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ANTIFUNGAL, ANTIBACTERIAL, AND GC-MS PHYTOCONSTITUENTS ANALYSIS OF N-HEXANE FRACTION OF *Parinari kerstingii* LEAVES IN VIEW OF HERBAL COSMETIC PRODUCTION

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ABSTRACT

Plant extracts are increasingly used in cosmetics, over-the-counter drugs, and food supplements. This study aims to establish the potential uses of *Parinari kerstingii* leaf extracts in skin care products and applications. The n-hexane fraction from the methanol extract was subjected to GC-MS, Phytochemical, antifungal, and antibacterial analysis. The GC-MS analysis revealed the presence of compounds including Eicosane; Phenol, 2,4-bis(1,1-dimethylethyl); 6-isopropyl-3-methylcyclohex-2-en-1-one; (9Z,12Z,15Z)-9,12,15-Octadecatrienoic acid; Nonanoic acid; Oleic acid; Palmitic acid, among others, were present in the n-hexane fraction. These compounds have been scientifically proven to possess antifungal activity via multiple mechanisms of action, including inhibition of fungal biofilm formation. Qualitative phytochemical screening showed the presence of Alkaloids, Phenolics, Tannins, Steroids, Sterols, Terpenoids, and Triterpenoids. The fraction showed antifungal activity against *Aspergillus niger*, *Aspergillus ustus*, *Aspergillus ochraceus*, *Candida albicans*, *Trichophyton rubrum*, and *Rizopus oryzae*. The fraction also showed activity against Gram-positive bacteria, including *Staphylococcus aureus*, *Streptococcus agalactiae*, *Bacillus cereus*, *Bacillus subtilis*, and *Bacillus anthracis*. No activity was recorded against gram-negative bacteria: *Escherichia coli*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Shigella flexneri*, and *Pseudomonas aeruginosa*. Conclusively, the n-hexane fraction obtained from the methanol extract of *Parinari kerstingii* leaves can be employed to produce skincare products or herbal cosmetics for managing fungal infections.

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INTRODUCTION

With annual growth in specialised, cutting-edge products, the skincare market is a highly valued

sector that accounts for a sizable share of the cosmetics market. Plant-based natural substances

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are among the most prized cosmetic ingredients [1]. Natural products, which include a wealth of medicinally effective components, are used to treat various skin conditions and infections, including eczema and acne [2], oxidative stress [3], and inflammation [4], as well as to protect the skin from UV rays [5]. Plants' natural products provide a wealth of cosmeceuticals. The waning faith in modern cosmetics has sparked recent consumer interest in herbal cosmetics, the conviction that plant cures are natural and, thus, superior to synthetic cosmetics manufactured by humans, and the mention of successful historical use by various cultures [6]. These factors have influenced the rising production of herbal cosmetics and increased acceptance. The cosmetic potential of many herbs has been thoroughly researched. However, there is a significant need for a systematic, in-depth analysis of scientific data supporting the use of specific herbs and their effectiveness as cosmetics [7].

Parinari kerstingii is widely distributed in tropical West Africa and is used in treating bronchopneumonia and feverish pains [8]. The phytochemical compound of *P. kerstingii* leaves showed the presence of alkaloids, flavonoids, steroids, terpenoids, phenols, glycosides, carbohydrates, reducing sugars, and saponins. The plant extract showed good anti-inflammatory properties [9], analgesic activity, and free radical scavenging activity [10]. The n-hexane extracts or fractions of plants are rich in fats and oils, essential oils, and other phytonutrients that can serve as preservatives, have antimicrobial, antioxidant, and anti-inflammatory activities, and thus are effective for skincare products. This study investigates the antifungal, antibacterial, and GC-MS Phytoconstituents of the n-hexane fraction obtained from the methanolic extract of *Parinari kerstingii* leaves, given its potential for the production of organic body lotions and creams (Herbal Cosmetic Production).

