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Evaluation of Antioxidant and Antimicrobial Activity of polyphenol and encapsulated polyphenol from the seeds of *Melia azedarach*

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Abstract – *Melia azedarach* contains several phytochemical compounds such as phenols, flavonoids, alkaloids, tannins, glycosides, steroids, saponins and terpenoids. *Melia azedarach* has attracted a lot of attention from researchers as one of the most versatile herbal medicines, having a wide range of biological activities, including anticancer, antimalarial, analgesic, anti-inflammatory, diuretic, astringent and gastric. Polyphenol from the seeds of *Melia azedarach* showed antibacterial activity against the bacteria tested (*Escherichia coli* and *Pseudomonas aeruginosa*) at all concentrations. For the encapsulated polyphenol from the seeds of *Melia azedarach*, we observed a decrease in the inhibitory effect for the bacteria tested. The biological study for the evaluation of the antioxidant activity of polyphenol and encapsulated polyphenol was evaluated by the DPPH method. The inhibition of the DPPH radicals of polyphenol from the seeds of *Melia azedarach* and of encapsulated polyphenol from the seeds of *Melia azedarach* was 85.58±0.5 % and 49.52 ±0.6 % respectively.

Key words: Antioxidant activity, antimicrobial activity, polyphenol, encapsulated polyphenol, seeds of *Melia azedarach*.

1. Introduction

Melia azedarach Linn commonly known Persian" Lilac or big lilac" is a tree belonging to the Meliaceae family. Native of Asia, it's presently used as tree for the reforestation in China, India, South and Central America (Ben Ghnaya *et al.*, 2013). *Melia azedarach* was successfully introduced and naturalized all over the world as decorative species because of its big tolerance to extreme environments as well as its high growth rate and its prolific

production of seeds (M'rabet *et al.,* 2017). *Melia azedarach* contains multiple phytochemical compounds as phenols, flavonoids, alkaloids, tannins, glycosides, steroids, saponines and terpénoïdes (Jaafar *et al.,* 2016). Litterature have been shown that *Melia azedarach* has a wide range of biological activities, such as antimicrobial, insecticidal, antioxidant, anti-inflammatory, antidiabetic and anti-aging compounds (Orhan *et al.*, 2012; Bahuguna *et al.,* 2009 ; Bullangpoti *et al.,* 2012; Aoudia *et al.*, 2012; Khan *et al.,* 2014; Aoudia *et al.*, 2013; Khan et al., 2018; Khan *et al.*, 2011) and also it has been *Melia azedarach* was known to fight numerous diseases such as rheumatism, leprosy, skin rashes, etc. This therapeutic power is due to the wide range of chemical compounds such as triterpenoïds, limonoïds, fatty acids and phenolic compounds (M'rabet *et al.,* 2017). These last compounds are recognized for their antioxidant potential; besides, polyphenols possess antibacterial, antifungal, antioxidant and antimicrobial activities (Aissani *et al.,* 2017). Unfortunately, polyphenols have a weak long-term stability, because they are sensible to the presence of metallic ions, to the light, the temperature, the oxygen and the enzymatic activities. Because of their low solubility in the water, they often present a weak bioavailability too (Aizpurua-Olaizola *et al.*, 2016). Furthermore, the taste and the unpleasant flavour of most of the phenolic compounds limit their uses in food or oral medicine. A wide range of technologies has been developed to encapsulate polyphenols, among which the drying by spraying, the coacervation, the trapping of liposomes, the inclusion complexation, the co-crystallization, the nanoencapsulation, the freeze-drying, the yeast encapsulation and the emulsion (Fang *et al.,* 2010). Cyclodextrins could be used for such purposes. The most known and used cyclodextrins are the β-cyclodextrin (β-CD), the β-cyclodextrin (β-CD) and the β-cyclodextrin (β-CD) constituted respectively of six, seven and eight glucose units. The molecules of cyclodextrins adopt a truncated cone with a hydrophobic cavity and a hydrophilic surface (Galvão *et al.*, 2015; Hill *et al.,* 2013; Rakmai et al., 2018). Cyclodextrins can form inclusion complexes with weakly water soluble molecules (such as polyphenols). The encapsulation of polyphenols in cyclodextrins leads to a bigger stability and a controlled liberation (Karageorgou *et al.,* 2018) and increases the liberation rate and the membrane permeability; it improves also the conservation of foodstuffs and masks or reduces their smell or their unsuitable taste (Kalogeropoulos *et al.,* 2010). Many works explored the use of the β-cyclodextrin (β-CD) and derivatives for the encapsulation of polyphenols (Vlaia et al.,2016). Encapsulated propolis

