https://doi.org/10.48047/AFJBS.6.14.2024.10046-10059

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Phytochemical screening and biological activities of *Pallenis hierochuntica* extracts during fructification in the Betita region, Algeria

Bouchra HARSA[1,*], Ratiba SERIDI[1], Ratiba MEKKIOU[2,*], Abdeldjalil KERKABOU[2], Nina SADOU[1], Chawki Bensouici[3] and Amina CHOUH[3]

[1]University of Badji Mokhtar-Annaba, Faculty of Sciences, Department of Biology, Option of Vegetal Biology and Pharmacognosy, Laboratory of Plant Biology and Environment (LBVE), Axis: Medicinal Plants and Natural Substances, Bp 12, 23000 Annaba, Algeria.bouchra_harsa@yahoo.com

[2]Unité de recherche Valorisation des ressources Naturelles, Molécules Bioactives et Analyses Physicochimiques et Biologiques (VARENBIOMOL), Université des Frères Mentouri Constantine 1, 25000, Constantine, Algérie.mekkiou_ratiba@yahoo.fr

[3]National Center of Biotechnology Research, Ali Mendjeli UV 03 BP E73, 25000 Constantine, Algeria.

Corresponding authors E-mail:mekkiou_ratiba@yahoo.fr* , *bouchra_harsa@yahoo.com*

+213776093506

Volume 6, Issue 14, Aug 2024

Received: 15 June 2024

Accepted: 25 July 2024

Published: 15 Aug 2024

doi: 10.48047/AFJBS.6.14.2024.10046-10059

Abstract

In this study, we investigated the phytochemical composition and biological activities of aerial parts, roots, and stems of *Pallenis hierochuntica* collected during the fructification period in the Betita region, Tebessa Province, Algeria. Chemical screening was carried out to highlight the presence of the main secondary metabolites groups. Total phenolic (TPC) and total flavonoid contents (TFC) were quantified. Antioxidant activity was evaluated using DPPH, ABTS, and CUPRAC assays; the antidiabetic potential was performed via the alpha-amylase test, while the sun protection factor (SPF) was determined to assess photoprotective properties. The phytochemical screening revealed the presence of many chemical groups. The highest TPC was recorded in roots extract; however the highest TFC was noted in aerial parts extract. Antioxidant activity assays indicated the roots had the highest antioxidant capacity. The alpha-amylase inhibitory activity was notably potent (IC50=136.77±0.19 µg/mL) in roots compared to aerial parts and stems extracts. All extracts demonstrated better alpha-amylase inhibition than the standard antidiabetic drugs. Furthermore, SPF values for all extracts were around 31.50 indicating strong UV protection. The preliminary results obtained through this study are encouraging and will contribute to further research on *P. hierochuntica* extracts and highlight its potential in developing natural therapeutic and cosmetic products.

Key Words: *Pallenis hierochuntica*, Phytochemical screening, Fructification, Antioxidant activity, Photoprotective activity, Alpha-amylase test.

Introduction

The *Inuleae-Inulinae* clade, belonging to the Asteraceae family (Anderberg et al., 2005), encompasses various species distributed in hot and dry environments, including eighteen Tell species and twelve species present in Algeria (Tahri et al., 2020). This clade represents a distinct group within the *Inuleae* tribe, which comprises more than 690 species (Nylinder and Anderberg, 2015). One of the species within this tribe is *Pallenis hierochuntica*

(Michon) Greuter, 1997 (Greuter, 1997), an annual herb characterized by its dicotyledone and therophyte life form (Fakhry and El-Kenany, 2019; Sherif and Betelmal, 2020), which is native to the Mediterranean region (El-Barasi and Gawhari, 2003; Steinbrinck and Schinz, 1907).

This plant, which is an understudied component of the Algerian flora, is typically found in clay soil, rockeries, and steppes, with a more common occurrence in the Northern Sahara sector and Western Sahara sector, but it is rare in the central Sahara sector (Quezel and Santa, 1963).

