PHYTOCHEMICAL PROPERTIES AND IN VITRO ANTIOXIDANT ACTIVITY OF *LAWSONIA INERMIS (*L) EXTRACTS.

Abstract:

The aim of this study was to determine the phytochemical composition and antioxidant activity of *Lawsonia inermis* extracts, a plant traditionally used in Mali. The sample (Leaves, bark roots and trunk bark) were collected in Kayes. The polyphenols, flavonoids, tannins and anthocyanins of the extracts were quantified using spectrophotometry and identified via HPLC. The antioxidant activity was assessed using 2, 2-azinobis-3-ethylbenzothiaz oline-6-sulfonic acid (ABTS) and 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radicals scavenging capacities. The leave extracts were mainly in flavonoids; Polyphenols, anthocyanins, however, they showed low tannins compositions. The values were 9.21 mg ECt; 7.77 mg EAG; 5.61 mg ECt; 0.74 mg ECG per 1 g of dry matter (DM) respectively for flavonoids, polyphenols, anthocyanins and tannins. The HPLC analysis of the extracts revealed the presence in Leave: of Apigenin, Chlorogenic and Lawsone acids were also found, in the bark of Roots: Protocatechic Acid.
All extracts showed good ABTS and DPPH radical scavenging capacities. The antioxidant activities of samples correlated with their phytochemical compositions.

Key words*Lawsonia inermis*, Extracts,Phytochemistry, Antioxidant-activities

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Introduction:-

Lawsonia inermis, or henna, is a thorny shrub 2 to 5 meters tall, native to Arabia and Iran, but also found in Mediterranean and subtropical Africa, Madagascar, Asia, and Australia. The plant has been cultivated in the Sahel and Sudanian regions near dwellings. *L. inermis* prefers sandy soils (Arbonnier, 2002).

According to Muhammad HS and Muhammad S in 2005, chloroform and aqueous extracts of the leaves inhibit the growth of bacteria responsible for wound infections. In vivo studies conducted on rats reportedly showed hepatoprotective activity of the ethanolic extract of the leaves (Ahmed et al., 2000). In vitro studies of the methanolic extract of the leaves reportedly showed immunomodulatory activity (Mikhaeil et al., 2004).
According to Kaplan and Hammerman in 2006, a deficiency in certain enzymes such as glucose-6-phosphate dehydrogenase causes side effects (hemolysis, anemia, reticulocytosis and hyperbilirubinemia).

Lawsonia inermis contains quinonoid derivatives of xanthones, flavonoids, coumarins, mannites, Gallic and tannic acids as well as lawsone which is thought to be responsible for the plant's colour (Kelmanson et al, 2002; Ong et al, 2009).
Siddiqui and Kardar isolated and identified two triterpenes (lawsonic acid and lawsonin) in the plant's leaves in 2001.

Chloroform extract revealed the presence of seven phenolic compounds in the leaves: p-coumaric acid, lawsone, apigenin, luteolin, 2-methoxy-3-methyl-1.4-naphthoquinone, cosmosin, and apigin. The ABTS [2.2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] assay of each of these isolated compounds showed antioxidant activity comparable to that of ascorbic acid (Mikhaeil et al., 2004).

The role of phenolic compounds in protecting against certain diseases is well established due to their potential interaction with numerous enzymes and their antioxidant properties (Zhang, 2003). Specifically, flavonoids are attributed with a variety of biological properties: antitumor, free radical scavenging, anti-inflammatory, analgesic, anti-allergic, antispasmodic, antibacterial, and anticancer.

Several in vitro and in vivo studies have focused on evaluating the antibacterial and antifungal properties of polyphenols. Currently, this effect is demonstrated by numerous experimental studies. Moroh et al., in 2008, demonstrated the antibacterial effect of acetic extracts rich in alkaloids and flavonoids of Morindamorindoides on eight strains of Escherichia coli, bacteria commonly found in diarrhea in infants and children up to the age of five.

The inhibitory activity of flavonoids on bacterial growth was studied by Katarzyna et al in 2007 who showed that many flavonoid compounds have an effect on different gram negative (Escherichia coli…) and gram positive (Staphylococcus aureus…) bacterial strains.