MATERIALS AND METHODS

Plant material, preparation of crude extract, and fractionation

Fresh leaves of *Parinari kerstingii* were collected from Ede-Oballa town, Nsukka Local Government Area, Enugu State, Nigeria. A voucher specimen (Interceded 0615 was deposited at the International Centre for Ethnomedicine and Drug Development, Nsukka. *Parinari kerstingii* leaves were air-dried at room temperature ($23\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$) and finely powdered using a mechanical grinder. The

powdered material (3000 g) was cold macerated with methanol for 3 days. The solution was filtered through Whatman No. 4 filter paper, and the filtrate was concentrated to a semi-solid using a rotary evaporator. The maceration was repeated twice to increase the yield. Fractionation was done by subjecting the crude extract (260 g) to column chromatography (with silica gel as the stationary phase) using n-hexane mobile phases. The filtrates were concentrated under reduced pressure, and the resulting fraction was stored in an airtight sample bottle for further use.

Quantitative analysis of the compositions of *Parinari kerstingii*

Quantitative phytochemical analysis of the n-hexane fractions of *Parinari kerstingii* was carried out using established standard methods [32].

GC-MS Analysis

GC-MS analysis of the n-hexane fraction of *P. kerstingii* leaves was performed using a Bruker Scion™ GC-MS with Scion 436 GC. Autosampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-1MS (95 % Dimethyl poly siloxane) fused capillary column (30 m x 0.25 mm ID x 0.25 μM). An electron ionization apparatus was used in electron-impact mode for GC-MS detection, with an ionization energy of 70eV. Helium gas (99.999 %) was used as the carrier gas at a uniform flow rate of 1 mL/min. An injection volume of 0.5 μL was used, with a split ratio of 50:1. 280 °C was the constant injector temperature, while the ion source temperature was 250 °C. The oven temperature was set from 80 °C (for 2 min), with an increase of 20 °C/min to 160 °C, then 5 °C/min to 280 °C, ending with a 10 min isothermal at 300 °C. Mass spectra were taken at 70 eV at a scanning interval of 0.5 seconds, and fragments from 50-500 Da. The solvent delay was 0-3.5 min, and the total GC-MS run time was 28 min. Comparing each component's average peak area to the total allowed area allowed us to determine the proportional percentage of each component. The Mass Spectrometer used in this analysis was a TQ Quadrupole Mass Spectrometer, and the software used to handle chromatograms and mass spectra was MS WorkStation 8.

Identification of Components

The relative percentage of each component shown was calculated by comparison of its average peak area to the total area. GC-MS detection and interpretation were performed using the NIST library

(National Institute of Standards and Technology) version 20. The spectra of the unknown compounds were compared with those of the known compounds of the test materials were ascertained.

Test Organisms

Antibacterial activity of n-hexane fractions was determined against five Gram-positive (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Bacillus cereus*, *Bacillus megaterium*, and *Bacillus subtilis*) and five Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella flexneri*, *Shigella dysenteriae*, and *Shigella sonnei*). Antifungal activity was determined against six fungi (*Candida albicans*, *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus ustus*, *Rhizopus oryzae*, and *Trichophyton rubrum*). Test organisms were the laboratory stocks of Microbiology Lab, Department of Pharmaceutical Microbiology and Biotechnology, University of Nigeria, Nsukka.

Growth media and culture conditions

Nutrient agar media (Yeast extract 1 %, Peptone 0.5 %, NaCl 0.5 %, agar 1.5 %) purchased from Difco. Sabouraud Dextrose agar media (Enzymatic digest of casein 0.5 %, an enzymatic digest of animal tissue 0.5 %, Dextrose 4 %, Agar 1.5 %) from Acumedia were used for antibacterial and antifungal activity assay, respectively. The strains were incubated at 37 °C overnight [33], [34].