For centuries, the people in the Betita Region in Tebessa Province, Algeria, have recognized the traditional therapeutic benefits of *Pallenis hierochuntica*. Despite this recognition, a significant research void persists regarding its phytochemical composition and biological activities, particularly during fructification. Our study aims to fill this gap by comprehensively investigating various aspects of the *Pallenis hierochuntica* species for the first time. In order to identify specific chemical groups such as flavonoids, coumarins, tannins, saponins, and terpenes that may be involved in the plant's medicinal qualities, a phytochemical screening was conducted. Additionally, assessing antioxidant potential using established assays, such as DPPH, ABTS, and CUPRAC, is pivotal for understanding its role in combating oxidative stress-related disorders (El-Ahmir et al., 2020) and exploring the antidiabetic effects through alpha-amylase inhibition assays to address the global burden of diabetes and related metabolic disorders. Furthermore, our investigation extends the evaluation of the extracts' SPF values, considering the increasing concerns about UV radiation-induced skin damage.

Material and Methods

Plant material

Pallenis hierochuntica (Michon) Greuter was harvested to preserve its ecological distribution for the management of bioresources during the fructification period in the Betita region (Bir El Ater, Tebessa Province) in February 2020. Following the identification by Mme., HARSA Bouchra from the Biological Department of Badji Mokhtar University, Annaba, based on the phenotypical features described by Quezel and Santa under the old scientific name (Quezel and Santa, 1963), the aerial parts, underground parts, and stem parts were separated. They were then dried in the shade for 14 days at room temperature and stored in the dark.

Preparation of extracts

A total of 30 g of the aerial parts, 30 g of the underground parts, and 50 g of the stem parts of *P. hierochuntica* were macerated for 72 h three times in a hydroalcoholic system ($MeOH/H_2O$, 50/50, v/v)

until the plant material was fully covered. The solutions were then filtered and dried using a rotary evaporator at 35°C under reduced pressure. The resulting crude extracts were stored for future use following the method outlined by Seidel, which states that dried plant material should be stored in sealed containers in dry and cool environments. Furthermore, extended storage should be avoided because some components may degrade (Seidel, 2005).

Phytochemical screening methods

The initial crucial step in this study is phytochemical screening, which makes it possible to detect important chemical components such flavonoids, leucoanthocyanins, coumarins, catechol tannins, gallic tannins, saponins, terpenes, sterols, and quinones.

The dried aerial parts, roots, and stems were subjected to preliminary phytochemical screening for the detection of major chemical groups using standard procedures, which consists incolor change or precipitate formation upon the addition of specific reagents to the tested crude extracts (Sofowora, 1993; Trease and Evans, 1989).

Quantitative analysis

Estimation of TPC

The amount of phenol in all extracts was determined by Folin-Ciocalteu reagent method with some modifications (Singleton *etal*., 1999). Mixture of each extract (0.5 mL) and 5mL of Folin–Ciocalteu reagent (1N) was neutralized with 4mL of saturated sodium carbonate (75 g/L), and kept at room temperature for 2 h. The absorbance was measured by spectrophotometry at 765nm. The TPC were expressed as mg Gallic acid equivalents per g of dry extract (mg GAE/g DE).

Estimation of TFC

The total flavonoid content was calculated using $AlCl_3$ method with slight modifications (Türkoğlu *et al.,* 2007). A 50 µL of the diluted extract solution was mixed with 1900 µL of methanol, 50 µL of 1 M potassium acetate, and 50 µL of 10% aluminum nitrate. Then, the obtained mixture was distributed in the wells of 96-well microtiter plates in triplicate (200 µL for each extract mixture). After incubation at room temperature in the dark for 40 min, the absorbance was measured at 415 nm by using a 96-well microplate reader (EnSpire, PerkinElmer, MA, USA). The experiment was done in triplicate and TFC was expressed as mg of quercetin equivalents per g of dry extract (mg QE/g DE).

Antioxidant activity

The evaluation of the antioxidant potential of all extracts was performed using DPPH, ABTS, and CUPRAC assays. Measurements were taken using a Multimode Plate Reader on a 96-well microplate apparatus. The IC50, and A0.5 (µg/mL) values represented the concentration of 50% inhibitionand compared to positive controls (BHA)under identical experimental conditions.