Given the limited number of scientific studies devoted to the plant and its use in traditional medicine, the objective of this study was to identify the polyphenolic compounds present in the leaves, trunk bark, and root bark, and to evaluate their antioxidant effects, which would justify the plant's uses in traditional medicine.

Material and Methods

Material

The samples (Leave, trunk bark, roots bark) of *Lawsonia inermis* were collected in Kayes (Mali). They have been transported and identified to the Department of Traditional Medicine (DMT) under the number (0903). Folin-Ciocalteu, Gallic, Protocatechic, Chlorogenic, Caffeic acids, Lawsone, Rutin, Apigenin, Quercetin, Kaempferol. ABTS and DPPH were provided by the companies SIGMA-Aldrich (France) and Across organics (Belgium). All other chemicals and solvents used were obtained from a commercial source and were of analytical grade.

Methods

Samples Preparation

After the initial cleaning process, samples (the different parts of the plant) were dried in the shade and at room temperature in the Natural Substances laboratory of the FST. After drying, the samples were pounded using a laboratory scale hammer miller and the resulting powder sieved until a fine powder was obtained.

Preparation of Extracts

The preparation of the sample extracts was carried out by the method describedby Abu Bakar et al., 2009, with slightly modified. To 5 g of plant powder were added2 times 20 mL of a 50/50 (V/V) hydromethanolic solution for UV-Visible Spectroscopyand HPLC. The mixtures were stirred for 6 hours, and then filteredthrough a 0.45 µmmillipore membrane. The filtrates collected were centrifuged at1500 ×g for 20 min. The extracts obtained have been cooled and conserved at(+4˚C) in bottles before analysis.

Total polyphenol content (TPC)

The total polyphenolic content was determined using the Folin-Ciocalteu method. The plant extract (0.1 ml) was mixed with 0.1 ml of the Folin-Ciocalteu reagent (1 N). The mixture was left at room temperature for 5 minutes. Then, 1 ml of 7% sodium carbonate (Na_2CO_3) was added to the mixture.
After 90 minutes of incubation at 23°C, absorbances were measured at 750 nm against a blank prepared with methanol. Note that a calibration curve was established before using gallic acid under the same conditions as the samples to be analyzed. The results obtained are expressed in mg gallic acid equivalent per g of dry matter (mg EGA/g). All samples were analyzed at least three times, and the mean was calculated

Total Flavonoid Content (TFC)

Total flavonoids were assessed colorimetrically. A 0.25 mL aliquot of the plant extract was taken, and 1 mL of distilled water and 75 µL of 5% sodium nitrate (NaNO3) were added. After 5 minutes, 75 µL of 10% aluminum trichloride (AlCl3) were added to the mixture. After 6 minutes, 0.5 mL of 1 M sodium hydroxide (NaOH) and 0.6 mL of distilled water were added to the mixture while stirring.
A calibration curve was developed using standard catechin solutions prepared at different concentrations. The absorbance of the mixture was determined by UV spectrometry at 510 nm relative to a blank prepared with water. The total flavonoid content was expressed as mg catechin equivalent per gram of dry matter (mgCE/g). All samples were analyzed at least three times, and the mean was calculated.

Total Anthocyanin Content (TAC)

Total anthocyanin compounds were evaluated colorimetrically using a UV spectroscope. The method used was based on the change in absorbance using two buffers: one containing 0.025 M potassium chloride (KCl) (pH 1) and the other 0.4 M sodium acetate (pH 4.5). 0.2 mL of the extract was mixed with 1.8 mL of each buffer solution. The absorbance of the solution was determined at 510 nm and 700 nm against a blank prepared with methanol.
The change in absorbance was calculated using the following formula:

$$\Delta A = [(A510-A700) \text{ x } pH1] - [(A510-A700) \text{ x } pH4] \quad (1)$$

The concentration of anthocyanin pigment in the extract is expressed in mg cyanidin-3-glycoside equivalent per liter of solution or in mg cyanidin-3-glycoside equivalent per gram of dry matter (mg CG/g) by comparison to a calibration curve established with cyanidin-3-glycoside. All samples were analyzed at least three times, and the average was calculated. The anthocyanin concentration was calculated using the following formula:

$$(mg/L) = \Delta A \text{ x } Mm \text{ x } Df \text{ x } (100/Ma) \quad (2)$$

With ΔA: the change of absorbances, Mm: molecular mass of cyanidin (449.2);

Df : dilution factor ; Ma : molecular absorptivity (26.900).

