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Original Research Article

GC-MS analysis, iron-chelating, total flavonoid, total phenolic, and antioxidant activities of ethanol extract from kwini mango (*Mangifera odorata* Griff) in central Java province

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ABSTRACT

The leaves *Mangifera odorata* Griff exhibit pharmacological effects, including antioxidant, iron-chelating, analgesic, and antibacterial . These effects are attributed to active compounds such as mangiferin and phenolic acids. Standardizing the extract *Mangifera odorata* Griff leaves is essential to ensure consistent therapeutic effects. This study aimed to evaluate the GC-MS profile, iron-chelation ability based on ferritin reduction test animals' blood, total flavonoid, phenolic content, antioxidant activity ethanol extract *Mangifera odorata* Griff leaves. extract was prepared using the maceration technique. The extract contained 108.99 ± 0.8321 mgQE/g of total flavonoids and 9.718 ± 0.0319 mgGAE/g of total phenolics. GC-MS analysis identified 103 potential compounds in the ethanol extract, including three bioactive polar compounds: 1, 6, 10, 14, 18, 22-Tetracosahexae, Isophytol, and 3, 7, 11, 15-Tetramethyl-2-hexadecanoic acid, along with Pentadecanoic acid, 14-methyl ester, and 8-Octadecenoic acid, methyl ester. Phytol was the predominant compound, known for its iron-chelating properties. The antioxidant assay showed an IC_{50} of 45.92 ppm for the ethanol extract, compared to gallic acid's IC_{50} of 25.20 ppm, indicating notable antioxidant activity. Additionally, the extract at a dose of 200 mg per 200 g body weight effectively reduced ferritin levels in the blood of test animals, highlighting its potential as an iron-chelating agent both in vitro and in vivo.

Keywords: Flavonoids, Phenolics, Iron Chelation, Antioxidants, *Mangifera odorata Griff* .

Introduction

Iron Overload (IO) refers to an imbalance in iron homeostasis, where the iron levels in the body exceed the necessary amount.[1] Excess iron increases the risk of heart failure, liver diseases, diabetes, and metabolic syndrome.[2] In 2021, the global prevalence of IO was estimated at around 176 million cases. In Indonesia, there were 7,029 reported cases in 2020.[3] A significant rise from 4,431 cases in 2015.[4] The current treatment for IO commonly involves the synthetic drug deferoxamine, which forms iron complexes that are excreted through urine.[5] However, deferoxamine can cause several side effects, such as visual and auditory impairments, cardiovascular issues, digestive problems, hematological, liver, neurological, and musculoskeletal disorders, along with increased liver transaminase enzymes.[6,7] Additionally, the drug is expensive and requires inconvenient subcutaneous infusion for 8-12 hours a day, 5-7 days a week. Therefore, there is a need for alternative iron-chelating agents derived from natural sources.[8] Secondary metabolites from natural sources, such as mangiferin, quercetin, epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate, have shown promise as potential anti-IO agents.[9,10] Flavonoids, alkaloids, and mangiferin are particularly effective due to their aromatic ring structures bound to glucose via C-C bonds.[11] Mangiferin, in particular, demonstrates strong biological activity, enabling it to neutralize free radicals and lower blood iron levels, which correlates with reduced serum ferritin levels.[12] Furthermore, mangiferin acts as a chelator for Fe3+, promotes Fe2+ oxidation, and prevents Fe3+ reduction, thus limiting the availability of iron ions involved in lipid peroxidation reactions.[13] Kweni mango leaves (*Mangifera odorata* Griff) are rich in flavonoids and polyphenolic compounds, which possess iron-chelating and antioxidant properties.[14] The specific chemical structure of these flavonoids allows them to bind iron, forming stable and soluble iron-flavonoid complexes.[15] The flavonoids have three potential sites for metal chelation: the 3-hydroxy-4-keto group on the C-ring, the 5-hydroxy group on the A-ring, and the 4-carbonyl group on the C-ring, as well as the 3,4-dihydroxy group on the B-ring.[16] As such, *Mangifera odorata* Griff presents a promising alternative approach to managing iron overload through its iron-chelating activity, thanks to its flavonoids, phenolic compounds, and the essential oil isophytol

Materials and Methods

Plant Collection and Identification

Leaves of *Mangifera odorata* Griff were gathered from Kebumen, Central Java Province, at coordinates 03°35'12.0"N-98°28'45.60"E in July 2023. The environmental conditions during collection included a pH of 5, light intensity of 397 cd, and soil temperature of 30°C. The plant identification was performed, and the specimens were authenticated at the Laboratory of Biology, Ahmad Dahlan University, Indonesia, on June 21, 2023 (No. 296/Lab.Bio/B/VI/2023). Ethical approval for the use of Wistar strain rats (ID 012306095) was obtained on July 4, 2023.