The free Radical Scavenging Test (DPPH)

The antioxidant activity was evaluated by monitoring its ability in quenching the stable free radical DPPH. First, the test proposed by Blois in 1958 with slight modifications, was employed to assess the scavenging activity of all extracts (Blois, 1958). In brief, 4 mg of DPPH was dissolved in 100 ml of methanol. Next, 160 µl of the DPPH solution was added to 40 µl of sample stock solutions of varying concentrations in a microplate. Following an incubation period of 30 minutes in darkness at room temperature (25 °C), the absorbance was measured at 517 nm. The inhibition percentage was calculated using the equation:

Inhibition percentage = [(Ac - As) / Ac] * 100, (Formula I)

where Ac represents the absorbance of the negative control (DPPH solution), and As represents the absorbance of the sample.

The ABTS Radical Cation decolorization Test

The ABTS assay, based on the ability of ABTS to act as a sensor for antioxidant activity, was conducted with few modifications according to the method proposed by Re et al. (Re et al., 1999). Initially, a solution of 7 mM ABTS in 5 ml of H_2O was prepared, along with 2.45 mM potassium persulfate ($K_2S_2O_8$) in 5 ml of H_2O, which was incubated in darkness for 16 hours. Subsequently, 160 µl of the ABTS solution was mixed with 40 µl of the extract's stock solution, containing different concentrations, followed by a 10min incubation period. The percentage of inhibition was calculated, using formula I and based on the absorbance measured at 734 nm.

Cupric ion reducing antioxidant capacity assay (CUPRAC)

CUPRAC assay was performed according to the method described by Apak et al. (Apak et al., 2004). A portion of40 µl of the sample stock solution or standard was combined with 60 µL of ammonium acetate aqueous buffer (pH 7), 50 µL of neocupronine alcoholic solution, and 50 µL of copper (2) chloride (CuCl2) aqueous solution. After a 60 min incubation period, the absorbance at 450 nm was measured. The results are given as A0.5, which corresponds to the concentration producing an absorbance of 0.5.

Alpha-amylase inhibitory activity

The alpha-amylase inhibitory activity was assessed using the method outlined by Zengin et al. (Zengin et al., 2014). This procedure involved preparing a reaction mixture in a 96-well microplate. The mixture consisted of α-amylase solution in sodium phosphate buffer (pH 6.9 with 6 mMNaCl) and 25 µl of the sample at various concentrations. After 10min of incubation at 37°C, 50 µl of a 1% starch solution was added to initiate the reaction. A control without the enzyme solution was used for comparison. Following an additional 20-minute incubation at 37°C, the reaction was stopped by adding 25 µl of 1 M HCl and 100 µl of iodine-potassium iodide solution. The absorbance was measured at 630 nm. The percentage inhibition of alpha-amylase was calculated using the following formula: I% = 1 - [(Absc - Abse) - (Abss - Absb) / (Absc - Abse)] × 100.

Here, Abse represents the absorbance of the solution containing the solvent volume, extract, enzyme, starch, HCl, and iodine-potassium iodide; Absb is the absorbance of the solution containing the extract, sodium phosphate buffer, and iodine-potassium iodide; Absc is the absorbance of the solution containing the solvent volume, extract, sodium phosphate buffer, starch, HCl, and iodine-potassium iodide; and Abss denotes the absorbance of the solution containing the extract, starch, enzyme, iodine-potassium iodide, and HCl.

Photoprotective activity

The sun protection factor (SPF) of *Pallenis hierochuntica* extracts was determined *in vitro* to evaluate their potential for UV protection. The method involves measuring the absorbance of extracts at seven specific wavelengths between 290 and 320 nm, at 5 nm intervals. The SPF value was calculated using the equation [SPF = CF * (I * Abs) / (EE × I)], with each measurement repeated three times (Sayre et al., 1979; Mansur et al., 1986; Zaidi et al., 2023).

In this equation, CF represents the correction factor (set at 10), I denotes the solar intensity spectrum, Abs corresponds to the absorbance of the sunscreen product at each wavelength, and EE×I is a constant provided by Sayre et al., as shown in Table 1. The values in the table represent the normalized product function (EE×I) associated with specific wavelengths from 290 to 320 nm, which are crucial for the SPF calculation.

Table 1. Values of EE (λ) × I (λ) function used to calculate the SPF

Wavelength λ (nm)	**290**	**295**	**300**	**305**	**310**	**315**	**320**	Total
EE × I (λ) (Norms)	0.0150	0.0817	0.2874	0.3278	0.1864	0.0837	0.018	1.0000

Results and discussion

Phytochemical Screening

The phytochemical screening of different parts of *Pallenis hierochuntica* reveals the presence of the most chemical groups in this species. Table 2 summarizes the obtained results.