Total tannin content (TTC)

In a test tube containing 1.5 mL of concentrated sulfuric acid, we added 50 µL of extract and 3 mL of a 4% methanol-vanillin solution. The mixture was left to stand for 15 minutes. Absorbance was measured at 500 nm against a methanol blank.

The calibration curve was developed using standard solutions of catechins prepared at different concentrations. The calibration curve has been established with a correlation coefficient $R^2 = 0.9899$. The results are expressed in mg equivalent catechins per 1 g of dry matter (mg ECt/1 g DM). All samples were analyzed at least three times.

HPLC Analysis

The HPLC analysis was carried out according to the method described by Muanda et al., 2011,with a slight modification, using an elution gradient consisting of three phases. Solvent A: 50 mM ammonium phosphate (NH4H2PO4) at pH 2.6 (adjusted with ortho phosphoric acid), solvent B: (80/20 (v/v)) acetonitrile/solvent A, and solvent C: 200 Mm ortho phosphoric acid (H3PO4) at pH 1.5 (pH was adjusted with 0.1 M NaOH). After preparation, the solvents were put in an ultrasonic device for 10 min for homogenization. The profile of the gradient used for 60 min is presented in Table 1. The elution flow rate was 1 mL/min and the injection loop capacity 20 µL. Detection was performed at 280 and 320 nm. Standard phenolic compounds (9 standards) were prepared by dissolving 2 mg/mL. In each sample, the phenolic compound was identified by the retention time of the corresponding standard and the concentration of the phenolic compound was calculated by comparing the peak areas. The samples were analyzed at least three times. After each cycle, the system was reconditioned 10 minutes before a new analysis.

Table 1. Profile of the gradient used for 60 min.

T (mn)	A%	B%	C%
0-4	100	0	0
4-10	92	8	0
10-22.5	0	14	86
22.5-27.5	0	16	84
27.5-50	0	25	75
50-55	0	20	80
55-60	100	0	0

Antioxidant Activity Assay

Scavenging capacity of ABTS radicals

The method developed by Kim et al., 2002,slightly modified has been used in this experiment. 1.0 mM AAPH was mixed with 2.5 mM ABTS using buffer. The buffer solution consists of 100 mM potassium phosphate (pH 7.4) containing 150 Mm NaCl. The mixture was heated in a water bath at 68˚C for 20 minutes until the concentration of the blue-green ABTS radical complex gives an absorbance of between 0.65 ± 0.02 at 734 nm. To 60 µL of the

sample has been added 2.94 mL of the radical blue-green solution of ABTS. The mixture was incubated in a water bath at 37˚C for 20 minutes. The control consists of 60 µL of methanol and 2.94 mL of ABTS and was checked for each series of samples. The absorbance was measured at 734 nm. Stable radical scavenging activity in the ABTS test of phenolic compounds was expressed in mg equivalent vitamin C (mg EVC/100 (DM). The radical solution was prepared daily. All samples were analyzed at least three times

Scavenging capacity of DPPH radicals

The DPPH radical scavenging activity was determined using the method devoloped byHoste et al., 2006,with some modifications. To 2.90 ml of an aqueous solution of 50% methanol (100 mM of DPPH), 100 µL of plant extract were added. The mixture has been heated in a water bath at 20˚C away from light for 40 min. The blank was prepared with (100 µL of 50% methanol and 2.90 mL of the DPPH solution) and checked for each series of samples. The decrease in absorbance was measured at 517 nm 40 minutes later. The free radical scavenging activity in the DPPH test of total phenolic compounds was expressed in mg equivalents vitamin C (mg EVC/100 g (DM). The radical solution has been prepared daily. All samples were analyzed at least three times.

Statistical Analysis

The results were processed with software such as: Excel version 2019 and Minitab 18.1, for analysis of variance (ANOVA) was used to compare the mean values of these varieties with the Fischer test at the probability threshold P = 0.05.