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Sample Preparation
A total of 7 kg of carefully sorted and washed leaves were dried at 50°C for two weeks. After drying, the leaves were ground into a fine powder and weighed. The powdered material was then macerated with 96% ethanol (EtOH pro analysis, Nury Lab, Indonesia) for 5 days in a dark environment. The crude extract was filtered using Whatman No. 1 filter paper, and the filtrate was evaporated to dryness using a rotary evaporator (Heidolph, Germany) at 40°C.[17]

GC-MS (Gas Chromatography-Mass Spectrometry) Analysis
Chemical components were identified using GC-MS analysis (GC Model SCION 436 and 456, Netherlands). Helium served as the carrier gas, and the sample was injected into the GC-MS through a capillary column under constant pressure. The filtered extract solution was introduced at a split ratio of 8:1 psi and a total flow rate of 1.2 mL/min. The operational temperatures were set as follows: 280ºC for the column, 140ºC for the oven, 250ºC for the injector, and 230ºC for the detector. A mass detector was used to identify the eluted components.[18,19]

Iron-Chelating Activity
Thirty-five Wistar strain rats (180–200 g, aged 8–10 weeks) were divided into seven groups:

Group 1 - received 0.5% CMC (normal control)
Group 2 - was given ferrous sulfate at 40 mg/kgBW (disease control)
Group 3 - received ferrous sulfate 40 mg/kgBW + deferoxamine 1 mg/200 gBW (positive control)
Group 4 - was treated with ferrous sulfate 40 mg/kgBW + mangiferin 0.5 g/kgBW (comparison control)
Group 5 - received ferrous sulfate 40 mg/kgBW + extract at 50 mg/200 gBW,
Group 6 - was treated with ferrous sulfate 40 mg/kgBW + extract at 100 mg/kgBW, and
Group 7 - received ferrous sulfate 40 mg/kgBW + extract at 200 mg/kgBW.[20]

Ferrous sulfate was administered over a specified duration, based on preliminary testing. The extract, comparator, and deferoxamine were administered for 28 days after inducing iron overload (IO) in the rats. Blood samples were collected via orbital vein on the day of IO induction and on day 29 post-treatment to measure biochemical parameters. The rats were euthanized, and their kidney and liver tissues were collected for histological examination. Serum ferritin levels were measured using the VIDAS® Ferritin ELISA method.[21]

Total Flavonoid Content
To prepare the quercetin standard solution, 25 mg of quercetin was dissolved in 25 mL of 96% ethanol. A 1 mL aliquot of this solution was diluted with 96% ethanol to 10 mL to make a 1000 ppm concentration. From this, a 100 ppm solution was prepared by diluting 5 mL with 50 mL of ethanol. Solutions of 2, 4, 6, 8, and 10 ppm were then prepared. Each sample was mixed with 4 mL of 96% ethanol, 0.2 mL of $AlCl_3$, 0.2 mL of 1M potassium acetate, and 5.6 mL of distilled water, then incubated for 30 minutes at room temperature. The absorbance was measured at 440 nm using a UV-Vis spectrophotometer.[22]

Total Phenolics Content
For the determination of maximum wavelength (λmax), 300 µL of 30 ppm gallic acid solution was mixed with 1.5 mL of Folin-Ciocalteu reagent (1:10). After resting for 3 minutes, 1.2 mL of 7.5% Na_2CO_3 solution was added, and the solution was shaken until homogeneous. The mixture was allowed to rest at room temperature, and absorbance was measured at 600–850 nm. A standard curve for gallic acid was created using varying concentrations (10, 20, 30, 40, and 50 ppm), and the total phenolic content of the ethanolic extract from *Mangifera odorata* Griff leaves was determined by mixing 300 µL of the extract with Folin-Ciocalteu reagent and Na_2CO_3 solution. The absorbance was measured using a UV-Vis spectrophotometer.[22]