polyphenol extracts in the 2-hydroxypropyl-β-cyclodextrin (2HP- β-CD) to obtain a stable active and easy to deal material. (Diamanti *et al.,* 2017) encapsulated the aqueous extract of the whole grenade fruit which is rich in phenolic compound by the β-CD to improve the efficiency of the aqueous extraction of this plant. Polyphenols extracts from green tea have been encapsulated in three different materials, maltodextrine (MD), β-cyclodextrin (β-CD) and a mixture of both to improve their stability (Pasrija *et al.,* 2015) as well as coffee leaves phenolic extracts (Ballesteros *et al.,* 2017). Similar encapsulation experiments have been achieved with polyphenols from apple in β-CD nanosponges (Ramirez-Ambrosi *et al.,* 2014) and polyphenols from *Clitoriaternatea* extract in alginate and arabicgums (Pasukamons *et al.,* 2016). In both cases, the stability and the biological activity were preserved. Recently, we prepared and characterized the inclusion complexes of *Melia azedarach L.* seed oil/β-cyclodextrin polymer and we proved that the encapsulation of *Melia azedarach* seed oil by lyophilization seems to be a good alternative to maintain the quality and the durability of the active compounds which have been protected against their environment (Benyacoub *et al.,* 2018). The objective of this present study is the valuation of polyphenol from the seeds of *Melia azedarach* and encapsulated polyphenol from the seeds of *Melia azedarach* by tests on the total content of phenolic compounds, antimicrobial activity and antioxidant activity.

2. Material and methods

2.1 Vegetal material

Fruits of *Melia azedarach* were collected in March 2016 at Ouled Yaich of Blida, Algeria; plant samples were identified and authenticated by the botanic laboratory of Agriculture department (University of Blida1, Algeria). These fruits were cleaned and immersed in water during 24 hours to separate the fleshy pulp of seeds. The seeds were air-dried in the shade and crushed. Next, the powder was kept at room temperature in the darkness.

2.2 Bacterial strains

All the strains tested were provided by the hygiene laboratory of Blida. To assess the antimicrobial activity of polyphenol from the seeds of *Melia azedarach* without and with encapsulation, two bacterial strains and one fungal strain were tested (*Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*).

2.3 Extraction of phenolic compounds from *Melia azedarach*

100 g of the dried powder of *Melia azedarach* seeds were extracted in Soxhlet apparatus with 600 mL petroleum ether (60-80 °C) for 8h. This process was firstly

adapted for delipidation in order to separate the hydrophobic components (M'rabet *et al.*, 2017). Then, the pretreated powder was dried and the phenolic compounds were extracted in a soxhlet apparatus with 300 mL methanol for 8 h. finally, the methanol is evaporated using a rotary evaporator.

2.4 Synthesis of the β-cyclodextrin polymer

The β-cyclodextrin-epichlorohydrin (β-CD –EP) polymer was obtained by polycondensation of β-cyclodextrin and epichlorohydrin under strongly alkaline conditions (NaOH). 0.88 mmol of β –CD and 5 mL of NaOH solution (10%(w/w)) is stirred for 24 h at 25◦C. and 12.7 mL of EP is addedrapidly and stirred vigorously with a magnetic stirrer for 4 h at 60◦C. Finally, the reaction is stopped by addition of acetone. After decantation, acetone is removed and the solution is kept at 50◦C overnight. After cooling, the solution is neutralized with 6 N HCl and dried at 50 °C during a night. 25 mL of acetone were added to the resulting residue leading to the formation of a white precipitate. The solution was decanted and the white product was dried in an oven at 50 ° C for 24 h (Benyacoub *et al.*, 2018).