Results and Discussion

Total Polyphenolic Content (TPC)

Figure 1 shows the results of the phenolic compound composition of *L. inermis* extracts analyzed by UV-visible spectroscopy. Analysis of the results shows that the different extracts are rich in phenolic compounds (flavonoids, anthocyanins, tannins, and polyphenols). The leave showed the lower in total tannins.

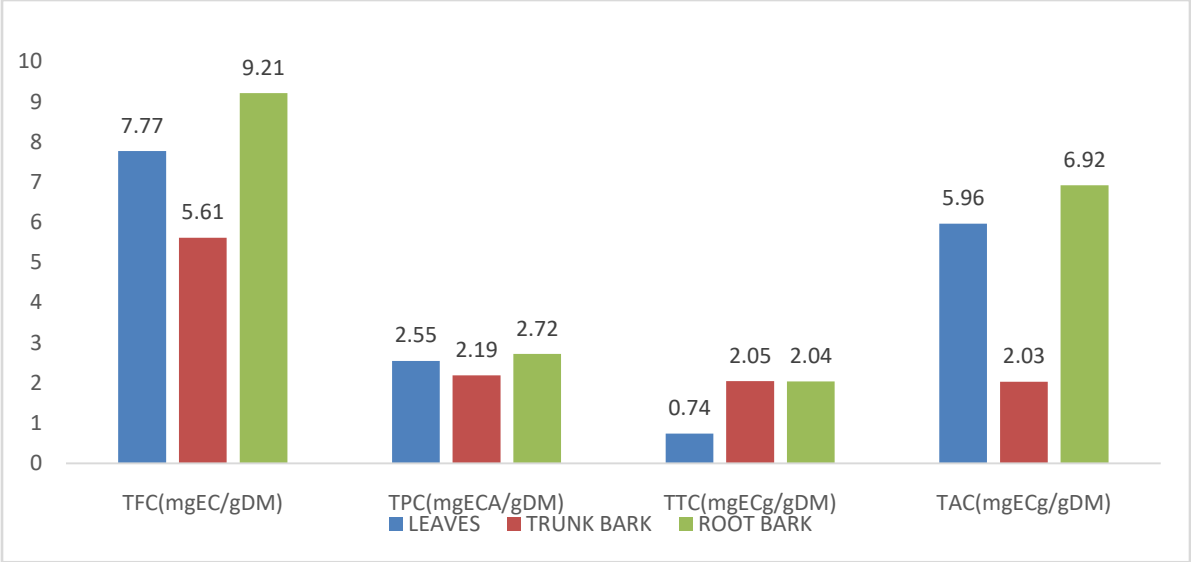


Figure 1: Total Polyphenolic Content (TPC) of the*L. inermis* extracts

HPLC Analysis

The results of HPLC analyses of *L. inermis* extracts are shown in Figure 2 (chromatograms of leave and root bark extracts). Analysis of this figure reveals the presence of some polyphenolic compounds in the both extracts.