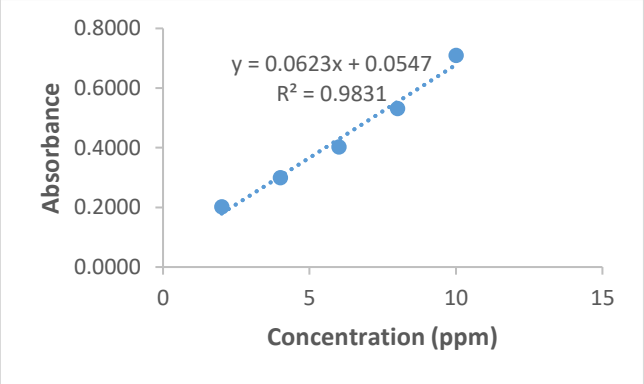

Figure 2: Standard curve of quersetin absorbance vs. concentration

Antioxidant Activity Testing
The antioxidant activity of the *Mangifera odorata* Griff ethanolic extract was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The extract was tested at various concentrations (10–60 ppm), and absorbance was measured at 516 nm. Gallic acid was used as the standard control. The inhibition percentage of DPPH radicals was calculated using the formula:

$$\text{Inhibition (\%)} = ([Ab - As] / [Ab]) \times 100$$

Where Ab is the absorbance of the blank, and As is the absorbance of the sample. The IC_{50} value was determined from the linear regression plot using the formula:

$$IC_{50} = ([50 - b] / [a]) \times 100$$

Where b is the y-intercept, and a is the slope of the regression line.

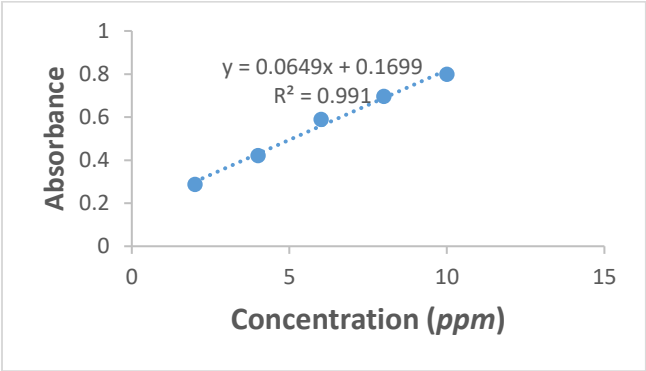

Figure 3: Standard curve of gallic acid absorbance vs. concentration

Statistical Analysis
The antibacterial activity of iron chelation, total flavonoid, total phenolic content, and antioxidants activity were analyzed using IC_{50} values derived from the experiments. The chemical composition of the *Mangifera odorata* Griff leaf extract was further evaluated using GC-MS

Results and Discussion

A total of 7 kg of fresh *Mangifera odorata* Griff leaves produced 650 g of simplicia powder and 214.5 g of concentrated crude extract. The analysis of the secondary metabolite groups in the simplicia and ethanol extract of *Mangifera odorata* Griff leaves aimed to identify phytochemical compounds with potential iron-chelating, total flavonoid, total phenolic, and antioxidant properties, as shown in Table 1. Both the simplicia and the ethanol extract of *Mangifera odorata* Griff leaves contain secondary metabolites, including saponins, tannins, flavonoids, and alkaloids, as presented in Table 2.

GC-MS Analysis

GC-MS analysis revealed that the ethanol extract of *Mangifera odorata* Griff leaves contains natural compounds such as 1,6,10,14,18,22-Tetracosahexae, Isophytol, 3,7,11,15-Tetramethyl-2-hexadeconic acid, pentadeconic acid, 14-methyl ester, and 8-octadeconic acid methyl ester (Table 6). stearate, n-hexadecanoic acid, 2' HydroxyCyclohexyl) Propanol, 2H-Pyrrol-2-one, 1,5-dihydro-1-methyl-, and indole[23] . The results of other studies using GC-MS were obtained of the derivatized ethyl acetate extract showed the presence of phenolics (benzoic acid, 4-hydroxybenzoic acid and 3,4- dihydroxybenzoic acid)[28]. These compounds have potential iron-chelating, antioxidant, and anti inflammatory effects. The most abundant compounds identified by GC.