Test for antibacterial activity

In vitro antibacterial activity was carried out on a Muller-Hinton agar plate by the disc diffusion method [35]. Both crude extracts were separately dissolved in 1 mL of their respective solvent. The filter paper discs (6 mm diameter) were

compounds stored in the NIST library. 20. The name, molecular weight, and molecular formula of

impregnated with known amounts of test substances to a final concentration of 500 µg/disc. Discs were placed on the agar plate culture of test organisms using sterilized forceps. Plates were kept at a low temperature (4 °C) for 24 h to allow maximum diffusion. The plates were then incubated at 37 °C overnight. After 18-20 h of incubation, the diameter (mm) of the zone of inhibition for each extract against each tested microorganism was measured. A standard antibiotic disc of Kanamycin (30 µg/disc, Hi-media, India) was used as a positive control. A blank disc impregnated with solvent and then dried was used as the negative control.

Test for antifungal activity

In vitro antifungal activity of the n-hexane fraction was assessed on Sabouraud Dextrose agar plates by the disc diffusion method against six pathogenic fungi at a concentration of 500 µg/disc, as described in the antibacterial screening section. A standard disk of antifungal agent Clotrimazole (10 µg/disc, Hi-media, India) was used as a positive control [36].

Statistical Analysis

The data were statistically evaluated using descriptive statistics. Results are presented as mean ± standard error of the mean (SEM), and differences were considered statistically significant at $P < 0.05$.

RESULTS

Qualitative and quantitative analysis of phytochemical constituents of the n-hexane fraction and the methanol extract of *P. kerstingii*.

Table 1-2: shows the qualitative and quantitative studies of the phytochemical constituents of the

methanol extract and its n-hexane fraction of *P. kerstingii* leaves. Table 3 shows the GC-MS analysis of phytochemical constituents of n-hexane fraction from methanol extract of *P. kerstingii*. Table 4 shows the antifungal analysis of phytochemical constituents of n-hexane fraction from methanol extract of *P. kerstingii*. Antibacterial analysis of phytochemical constituents of n-hexane fraction from methanol extract of *P. kerstingii*

Table 1: Qualitative studies of the phytochemical constituents of the methanol extract and its n-hexane fraction of *P. kerstingii* leaves

Phytochemical	Methanol extract	n-Hexane fraction
Alkaloids	+	+
Anthocyanin	+	-
Anthraquinone	+	-
Coumarins	+	-
Flavonoids	+	-
Phenolics	+	+
Tannins	+	+
Steroids	+	+
Sterols	+	+
Terpenoids	+	+
Triterpenoids	+	+
Saponins	+	-
Glycosides	+	-
Reducing sugar	+	-

+ means present, while - means not detected.

Table 2: Quantitative studies of the phytochemical constituents of the methanol extract and its n-hexane fraction of *P. kerstingii* leaves

Phytochemical (mg/100g)	Methanol extract	n-Hexane fraction
Alkaloids	1106.000± 0.018	292.300 ± 0.023
Flavonoids	29844.891± 0.536	ND
Phenolics	9726.320 ± 0.530	392.021 ± 0.500
Tannins	852.063 ± 0.204	107 ± 0.890
Glycosides	289.738 ± 0.825	ND
Steroids	321.000 ± 0.003	220.640 ± 0.038
Terpenoids	4118.110 ± 0.814	429.722 ± 0.106
Saponins	305.105 ± 0.404	ND
Reducing sugar	1219.713 ± 0.112	ND