2.5 Total phenolic compounds (TPC)

The total content in phenolic compounds of polyphenols and encapsulated polyphenols was determined with the Folin-Ciocalteau (FC) method (M'rabet *et al.*, 2017). 100 µL of extract (1 mg/mL) were added to 500 µL of the Folin-Ciocalteure active. After 5 min, 400 µL of Na_2CO_3 (7.5 % w) were added. After 30 minutes of incubation in the darkness at room temperature, the absorbance of samples was measured at 758 nm by UV/VIS. The amount of phenolic compounds is determined by comparison with the calibration curve obtained with the gallic acid. The values of GPC were expressed in mg of gallic acid equivalent per g of extract (mg of GAE / g of extract).

2.6 Antimicrobial activity

The antimicrobial activity of polyphenol and encapsulated polyphenol from the seeds of *Melia azedarach* is assessed by the disk diffusion method to test the susceptibility of microbial strains. The aromatogram method was used to assess the antimicrobial activity of polyphenol from the seeds of *Melia azedarach* and encapsulated polyphenol from the seeds of *Melia azedarach* (Benyacoub *et al.*, 2019).

2.7 Antioxidant activity

The antioxidant activity of the samples was determined by using the test of DPPH radicals trapping according to the method described by (Hajji *et al.*, 2018) with some slight modifications. Various solutions of polyphenols and encapsulated polyphenols in methanol (0.2-1 mg/mL) were prepared.

25 µL of each solution were added to 1 mL of a solution of DPPH (6×10-5mol/L in methanol). After 20 min at room temperature in the darkness, the decreases of the absorbance of DPPH were recorded by UV-VIS (Spectrometer Shimadzu UV 1800 Japan) at 517 nm during 20 minutes. The percentage of inhibition of the DPPH radical was calculated with the butyl Hydroxyanisole (BHA) using the same method for different concentrations (0.2-1 mg / ml).

3. Results and discussion

3.1 Total phenolic compounds (TPC)

The content in phenolic compounds of *Melia azedarach* seed polyphenol and encapsulated *Melia azedarach* seed polyphenol was determined by the method of Folin-Ciocalteu by using the gallic acid as standard. The total content in phenolic compounds of polyphenols of seeds of *Melia azedarach* was evaluated to 71.085 ± 2.657 mg GAE/g, while the content in phenolic compounds of encapsulated polyphenols of seeds *Melia azedarach* was 26.72±3.527 mg GAE/g. The total content of polyphenol compounds of encapsulated polyphenols of seeds *Melia azedarach* is lower than that of polyphenols of seeds *Melia azedarach* (Figure.1). It is estimated that the total phenolic content of polyphenol was protected in the capsule. (Teixeira *et al.*, 2013) encapsulated black pepper oleoresin by HP-β-CD and stated that the total phenolic content of pepper was protected in the inclusion complex black pepper oleoresin-HP-β-CD as (Li *et al.*, 2017) encapsulated the phenolic extract of plum and they showed that phenol-rich phenolic extract. But the coating agents reduced the concentration of the active ingredient in the capsule. The results of this study shows that the total phenolic content of polyphenols of seeds *Melia azedarach* is protected in the capsule.

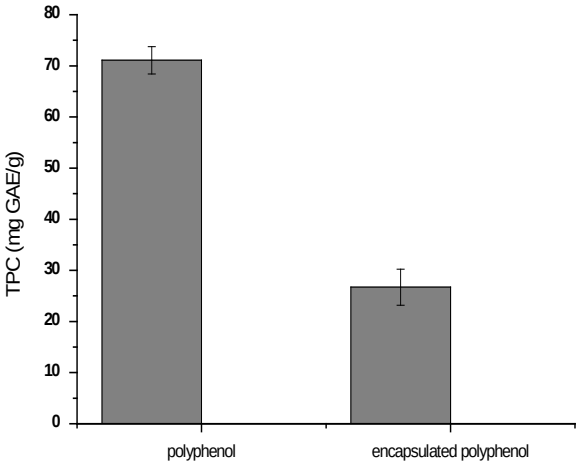

Figure 1. The total phenol content in the polyphenol and in the encapsulated polyphenol from the seeds of *Melia azedarach.*

3.2 Antimicrobial activity

polyphenol from the seeds of *Melia azedarach* showed antibacterial activity against the bacteria tested (*Escherichia coli* and *Pseudomonas aeruginosa*) at all concentrations. Where antifungal activity (*Candida albicans*) was observed only at 10 $mg.mL^{-1}$. The greatest inhibition is

observed for *Escherichia coli* with a zone of inhibition (15 mm for the concentration of 10 mg.mL^{-1}).