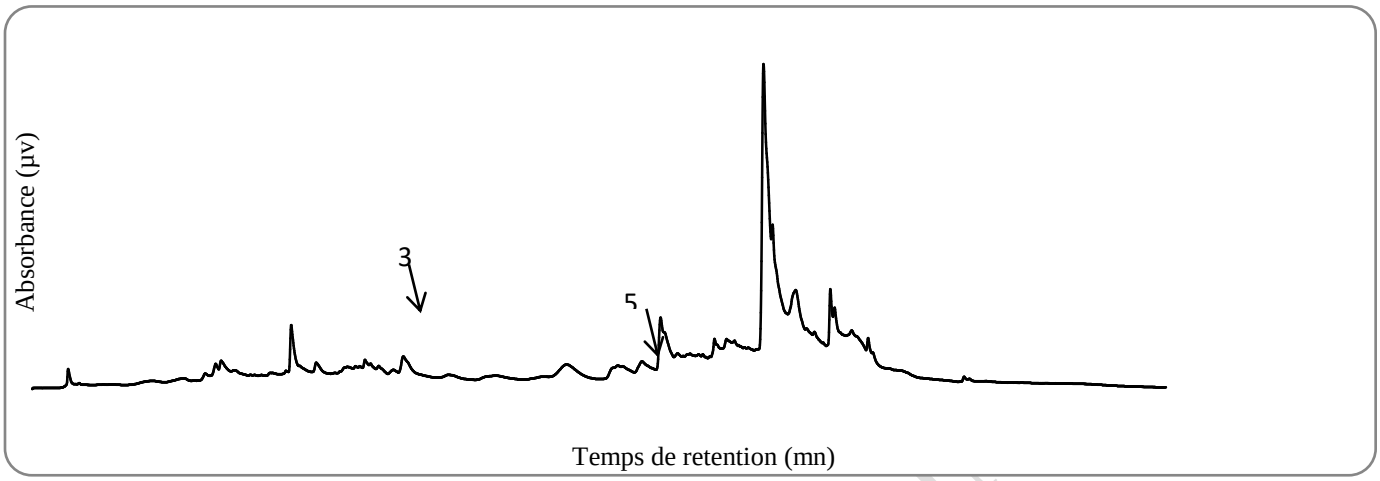
However theresults of qualitative and quantitative analyses of the different *L. inermis* extracts are given in Table 2. Analysis of these results shows that Protocatechic acid (1503.54 µg/ml) is the predominant compound in the root extracts, while apigenin (166.40 µg/ml) is the predominant compound in the leaf extracts. However, the leave extracts also contain chlorogenic acid (60.17 µg/ml) and lawsone (9.07 µg/ml). Our results are comparable to those of Handa et al, in 1997 and Mebe et al, in 1998 on the extract of the leaves of *L.inermis*, in particular by the presence of flavonoids, coumarins, naphthoquinones and triterpenes.In contrast, the instrument did not detect any of our standards in the trunk bark extracts. It should be noted that not all compounds were identified during our analyses due to a lack of suitable standards.

Table 2. HLPC Analysis results of the *L. inermis* extracts.

Names of compound	**Rt**(min)	**Leaves** µg/mL	**Trunk Bark** µg/mL	**Bark of Roots** µg/Ml
Gallic Acid	09.65	Nd	Nd	Nd
Protocatechic Acid	11.85	Nd	Nd	1503.54 ±40.80
Chlorogenic Acid	16.72	60.17±0.4.07	Nd	Nd
Caffeic Acid	19.55	Nd	Nd	Nd
Lawsone acid	25.98	09.07±0.12	Nd	Nd
P-Cumaric Acid	33.90	Nd	Nd	Nd
Rutin	35.50	Nd	Nd	Nd
Quercetin	39.82	Nd	Nd	Nd
Apigenin	41.41	166.40±0.4. 34	Nd	Nd
Kaempferol	42.52	Nd	Nd	Nd

Results reported are means of triplicate samples ± standard deviation. Rt: Retention time,

Nd: no detected.



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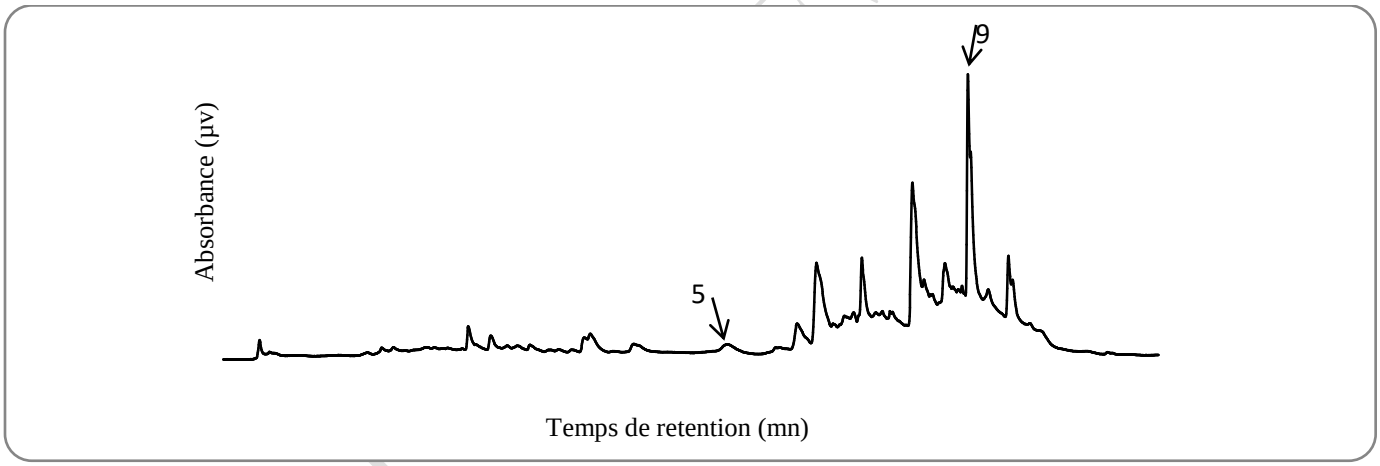