Table 1: Standardization of *Mangifera odorata* Griff non-specific parameters

Non-specific parameter	Value	Standard	Compliance
Extract yield	33 %	>30	Yes
Water content	8.80±0.58%	>10%	Yes
Water-soluble essence	69.78%	>30	Yes
Ethanol-soluble essence	75.51%	>10%	Yes
Specific gravity	1.0230 g/ml	1.055	Yes
Total content	3.35%	3–5%	Yes
Acid-insoluble ash content	1.84%	>0.9%	Yes
Organoleptic properties:			Yes
Odor	Specific	Specific	Yes
Taste	Bitter	Bitter	Yes
Color	Brownish-black	Brownish-black	Yes
Viscosity	Thick	Thick	Yes

Table 2: Standardization of *Mangifera odorata* Griff extract on specific parameters (phytochemical screening).

Specific parameter	Phytochemical screening result	Compliance
Saponin	Stable foam (positive)	Yes
Tannin	Blackish-green color (positive)	Yes
Flavonoid	Amyl alcohol layer with orange color (positive)	Yes
Alkaloid	Orange color (positive)	Yes

Table 6: GC-MS analysis results of EtOH extract of *Mangifera odorata* Griff

No	RT (Min)	Peak Name	Compound Nature	Molecule Formula	Molecular Weight (g/mol)	Amount	Ref.
1	25.07	1,6,10,14,18,22-Tetracosahexae	Squalene	C30H50	410	10.25	antioxidant
2	19.28	Isophytol	Phytol	C20H40O	296	5.46	Chelating ion
3	16.57	3,7,11,15-Tetramethyl-2-hexadecanoic	Neophytadiene	C20H38	278	4.93	
4	17.47	Pentadecanoic acid, 14-metyl ester	Hexadecanoic acid, methyl ester	C17H34O2	270	5.27	antibacterial
5	19.15	8-Octadecenoic acid, methyl	Heptadecanoic acid, 16-methyl-, methyl ester	C19H38O2	298	5.7	antiinflamatory