Table 2. The main chemical groups detected in different parts of *Pallenis hierochuntica* fructification

Chemical groups	Aerial Parts	Roots	Stems
Flavonoids	+++	+++	+++
Leucoanthocyanins	+	+	+
Coumarins	+++	+++	+++
Catechol tannins	+++	+++	+++
Gallic tannins	++	++	+++
Saponins	-	+	-
Terpenes	+++	+++	+
Triterpenes	+++	+++	+++
Sterols	+++	+++	+++
Quinones	+	-	-

'-': Negative reaction,'+': Slight presence, '++': Moderate presence, '+++':Strong presence

This study is the first report on the phytochemical investigation of aerial parts, roots, and stems of *Pallenis hierochuntica* growing in the Betita region (Eastern Algeria) during the fruiting period.

As shown in Table 2, all parts of *P. hierochuntica* are rich in flavonoids, coumarins, catechol tannins, terpenes, and sterols. In fact, a literature survey indicates that *P. spinosa,* a related speciesfrom Algeria has been widely studied. It contains various classes of flavonoids, particularly flavones such as luteolin, and apigenin, and flavonols such as quercetin, and patuletin, and their derivatives (Khettaf and Dridi, 2022). In addition, epicatechin, epigallocatechin, phlorizin, kaempferol, catechin, rutin, naringenin, apigenin and apigenin glucoside), have been detected in this species using HPLC (Amrani-Allalou et al. 2021). In addition, two mono- and diglycosides of patuletin, a quercetin monoglycoside, two tricin monoglycosides, and four methoxylated flavonols were reported from *P. spinosa* growing in Egypt (Ahmed et al., 1992). However, coumarins such as herniarin, umbelliferone, esculetin, scopoletin, and isoscopoletin, have been isolated from different plants within the subtribe *Inuleae-Inulinae*, including *Pallenis* genus which showed antioxidant properties (Hoult, and Paya, 1996). It is important to mention that umbelliferone is used as a sunscreen agent and optical brightener in textiles (Lino et al., 1997; Leal et al., 2000).

Furthermore, terpenes and triterpenes are strongly present in both the aerial parts and roots of *P. hierochuntica,* indicating a broader distribution and potentially diverse functions throughout the plant. This finding is in good agreement qith the study reported by Adoui (2023) that *P. spinosa*, contains various terpenes, including α-cadinol, γ-cadinene, β-pinene, β-caryophyllene, and p-cymene (Adoui et al., 2023). Besides, sterols are present across all parts of the plant, while quinones are absent in the

roots and stems. Saponins were found only in the roots. The aerial parts, roots, and stems all exhibit these compounds, indicating the overall rich phytochemical profile of *Pallenis hierochuntica*. These results suggest that this species, like other plants in the subtribe, maybe a rich source of bioactive compounds, further enhancing its potential as a natural source of bioactive compounds for developing treatments for diseases related to oxidation and inflammation (Malarz, et al., 2024).

Total phenolic and flavonoid contents

According to Table 3, the current study found that *P. hierochuntica*'s methanolic root extract had the highest TPC (86.34±1.11 mgGAE/g extract) when compared to the extracts from the stems (73.18±1.42 mgGAE/g extract) and aerial parts (47.65±0.72 mgGAE/g extract). The aerial parts extract had the highest TFC, measuring 12.38 ± 0.72 mg QE/g of extract, and representing 25.98% of the total polyphenol content. The TFC content of the roots and stems extracts was similar, ranging from 10.10±0.65 to 10.87±0.55 mgQE/g of extract, or 11.70% and 14.85%, respectively.

Table 3. TPC, TFC, and Flavonoid percentage in different extracts.

Sample	**TPC**(mgGAE/g E)	**TFC**(mg QE/g E)	**Flavonoid** (%)
Aerial parts	47.65±0.72	12.38±0.45	.2598
Roots	86.34±1.11	10.10±0.65	11.70
Stems	73.18±1.42	10.87±0.55	14.85

Antioxidant activity

Table 4 provides the antioxidant activity of different parts of *P. hierochuntica* fructification measured through various assays. The antioxidant activity, assessed by DPPH, ABTS, and CUPRAC assays, consistently shows that the roots of the plant exhibit the highest antioxidant capacity, followed by stems and aerial parts.