Table 3: Phytoconstituents identified through GC-MS

No.	RT	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area %
1.0	9.3	6-methoxy-1-methyl-4-propan-2-ylcyclohexene	C ₁₁ H ₂₀ O	168	0.39
2.0	9.6	Eicosane	C ₂₀ H ₄₂	282	1.26
3.0	11.2	Phenol, 2,4-bis(1,1-dimethylethyl)	C ₁₄ H ₂₂ O	206	0.72
4.0	12	6-isopropyl-3-methylcyclohex-2-en-1-one	C ₁₀ H ₁₆ O	152	0.32
5.0	12.9	Octane, 1-propoxy	C ₁₁ H ₂₄ O	172	2.81
6.0	14.3	(Z)-3,7,11,15-tetramethylhexadec-2-en-1-ol	C ₂₀ H ₄₀ O	296	0.34
7.0	15.2	1-Octadecyne	C ₁₈ H ₃₄	250	0.36
8.0	16.9	1-Dodecanol	C ₁₂ H ₂₆ O	186	0.41
9.0	17.9	(E)-1,2,4-trimethoxy-5-(prop-1-en-1-yl)benzene	C ₁₂ H ₁₆ O ₃	208	21.61
10	18.1	(Z)-1,2,4-trimethoxy-5-(prop-1-en-1-yl)benzene	C ₁₂ H ₁₆ O ₃	208	2.87
11	18.7	(9Z,12Z,15Z)-9,12,15-Octadecatrienoic acid	C ₁₈ H ₃₀ O ₂	278	2.01
12	19.7	Nonanoic acid /Allylnonanoate	C ₁₂ H ₂₂ O ₂	198	1.06
13	20.1	2-(dimethylamino)ethyl (Z)-2-(dimethylamino)-N-hydroxy-2-oxoethanimidothioate	C ₈ H ₁₇ N ₃ O ₂ S	219	1.86
14	20.8	oleic acid	C ₁₈ H ₃₄ O ₂	282	42.2
15	21.0	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	12.03
16	21.8	Ethyl palmitate	C ₁₈ H ₃₆ O ₂	282	5.6

17	22.4	Eicosanoic acid	C ₂₀ H ₄₀ O ₂	312	0.49
18	23.3	(Z) 9-Octadecenamide	C ₁₈ H ₃₅ NO	282	2.01
29	23.7	[(Z)-nonadec-17-enyl] acetate	C ₂₁ H ₄₀ O ₂	324	8.65
20	24.2	Oxalic acid, hexadecyl ester	C ₂₄ H ₄₆ O ₄	398	2.72
21	24.4	9-Octadecenal	C ₁₈ H ₃₄ O	266	2.43
22	25.3	Diisooctyl phthalate	C ₂₄ H ₃₈ O ₄	390	5.11
23	26.1	Hexadec-9-enal	C ₁₆ H ₃₀ O	238	2.6

Table 4: Mean antifungal activities of the n-hexane fraction of *Parinari kerstingii* leaves

Fungal strain tested	Diameter of the zone of inhibition	
	N-hexane Fraction (500µg/disc)	Clotrimazole
<i>Aspergillus niger</i>	26.0	24.8
<i>Aspergillus ustus</i>	26.5	22.7
<i>Aspergillus ochraceus</i>	16.9	23.7
<i>Candida albicans</i>	28.6	28.5
<i>Trichophyton rubrum</i>	18.0	11.8
<i>Rizopus oryzae</i>	23.6	23.7

n = 3

Table 5: Mean antibacterial activities of the n-hexane fraction of *Parinari kerstingii* leaves against bacterial strains

Gram positive bacteria	n-Hexane Fraction	Kanamycin (30µg/disc)
<i>Staphylococcus aureus</i>	14.9	24.6
<i>Streptococcus agalactiae</i>	7.1	24.5
<i>Bacillus cereus</i>	13.0	22.8
<i>Bacillus subtilis</i>	11.7	26.1
<i>Bacillus anthracis</i>	7.17	21.6
Gram-negative bacteria		
<i>Escherichia coli</i>	-	22.8
<i>Salmonella typhimurium</i>	-	25.6
<i>Shigella dysenteriae</i>	-	24.3
<i>Shigella flexneri</i>	-	25.6
<i>Pseudomonas aeruginosa</i>	-	23.8