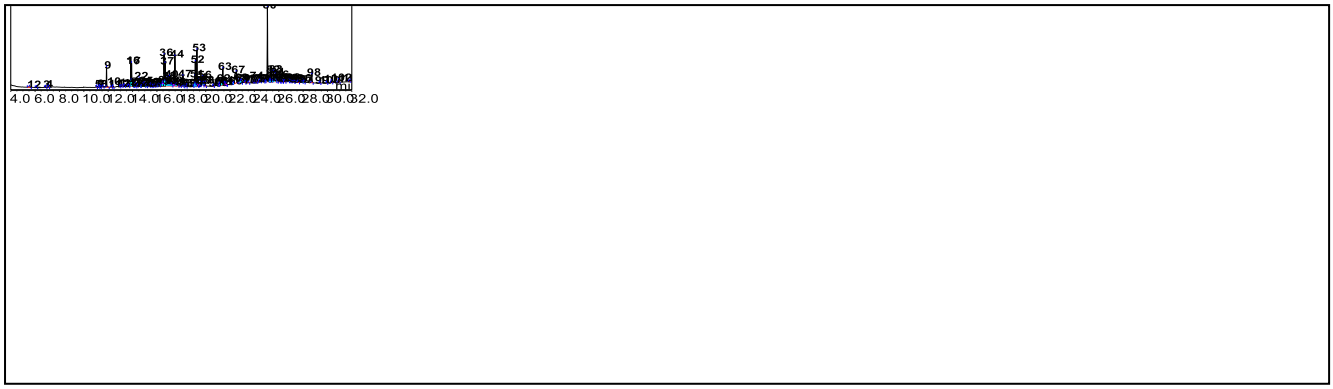

Figure 1: Analysis GC-MS of *Mangifera odorata* Griff

As shown in Figure 1, the GC-MS results displayed 103 peaks from the mangiferin extract with ethanol as the solvent, while in the research[23] the results were obtained butyl glyoxylate, ethylbenzene, cyclohexane, 1,2-dimethyl-(cis/trans), 2,3-butanediol, retina, methyl -MS were phytol and isophytol, which have been identified as Fe ion chelators in the extract of *Mangifera odorata* Griff. Phytol, a diterpene, is known to reduce the production of free radicals due to its branched chain saturated alcohol structure. Ferritin concentration in plasma is a key indicator of iron overload, with levels above 1000 ng/mL signaling excess iron. This overloadoccurs when excessive Fe^{3+} in the body binds to porphyrin, forming ferritin to store iron. Table 3 demonstrates a reduction in ferritin levels, as well as liver and kidney damage indicators, across all test groups. The study suggests that higher doses of mango kweni leaf extract lead to a more significant reduction in ferritin levels. The ferritin levels, along with indicators of liver and kidney damage, were analyzed using statistical methods. The Shapiro-Wilk test and variance homogeneity test were initially conducted to check for data normality. If the data were normal and homogeneous, a parametric One-Way ANOVA was performed; otherwise, the Kruskal-Wallis test was applied. Mangiferin, an iron chelator, binds with plasma transferrin or ferritin to prevent the release of iron ions and reduce the production of Non-Transferrin-Bound Iron (NTBI). Previous studies have shown that Mangifera foetida leaf extract reduces ferritin serum levels in thalassemia patients, and an in vivo study indicated that Mangifera foetida leaf extract can prevent iron overload in rats. Polyphenols in the extract help neutralize free radicals, lowering serum iron levels and ferritin in the blood.

Table 3: Activity of Reducing Blood Ferritin of *Mangifera odorata* Griff Levels in Test Animals

Group	Average ferritin level
Normal	140.45 ± 8.06
FeSO4	163.17 ± 3.33
Mangiferin	125.58 ± 5.93
Deferoxamin	127.60 ± 4.30
Extract dose 50 mg/200kgBW	158.25 ± 4.48 ^{bc}
Extract dose 100 mg/200kgBW	136.73 ± 4.94 ^{abc}
Extract dose 200 mg/200kgBW	103.05 ± 4.82 ^{a}

Total Flavonoids

Mangifera odorata Griff extract is known for its iron-chelating activity, which is likely attributed to the presence of mangiferin, flavonoids, and phenolic compounds. Mangiferin chelates Fe^{3+}, induces Fe^{2+} oxidation, and prevents Fe^{3+} reduction, limiting the availability of iron ions involved in lipid peroxidation. Flavonoids also exhibit iron chelation and antioxidant properties, reducing damage caused by iron overload. The total flavonoid content in the ethanol extract of *Mangifera odorata* Griff leaves was quantified using a quercetin standard curve (Figure 2), yielding a concentration of 108.99 ± 0.8321 mg QE/g, equivalent to 3.589% flavonoid content (Table 4). Previous studies have found varying flavonoid content in different mango varieties, with differences potentially arising from factors such as plant location and harvest time.

Table 4: Total Falavonoid of *Mangifera odorata* Griff Ekstrak

Replication	Absorbance	Flavonoid Total(mgQE/g)	Means±SD
1	0.325	108.43	
2	0.324	108.03	108.99±0.8321
3	0.328	109.63	
4	0.326	108.83	
5	0.329	110.03	

Total Phenolic Compounds: Phenolic compounds, which contain hydroxyl groups, react with the Folin-Ciocalteu reagent to form a blue-colored complex detectable by spectrophotometry. The total phenolic content was measured using a gallic acid standard solution (Figure 3). The measurement showed that the total phenolic level in the *Mangifera odorata* Griff leaf ethanol extract was 63.11 ± 1.621 GAE (mg/g sample), as shown in Table 5.