The roots extract has been shown to have the highest DPPH and ABTS quenching activities compared to stems and aerial parts extracts. The DPPH and ABTS IC50 of roots extract were 40.21±1.11 and 20.05±0.54µg/mL respectively and were more than 7 and 11 times higher than the standard BHA used as a reference (IC50 = 05.73±0.41 and 01.81±0.10µg /mL in DPPH/ABTS tests respectively). Although its activity was lower than the roots extract, the stems extract showed perfect antioxidant activity, as revealed by DPPH and ABTS assays with IC50 values of 48.98±1.42 and 25.21±0.75µg/mL respectively. On the other hand, the same trend was also observed in the CUPRAC test, in which the roots extract (A0.5 = 29.14±1.65µg/mL) showed better activity than the stems extract (A0.5 =

35.19±1.53µg /mL), 6 and 7 times higher than that of BHA (A0.5 = 05.35±0.71 µg/mL). In the three antioxidant tests, the extract of the aerial parts showed the weakest activity.

These findings are consistent with the report on the methanolic extract of *P. spinosa,* which also shows strong activity against the DPPH radical, with IC50 values of 1.24 ± 0.03 mg/mL for flowers, 1.68 ± 0.05 mg/mL for leaves, and stems extracts (Amrani-Allalou *et* al., 2021).It's important to note that all extracts of *P. spinosa* displayed the lower antioxidant capacity against the ABTS radical (Amrani-Allalou *et* al., 2021).

In this study, there was a clear correlation between the results of the antioxidant activity through DPPH, ABTS, and CUPRAC assays for all extracts and their TPC and TFC contents. This observation is in good agreement with other studies that showed a relationship between the antioxidant power of extracts and the content of phenolic compounds, along with their structural diversity (Dudonné, et al., 2009; Piluzza and Bullitta, 2011) suggesting that the roots, stems and aerial parts of *Pallenis hierochuntica* species are particularly rich in antioxidant compounds, underscoring their potential as a valuable source of natural antioxidants.

Table 4. The IC50 (µg/mL) values of different parts of fructification within DPPH, ABTS, and CUPRAC assays

Sample	**DPPH**	**ABTS**	**CUPRAC**
Aerial parts	65.98±0.72	34.16±0.76	42.26±2.16
Roots	40.21±1.11	20.05±0.54	29.14±1.65
Stems	48.98±1.42	25.21±0.75	35.19±1.53
BHA	05.73±0.41	01.81±0.10	05.35±0.71

Alpha amylase activity

Table 5 provides the alpha-amylase inhibitory IC50 values of the different extracts of *P. hierochuntica,* highlighting the potential antidiabetic properties of the plant. The roots extract shows the most potent alpha-amylase inhibitory activity with an IC50 value of 136.77±0.19 µg/mL, suggesting strong potential for managing diabetes. The aerial parts extract has a moderately high inhibitory activity with an IC50 value of 193.11±2.77 µg/mL. Conversely, the stems extract exhibited the highest IC50 value (214.99±1.24 µg/mL), indicating the lowest inhibitory activity among the plant parts tested. When compared to acarbose, a standard antidiabetic drug with an IC50 value of 3650.93±10.70 µg/mL, *P. hierochuntica* extracts demonstrate significantly more effective alpha-amylase inhibition. This indicated that these extracts could be potent natural alternatives for managing alpha-amylase activity.

Consistent with this, extracts from *Pallenis spinosa*, a related species, showed strong activity against alpha-amylase at a concentration of 10 mg/mL, with inhibition percentages ranging between 68.54 ± 1.32% and 91.21 ± 0.40%. However, it is notable that the inhibition of alpha-amylase activity was significantly reduced after gastrointestinal digestion, with inhibition dropping to 8.96% for flowers extract, 5.73% for stems extract, and 5.28% for leaves extract. This reduction highlights the importance of considering the bioavailability and stability of bioactive compounds after digestion in the development of antidiabetic treatments (Amrani-Allalou et al., 2021).

The strong alpha-amylase inhibitory activity of *Pallenis hierochuntica* roots and the notable but reduced activity post-digestion of *Pallenis spinosa* extracts underscore the potential of these plants as sources of natural antidiabetic agents. Further research into optimizing the stability and bioavailability of these extracts could enhance their therapeutic efficacy.