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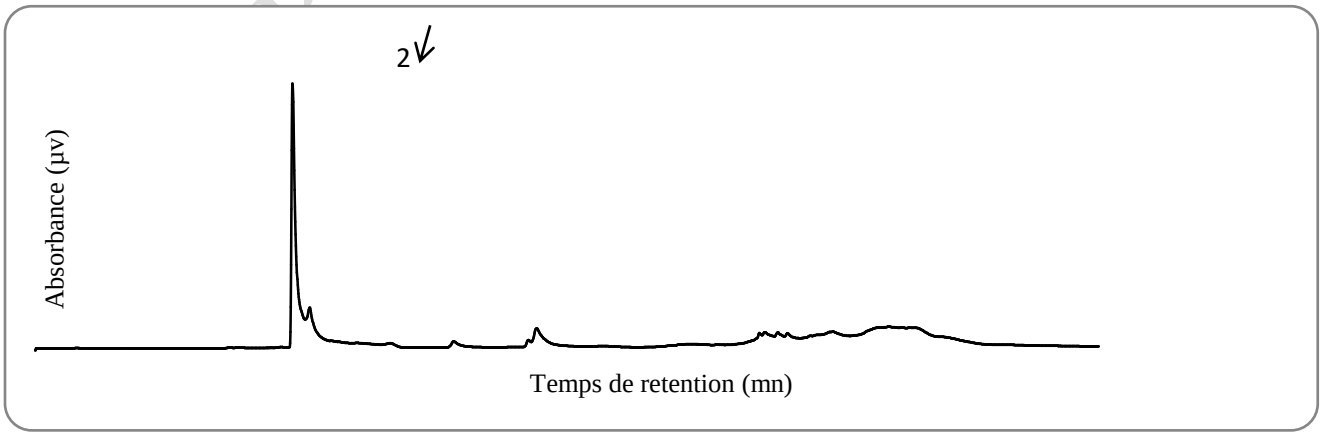

Figure 2: Chromatogram profiles of the extracts of Leave (a and b), of root bark (c).

Antioxidant Activity

The results of the antioxidant activity analysis of extracts from different parts of *L. inermis* are reported in Table 3. The result showed that the extract of *L. inermis* leaves exhibits an antioxidant activity of 2.49 mg VCE by ABTS, which could explain the presence of apigenin (166.4 µg/g dry matter) in *L. inermis* leaves. Mikhaeil*et al*. demonstrated in 2004 that the flavonoids contained in *L. inermis* leaves have antiradical activity comparable to that of ascorbic acid; in particular, apigenin-7-apiosyl glucoside showed a high potential for scavenging free radicals. These compounds act as free radical scavengers by preventing the peroxidation of thephospholipid membrane and protecting immunomodulatory cells. This could explain the hepatoprotective effect of henna (Anaad et al., 1992).

Table 3.ABTS and DPPH radical scavenging activities of *L. inermis extract*

Samples	ABTS-Test (g EVC/100 g (DM))	DPPH-Test (g EVC/100 g (DM))
Leaves	2.49±0/06	2.23±0.002
Trunk Bark	1.74±0/004	2.0±0/01
Bark of Roots	0.96±0..01	2.15±0.003

Results reported are means of triplicate samples ± standard deviation

4. Conclusion

The results of this study revealed the presence of Apigenin and Protocatechic Acid with considerable quantity in the extracts of our studied plant. These chemical constituents are known to have the ability to scavenge free radicals from ABTS and DPPH. This explains the best antioxidant activities of our plant extracts. In sum, the results of phytochemistry and antioxidant activity analysis of *L. inermis* extracts revealed that it is a medicinal plant.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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