Pseudomonas aeruginosa presents a zone of inhibition (10 mm for the concentration of 20 mg.mL^{-1}. For *Candida albicans*, presents a zone of inhibition (10 mm for the concentration of 10 mg.mL^{-1}). encapsulated polyphenol from the seeds of *Melia azedarach*, we observed a decrease in the inhibitory effect for the bacteria tested. (Pinho *et al.,* 2014) encapsulated gallic acid by β-CD and showed that the antibacterial activity of gallic acid is reduced during encapsulation by β-CD. The results of this study show that the β-CD polymer can protect the polyphenols.

Table 1: Results of antimicrobial activity test of polyphenol and encapsulated polyphenol from the seeds of *Melia azedarach*

C (mg. mL^{-1})	Zone of inhibition (mm)					
	Candida albicans ATCC 24433		*Pseudomonas aeruginosa* ATCC 27853*G* (-)		*Escherichia coli ATCC 25922 G(-)*	
	Polyphenol	Encapsulated polyphenol	Polyphenol	Encapsulated polyphenol	Polyphenol	Encapsulated polyphenol
40	-	-	7	7	07	07
20	-	-	10	7	08	08
10	10	9	8	7	15	10
05	-	-	8	7	10	09
2.5	-	-	8	8	08	-

3.3 Antioxidant activity

The Figure. 2 represents the antioxidant activity by the trapping capacity of DPPH contained in polyphenol and encapsulated polyphenol from the seeds of *Melia azedarach* with regard to the butyl hydroxyanisole (BHA) at various concentrations [0.2-1 mg/mL].The polyphenols from the seeds of *Melia azedarach* present a strong antioxidant activity (85.58± 0.5 % of DPPH radicals trapping at 1mg/mL); the encapsulated polyphenol from the seeds of *Melia azedarach* for their part present a lower antioxidant activity (49.52±0.6 % of DPPH radicals trapping at 1mg/mL) (Figure.2). (Ballesteros *et al.,* 2017) encapsulated polyphenol extracted from spent coffee grounds by two different methods using three different membranes such as maltodextrin, gum arabic and combination of two membranes and showed that the polymers used are preserving the active components phenolic compounds extracted from coffee grounds. This study shows that the β-CD-EP polymer constitutes a barrier and can block the active polyphenol compounds from the seeds of *Melia azedarach* during the reaction with the DPPH radicals. So, we could conclude that the encapsulation method could protect the active components of polyphenol against the effect of temperature and light.

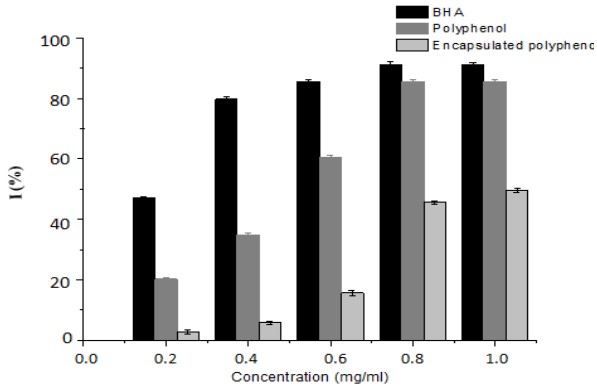

Figure 2. DPPH radical scavenging (%) for BHA (■), polyphenols (■) and encapsulated polyphenols from the seeds of *Melia azedarach* (■) at various concentrations [0.2-1 mg/mL].

