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Evaluation of antifungal, antioxidant activities and determination of total phenolic compounds of ethanolic extract from *Juglans regia* bark

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INTRODUCTION & AIM

Invasive fungal infections pose an ongoing and serious threat to human health and are associated with at least 1.5 million deaths worldwide annually [1]. The global emergence of multidrug resistant microorganism has limited the effectiveness of existing drugs. One of the proposed strategies to overcome this health care problem is the search for new antifungal agents from medicinal plants.

Although there are many antifungal agents in clinical use, none of them fulfills all the requirements to be a fully effective, nontoxic antifungal drug, and all have significant drawbacks. Fungal resistance leads to clinical failures of antifungal chemotherapy that render fungal infections extremely difficult to eradicate

As a result, we will need to look for new antimicrobial drug sources elsewhere, and plant-based material is an obvious choice. On a global scale, phytochemicals have been tested as possible sources of new antimicrobial compounds, food preservation agents, and alternatives for treating infectious disorders because of their antifungal, antibacterial, antioxidant and antiviral properties.

The genus Juglans (family Juglandaceae) includes various species and is widespread throughout the world. *J. regia L*., commonly known as walnut, is an important tree species, producing edible wood and nuts [2]. It is on the FAO's list of priority plants and is considered a strategic species for human nutrition. *J. regia* is an herb that has been used traditionally in medicine for its antibacterial, antiviral, hepatoprotective, anthelmintic, and antidiarrheal properties. It also has anticancer properties. Additionally, antifungal activity has been reported [3]

The purpose of the present work was to evaluate the *in-vitro* antifungal and antioxidant activity of ethanolic extracts prepared from *Juglans regia* bark using maceration and soxhlet methods and to determine the total phenolic compounds of the prepared extracts

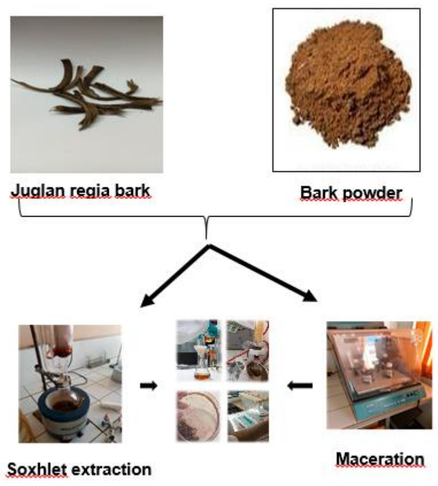
METHOD

1. Plant material

The barks of *J.regia* were harvested from Nedroma, Tlemcen (Algeria) during the period extending from October to November 2021. The plant material was identified, washed and then dried in the shade at room temperature. After drying, the plant materials were ground well into a fine powder using a mechanical blender.

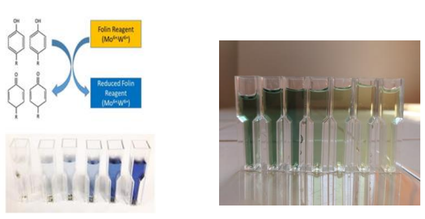
2. Preparation of bark extract

In the current study, the ethanolic extract from *J.regia* barks was prepared in ethanol by two distinct methods: maceration at 37°C for 72h at 100 RPM and soxhlet extraction for approximately 4 hours. After that, the extract was filtered using Whatman filter paper No. 1 and concentrated under vacuum on a rotary evaporator at 40 °C. The crude extracts were weighted in order to calculate the yield and then stored at 4 °C.



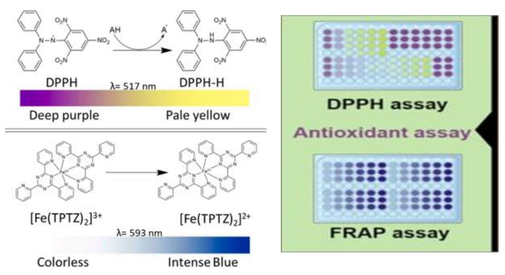
3. Total phenolic contents

Total phenolic content of the studied extracts was assessed using the the Folin-Ciocalteu colorimetric reagent using gallic acid as a standard.



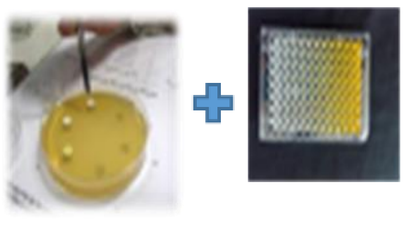
3. Antioxidant activity

The antioxidant activity was evaluated using DPPH Free Radical Scavenging Assay and Ferric-reducing antioxidant power assay (FRAP). Ascorbic acid was used as standard.