n = 3

DISCUSSIONS

From the qualitative phytochemical analysis shown in Table 1, Alkaloids, Phenolics, Tannins, Steroids, Sterols, Terpenoids, and Triterpenoids were present in the n-hexane fraction. Alkaloids, Anthocyanin, Anthraquinone, Coumarins, Flavonoids, Phenolics, Tannins, Steroids, Sterols, Terpenoids, Triterpenoids, Saponins, and Glycosides were present in the methanol extract. Table 2's quantitative estimation of the phytochemical constituents of *Parinari kerstingii*'s methanol extract and n-hexane fraction shows that the plant's

methanol extract contains high concentrations of alkaloids (1106.000 ± 0.014), flavonoids (29844.891 ± 0.536), phenolics (9726.320 ± 0.530), tannins (852.063 ± 0.204), steroids (321.000 ± 0.003), terpenoids (4118.110 ± 0.814), saponins (305.105 ± 0.404) glycosides (289.738 ± 0.825) and reducing sugar (1219.713 ± 0.112). Quantitatively, the n-hexane fractions showed the presence of alkaloids (292.300 ± 0.023), steroids (220.640 ± 0.038), phenolics (392.021 ± 0.500), tannins (107 ± 0.890), and terpenoids (429.722 ± 0.106). Alkaloids are recognized to provide medicinal benefits. Alkaloids are used to make anti-aging

treatments, tonics, creams, lotions, face, and hair masks, compresses for skin issues with many inflammations and discolorations, and anti-cellulitis remedies. Additionally, alkaloids are used in the manufacture of ampoules for aestheticians and cosmetologists [11]. Alkaloids such as Vincristine and Vinblastine have been widely used by dermatologists and cosmetics [12]. Other plants' secondary metabolites, such as fat-soluble flavonoids tocotrienol and tocopherol, have similar activities to vitamin E [13], [14]. Additionally, Tannins have antioxidant, antimicrobial, and anti-inflammatory properties suitable for skin care products [15], [16].

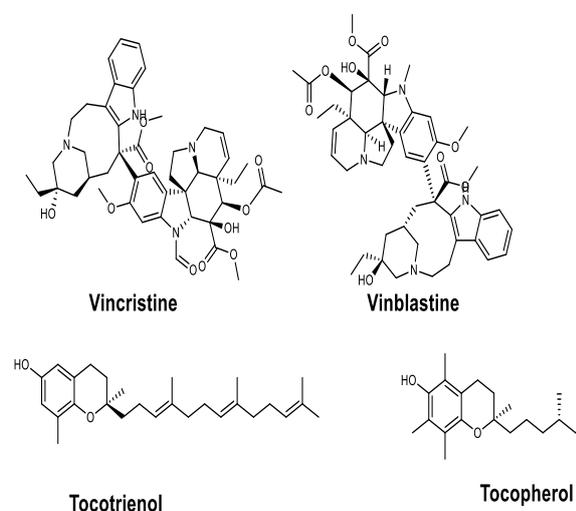


Figure 1. Natural products that have been used in skincare products

Many secondary metabolites identified through GC-MS are used for skin care products in the cosmetics industry. These include: Eicosane; Phenol, 2,4-bis(1,1-dimethylethyl); 6-isopropyl-3-methylcyclohex-2-en-1-one; (9Z,12Z,15Z)-9,12,15-Octadecatrienoic acid; Nonanoic acid; Oleic acid, Palmitic acid and Ethyl palmitate.

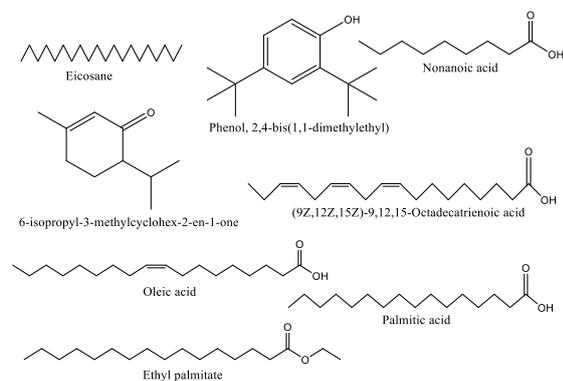


Figure 2. Compounds identified in the n-hexane fraction using GC-MS.