4. Conclusion

This study showed that the polyphenol of *Melia azedarach* seed very rich in phenolic compounds. polyphenol from the seeds of *Melia azedarach* confers significant antimicrobial and antioxidant properties. The results of this study showed that the use of the β-cyclodextrin-epichlorohydrin polymer as a matrix can create a protective barrier around the polyphenols of *Melia azedarach* seeds to protect the most active compounds from environmental damage and ensure long shelf life.

5. References

Aissani N, Balti R. and Sebai H. (2017) : Potent nematicidal activity of phenolic derivatives on Meloidogyne incognita. J. Helminthol.11: 1-6.

Aizpurua-Olaizola O, Navarro P, Vallejo A, Olivares M, Etxebarria N. and Usobiaga A. (2016): Microencapsulation and storage stability of polyphenols from Vitis vinifera grape wastes. Food Chem.1: 614–621.

Aoudia H, Oomah B.D, Zaidi F, Zaidi-Yahiaoui R, Drover J.C.G. and Harrison JE: (2013). Phenolics, antioxidant and anti-inflammatory activities of *Melia azedarach* extracts. Int. J. Appl. Res. Nat. Prod. 6 : 19–29.

Aoudia H, Ntalli N, Aissani N, Yahiaoui-Zaidi R. and Caboni P. (2012): Nematotoxic Phenolic Compounds from *Melia azedarach* Against Meloidogyne incognita. J Agric Food Chem. 28: 11675–11680.

Bahuguna Y, Patil K, Rawat MSM, Jalalpure S. and Uniyal S. (2009): Antiulcer activity of *Melia azedarach* L in aspirin induced and pylorus ligated rats. J Pharm Res. 2: 1456–1459.

Ballesteros LF, Ramirez MJ, Orrego C.E, Teixeira J.A. and Mussatto S.I. (2017): Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials. Food Chim. 237:623–631.

Ben Ghnaya A, Hamrouni L. and Hanana M, (2013): Notes ethnobotanique et phytopharmacologique sur *Melia azedarach* L. Phytotherapie.11 : 284-288.

Benyacoub A, Skender A, Boutemak K. and Hadj-Ziane-Zafour A. (2018): Inclusion complexes of *Melia azedarach*

L. seed oil/β-cyclodextrin polymer: preparation and characterization. Chem Pap. 1-10.

Benyacoub A, Skender A, Boutemak K. and Hadj-Ziane-Zafour A. (2019): Antioxidant, antimicrobial and insecticidal activities of *Melia azedarach* seed oil and encapsulated *Melia azedarach* seed oil. *PhytoChem & BioSub Journal :13-3.*

Bullangpoti V, Wajnberg E, Audant P. and Feyereisen R. (2012) : Antifeedant activity of Jatropha gossypifolia and *Melia azedarach* senescent leaf extracts on Spodoptera frugiperda (Lepidoptera: noctuidae) and their potential use as synergists. Pest Manag. Sci. 68: 1255–1264.

Diamanti A.C, Igoumenidis P.E, Mourtzinos I, Yannakopoulou K. and Karathanos V.T. (2017) : Green extraction of polyphenols from whole pomegranate fruit using cyclodextrins. Food Chim. 214: 61–66.

Fang Z. and Bhandari B. (2010): Encapsulation of polyphénols-a review. Trends Food Sci Technol. 21: 510–523.

Galvão J.G, Silva V. F, Ferreira S.G, França F.R.M, Santos D.A, Freitas L.S, Alves P. B, Araújo A. A. S, Cavalcanti S.C.H. and Nunes R.S. (2015) : β-cyclodextrin inclusion complexes containing Citrus sinensis (L.) Osbeck essential oil: An alternative to control Aedes aegypti larvae. Thermochim Acta. 608: 14–19.

Hajji T, Mansouri S, Vecino-Bello X, Cruz-Freire J.M, Rezgui S. and Ferchichi A. (2018): Identification and characterization of phenolic compounds extracted from barley husks by LC-MS and antioxidant activity in vitro. J. Cereal Sci. 81: 83–90.