4. Antifungal activity

The antifungal activity was determined using the disc diffusion and the micro-well dilution methods. The yeast strains used in this study are *Candida albicans (ATCC 26790*), *Candida albicans (ATCC 10 231), Candida albicans (IP444)*.



RESULTS & DISCUSSION

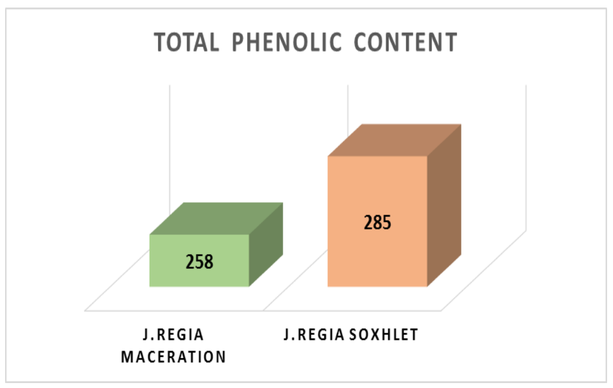
1. Yield and organoleptiques characteristics

J.Regia ethanolic extract by maceration method: Dark brown, solid
Yield : 0.9%

J.Regia ethanolic extract by soxhlet method: Dark Dark brown, viscous
Yield : 4.2%

2. Total phenolic contents

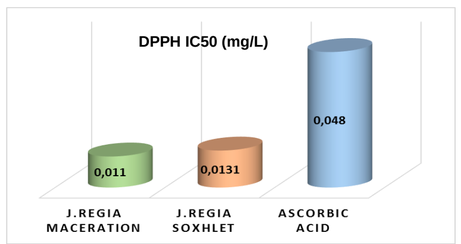
The obtained results showed that the ethanolic extract of Juglans regia bark prepared by the soxhlet method has a higher total polyphenol content(285±0.022 mg AGE/g extract) than the ethanolic extract of the same plant prepared by maceration (258 ± 0.018 mg AGE/g extract). These differences in phenolic compound content can probably be explained by differences in origin, variety, harvesting season, geographical location, maturity, solvent and extraction method. .

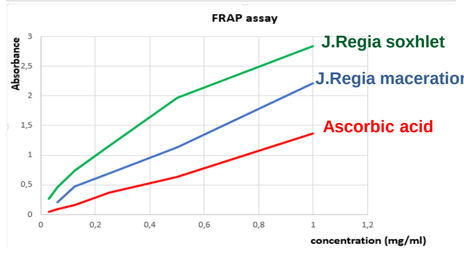


3. Antioxidant activity

The studied extracts prepared by maceration and soxhlet extraction were able to reduce the stable, purple-colored radical DPPH into yellow-colored DPPH-H, reaching 50% of reduction with an IC_{50} of 0.011 and 0.0131 mg /ml respectively.

the reducing power of both tested extracts was positively correlated with their concentrations, with absorbance increasing as the concentration increased





4. Antifungal activity

The antifungal activity of the J. regia ethanolic extracts were evaluated against three yeast strains: *C. albicans (ATCC 26790*), *C. albicans (ATCC 10 231),* and *C. albicans (IP444)*.. The results are summarized in table 1. Based on the results, we can conclude that the *J. regia* bark extract prepared using the Soxhlet method exhibited the highest antifungal activity against all three C. albicans strains, with a minimum inhibitory concentration (MIC) ranging from 39 to 78 µg/mL.

Yeast	Extraction method			
	maceration		Soxhlet	
	Zone of inhibition[1]	MIC[2]	Zone of inhibition	MIC
C. albicans (ATCC 26790)	12±0.57	0,156±0.00	14±1	0.078±0.00
C. albicans (ATCC 10 231)	10,33 ±0.88	0,156±0.00	13,66±0.57	0.078±0.00
C. albicans (IP444)	14± 0.33	0.078±0.00	16± 0.66	0.039±0.00

[1] Zones of inhibition are expressed as mm.
[2] MIC are expressed as mg/mL

CONCLUSION

The obtained data confirmed the correlation between the total phenolic compounds and biological activities of medicinal plants extract. In addition, Juglans regia bark extracts demonstrate notable antifungal activity, which explains their use in traditional medicine. This makes them suitable for use in pharmaceutical preparations and represents an effective solution for treating multidrug-resistant fungal infections and related disorders.

FUTURE WORK / REFERENCES

REFERENCES:

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