Table 5: Total Phenolics of *Mangifera odorata* Griff Ekstrak

Replication	Absorbance	Flavonoid Total(mgQE/g)	Means±SD
1	0.802	9.740	
2	0.798	9.678	
3	0.801	9.724	9.718±0.0319
4	0.803	9.755	
5	0.799	9.693	

A study by[27] reported that bachang mango leaves had a lower phenolic content, While in the study [24] the total phenolic results were obtained the ethyl acetate fraction from the methanol extract of Kasturi bark contains a total phenolic level of 3.92 mg GAE/g fraction and a total flavonoid level of 5.14 mg QE/g fraction [24]. The total flavonoid and total phenolic research on the Musa balbisiana Colla plant were found to be 454.87 mg QE/g and 16.14 mg GAE/g, respectively[29].The antioxidant mechanism of phenolic compounds involves redox reactions wherephenolics act as reducing agents, neutralizing free radicals and preventing or repairing tissue damage.In Vitro Antioxidant Activity of *Mangifera odorata* Griff: The antioxidant activity of *Mangifera odorata* Griff leaves was tested using the DPPH method, known for its simplicity, rapidity, and sensitivity in evaluating natural compounds. The ethanol extract of *Mangifera odorata* Griff leaves showed moderate antioxidant activity, with an IC_{50} value of 45.92 ppm, as shown in Tables 7 and 8.

This value is similar to the IC_{50} of 88.92 ppm found in previous studies using an aqueous extract of *Mangifera odorata* Griff leaves. The IC_{50} value for the gallic acid standard was 25.20 ppm, meanwhile, in the study Adedayo et al, (2020) an IC_{50} value of 1.67 ppm [25]. Research by stating that the antioxidant yield with an IC_{50} value of 2.11 ± 0.09 and 4.67 ± 0.06 µg/Ml [28]. Research Chukwuma et al, (2020) methanol fraction portrayed better antioxidant potential in all the antioxidant models used as revealed from its IC_{50} values of 19.10 ppm[26]. The presence of secondary metabolites, particularly flavonoids, in the ethanol extract contributes to its antioxidant activity.

Tabel 7: Antioxidant Activity of Gallic acid standart

Replication	%Inhibisi (*ppm)*						Regressio n Equations	R^2	IC_{50}
	10	20	30	40	50	60			
1	34.161	45.134	55.748	64.875	72.680	83.950	y = 0.9735x + 25.353	0.9973	25.32
2	34.468	45.544	55.758	64.896	72.669	84.002	y = 0.9662x + 25.738	0.9973	25.11
3	34.263	45.647	55.748	64.865	72.823	83.797	y = 0.9666x + 25.692	0.9973	25.15
4	34.366	45.441	55.779	64.619	72.516	83.991	y = 0.9663x + 25.633	0.9971	25.22
5	34.468	45.236	55.799	64.875	72.618	84.104	y = 0.9697x + 25.577	0.9973	25.19
Abs control					0,9751				
Means									25.20
SD	0.0790								
KV	0.31%								

Table 8: Antioxidant Activity of *Mangifera odorata* Griff Exstrax

Replication	%Inhibition (*ppm)*						Regression Equations	R^2	IC_{50}
	10	20	30	40	50	60			
1	43.4 32	45.3 08	46.7 83	48.2 57	50.5 36	52.5 47	y = 0.1792x + 41.,537	0.99 41	47. 23
2	43.0 29	45.7 10	46.9 17	48.7 94	51.2 06	52.8 15	y = 0.1923x + 41.349	0.99 25	44. 99
3	42.8 95	45.4 42	47.0 51	49.1 96	51.4 75	52.6 81	y = 0.1976x + 41.206	0.99 31	44. 50
4	42.6 27	45.3 08	47.0 51	49.0 62	51.3 40	52.8 15	y = 0.203x + 40.29	0.99 49	44. 68
5	43.2 98	45.1 74	46.9 17	48.2 57	50.5 36	52.0 11	y = 0.1743x + 41.6	0.99 69	48. 19
Abs Control					0.746				
Means	45.92								
SD	1.6788								
KV	3.66%								

Conclusion

The use of *Mangifera odorata* Griff balsamifera leaves as an iron chelating medicine by ethnic groups in Central Java, Indonesia, may be attributed to its strong antioxidant activity. The in vitro antidiarrheal effects of the extract could be related to the chemical compounds identified through GC-MS analysis, as well as the total flavonoid and total phenolic content in the ethanol extract of *Mangifera odorata* Griff leaves. These findings suggest that this extract has potential as a candidate for an iron-chelating treatment. Further research is required to evaluate its effects on SGPT, SGOT, BUN, creatinine levels, and hematological parameters.

Conflict of interest

The author reports no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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