Table 5. Alpha-amylase inhibitory IC50 values of different extracts of *P. hierochuntica*

Extracts	**IC50 (µg/mL)**
Aerial parts	193.11±2.77
Roots	136.77±0.19
Stems	214.99±1.24
Acarbose	3650.93±10.70

Sun Protection Factor (SPF) Values and Sunscreen Potential

Table 6 provides the SPF values for different extracts of *P. hierochuntica*, indicating the plant's potential use in sunscreen formulations. All parts exhibit high SPF values, suggesting that extracts from aerial parts, roots, and stems provide significant protection against UV radiation.

Table 6. SPF values of different extracts of *Pallenis hierochuntica*

Extracts	**SPF value**	**Category specified**
Aerial parts	31.47+0.31	High protection
Roots	31.45+0.06	High protection
Stems	31.48+0.73	High protection

These high SPF values indicate that *Pallenishierochuntica* extracts can be effective in sunscreen formulations, offering strong protection against harmful UV rays.

Indeed, it has been reported that several plant extracts or natural products, such as flavonoids or polyphenols, exhibit antioxidant and anti-inflammatory properties, and possess the ability to absorb UV rays and protect against sunburn (Saewan and Jimtaisong, 2013). Extracts from Asteraceae species have been widely reported, in literature to present photoprotective properties such as *Silybum marianum* in which the SPF value ranging from 2.01 to 6.07 (Vostálová, et al., 2019), and Calea fruticosa with a SPF value of 9.66 (Seregheti, et al., 2020). In contrast, Mishra et al. (2012) discussed the SPF of a specific essential oil formulation from *Calendula officinalis* L., which is a member of the Asteraceae family. The in vitro SPF of this essential oil formulation was evaluated using an ultraviolet spectrophotometric method and found to be 14.84 ± 0.16 (Mishra, et al., 2012). This comparison highlights the fact that SPF values can vary among different species within the same plant family. *Calendula officinalis* showed moderate SPF, whereas *Pallenis hierochuntica* demonstrated significantly higher SPF values, underscoring the exceptional photoprotective potential of its extracts.

This substantial difference in SPF values between species suggests that *P. hierochuntica* may contain unique compounds or higher concentrations of UV-absorbing phytochemicals that enhance its effectiveness as a sunscreen agent. The high SPF values of extracts emphasize their potential application in natural and effective sunscreen formulations, offering a promising alternative to synthetic UV filters.

CONCLUSION

This study examined the phytochemical profiles and biological properties of different parts of P. *hierochuntica* during the fruiting stage. This study included chemical screening to highlight the presence of several classes of secondary metabolites, antioxidant activity, alpha-amylase inhibitory activity, and sun protection factor values for the aerial parts, roots, and stems. Phytochemical screening revealed the presence of flavonoids, coumarins, catechol tannins, terpenes, triterpenes, and sterols in all examined plant parts, indicating plant richness. In contrast, saponins were only found in the roots. Regarding antioxidant activity, the results showed that the roots extract was the most effective, recording the highest values in DPPH, ABTS, and CUPRAC assays, followed by the stems and aerial parts extracts. Furthermore, the roots extract exhibited the strongest alpha-amylase inhibitory activity, suggesting potent anti-diabetic properties that significantly surpassed those of the standard drug acarbose. However, all studied parts demonstrated high SPF values, indicating high UV protection potential, paving the way for their use in sunscreen formulations. Therefore, this work is the first contribution to the study of the biological potential of this plant and further research is needed to

provide valuable insights into the medicinal properties of *P. hierochuntica* and to highlight its significance as a source of bioactive compounds with potential pharmaceutical applications.

Acknowledgement

Authors extend their heartfelt appreciation to **Mr. AiadAbd El Moutalib** for his invaluable financial and moral support, which greatly bolstered the success of this research.

Furthermore, authors express their thanks to **Mr. Prof. Belfar Mohamed Lakhdar** from VPRS Laboratory for his crucial administrative support, aiding in the organization and coordination pivotal to the accomplishment of this scientific endeavor.

Conflict of interest

The authors declare that there are no conflicts of interest. We confirm that all authors have agreed to submit this manuscript to the journal and will complete any necessary forms and disclosures.

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