Hill L.E, Gomes C. and Taylor T.M. (2013) : Characterization of beta-cyclodextrin inclusion complexes containing essential oils (trans cinnamaldehyde, eugenol, cinnamon bark, and clove bud extracts) for antimicrobial delivery applications. LWT-Food Sci. Technol, 51: 86–93.

Jaafar N. S, hamad M. N, abbas I.S. and jaafar I.S. (2016) : Qualitative phytochemical comparison between flavonoids and phenolic acids contents of leaves and fruits of *Melia azedarach* (family: meliaceae) cultivated in iraq by hplc and hptlc. Int. J. Pharm. Pharm. Sci. 8: 242–250.

Kalogeropoulos N, Yannakopoulou K, Gioxari A, Chiou A, Makris DP. (2010) : Polyphenol characterization and encapsulation in β-cyclodextrin of a flavonoid-rich Hypericum perforatum (St John's wort) extract. LWT-Food Sci. Technol.43:882–889.

Karageorgou I, Grigorakis S, Lalas S, Mourtzinos I, Makris DP. (2018) : Incorporation of 2-hydroxypropyl β-cyclodextrin in a biomolecule-based low-transition temperature mixture (LTTM)

boosts efficiency of polyphenol extraction from Moringa oleifera Lam leaves. J. Appl. Res. Med.Aromat. Plants. 9: 62–69.

Khan M.F, Rawat AK, Pawar B, Gautam S, Srivastava AK, Negi DS. (2014) : Bioactivity chemical analysis of *Melia azedarach L.* (Meliaceae): displaying antidiabetic activity. Fitoterapia.98: 98–103.

Khan M.F, Rawat A.K, Khatoon S, Hussain M.K, Mishra A, Negi D.S. (2018) : In vitro and in vivo Antidiabetic Effect of Extracts of *Melia azedarach*, Zanthoxylum alatum, and Tanacetumnubigenum. Integr. Med. Res.7: 176-183.

Khan AV, Ahmed QU, Mir MR, Shukla I, Khan A.A. (2011): Antibacterial efficacy of the seed extracts of *Melia azedarach* against some hospital isolated human pathogenic bacterial strains. J. Trop. Biomed.1: 452–455.

M'rabet Y, Rokbeni N, Cluzet S, Boulila, A, Richard T, Krisa S, Marzouki L, Casabianca H, Hosni K. (2017) : Profiling of phenolic compounds and antioxidant activity of *Melia azedarach* L. leaves and fruits at two stages of maturity. Ind. Crop Prod. 107: 232–243.

Pasrija D, Ezhilarasi P.N, Indrani D. and Anandharamakrishnan C. (2015): Microencapsulation of green tea polyphenols and its effect on incorporated bread quality. LWT-Food Sci.Technol. 64: 289–296.

Pinho E , Soares G and Henriques M.(2014) : Cyclodextrin modulation of gallic acid in vitro antibacterial activity. J Incl Phenom Macrocycl Chem, **81 :** 205–214 .

Rakmai J, Cheirsilp B, Mejuto J, Simal-Gándara J. and Torrado-Agrasar A. (2018) : Antioxidant and antimicrobial properties of encapsulated guava leaf oil in hydroxypropyl-beta-cyclodextrin. Ind. Crop. Prod, 111: 219–225.

Ramirez-Ambrosi M, Caldera F, Trotta F, Berrueta L.A. and Gallo B. (2014) : Encapsulation of apple polyphenols in β-CD nanosponges. J Incl Phenom. Macrocycl. Chem, 1: 85-92.

Teixeira BN, Ozdzmir N, Hill E. and Gomes C.L. (2013) : Synthesis and Characterization of Nano-Encapsulated Black Pepper Oleoresin using Hydroxypropyl Beta-Cyclodextrin for Antioxidant and Antimicrobial Applications. J Food Sci.78: 1913-1920.

Vlaia L.L, vlaia V, olariu I.V, mut A.M, gafitanu C.A, dehelean C, navolan D, lupuleasa D. and georgeta, H.C.(2016) : Preparation and characterization of inclusions complexes between propolis ethanolic extracts and 2-hydroxypropyl-b-cyclodextrin. Rev Chim. 67:378–381.