rosmarinus officinalis extract may possess promising antifungal properties against FOL, particularly at higher concentrations.

Antioxidant activity

Figure 3 represent the inhibition percentage of Rosmary extract for different concentration with 800 µg as an initial concentration the results for DPPH and ABTS gave inhibition percentage of 87.54%, 85.64% respectively, we notice that there's a proportional relationship between the concentration and inhibition %, Using BHA and BHT as standards, our extract showed excellent results for both DPPH and ABTS, with an IC50 of (µg/mL) 13.7±1.16 compared to IC50 (BHA) 6.14±0.41(µg/mL). and ABTS of 16.26±0.79 (µg/mL) compared to IC5O (BHT) 1.29±0.30(µg/mL).

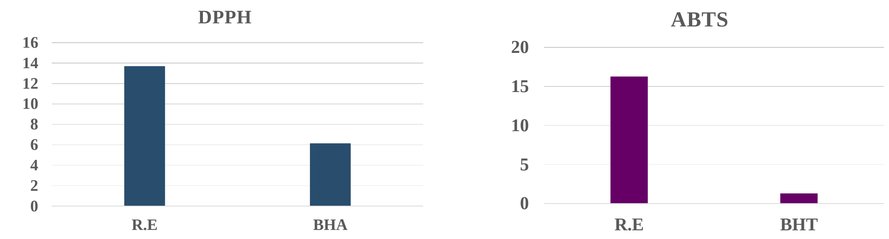


Figure3. Scavenging activity of ABTS and DPPH radicals by R.E .

Figure 4 displays the absorbance of Rosmary extract at various concentrations, starting at 200 µg. The absorbance values for phenanthroline and reducing power were 0.77±0.06 and 3.83±0.03, respectively. It is evident that the absorbance decreases with concentration, exhibiting a proportional relationship between the two. With an A0.5 of 48.93±1.35 (µg/mL) compared to A0.5 (ascorbic acid) of 6.77±1.15 (µg/mL), our extract shown good results for phenanthroline activity, and showed an outstanding performance with reducing power an A0.5 of 1.69±0.13 (µg/mL) as opposed to A0.5 (BHA) of 0,93±0,07 (µg/mL).

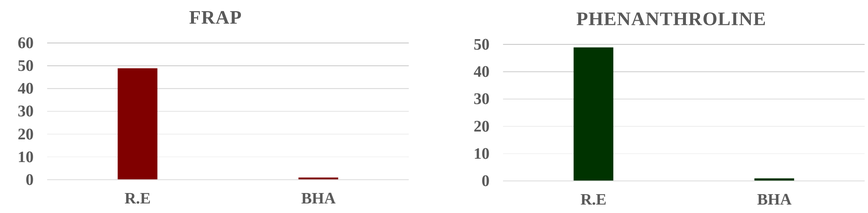


Figure4. Scavenging activity of FRAP and Phenanthroline radicals by R.E .

Conclusion

Antimicrobial packaging is an innovative way of inhibiting microbial growth on the foods while maintaining quality, freshness, and safety. Although there has been a rising interest in the researches in this field, availability of antimicrobials and new polymeric materials, regulatory concerns, and appropriate testing methods [2]. The aim of this research is to utilize natural compounds, particularly as preservatives, within the realm of food, The antifungal and antioxidant assessments of the extract yielded highly promising outcomes. Our next objective is to investigate its antibacterial properties and explore potential applications of the Rosmarinus officinalis extract as a food preservative