Eicosane has been reported to have antifungal properties [17]. Eicosane is also a promising antibiofilm agent against *Candida albicans* biofilm via molecular docking and in vitro (100 µg/mL), inhibiting 60 % of *C. albicans* biofilm. Moreover, light microscopic investigation revealed a significant reduction in yeast cell adhesion and colonisation of the matrix in Eicosane-treated samples [18]. It is also used as an emollient, fragrance, fragrance component, masking, perfuming, softener, and conditioner in skincare and cosmetic products [19]. Phenol, 2,4-bis(1,1-dimethylethyl), has also shown suitable antifungal activities against uropathogenic *Serratia marcescens* [20]. In silico docking studies showed that phenol, 2,4-bis(1,1-dimethylethyl)-, effectively binds and inhibits the active site of mitochondrial F1F0 ATP synthase in *Pithomyces atro-olivaceus* [21]. Phenol-2,4-bis (1,1-dimethylethyl) has also been known to reduce germinative tube length in *Aspergillus* and radial growth of *P. cinnamomic* [22]. 6-isopropyl-3-methylcyclohex-2-en-1-one is used as a flavouring and fragrance in cosmetics [23].

(9Z,12Z,15Z)-9,12,15-Octadecatrienoic acid has been extensively used in cosmetics as an antistatic agent, emollient, lip balm, hair conditioning, skin conditioning, and fragrance [24]. Nonanoic acid has been reported to inhibit spore germination and mycelial growth of pathogenic fungi [25]. In cosmetics, Oleic acid is used as an emollient and emulsifying agent. Oleic acid was revealed to effectively inhibit biofilm formation, hence reducing the virulence of *Candida albicans* [26]. Oleic acid has also been widely used as a delivery agent for antifungal drugs [27], [28], [29], [30]. Palmitic acid acts as a viscosity builder, emollient, and co-emulsifier. It also acts as a super-fattening agent and opacifier, widely used to improve emollience and emulsion thickness (Kalustian, 1985). Palmitic acid has been shown to possess antifungal activities [27]. Ethyl palmitate is a naturally occurring fatty acid ester reported to inhibit the activity of inflammatory cells. Ethyl palmitate reduced carrageenan-induced rat paw oedema and diminished prostaglandin E2 (PGE2) level in the inflammatory exudates. In lipopolysaccharide (LPS)-induced endotoxemia in rats, ethyl palmitate reduced plasma levels of tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6). It also decreased NF-κB expression in liver and lung tissues and ameliorated histopathological changes caused by LPS. Topical application of ethyl palmitate reduced ear oedema induced by croton oil in rats.

Ethyl palmitate inhibited neutrophil infiltration in the same animal model, as seen by a decline in myeloperoxidase activity [31].

Six fungi were used for the antifungal activity test of the n-hexane fraction, as shown in Table 4. The n-hexane fraction was most effective against *Candida albicans* and least effective against *Aspergillus ochraceus*. The n-hexane fraction showed more activity on *Aspergillus niger*, *Aspergillus ustus*, and *Trichophyton rubrum* than the commercial drug, Clotrimazole. In contrast, Clotrimazole was more active against *Aspergillus ochraceus*.

The n-hexane fraction was tested for antibacterial activity against 10 pathogenic bacteria at a 500 µg/disc dose by disc diffusion. The n-hexane fraction showed no activity against gram-negative bacteria, but was active against gram-positive bacteria, though at a level lower than the control.

CONCLUSION

In this study, the n-hexane fraction was obtained from the methanol extract of *Parinari kerstingii* leaves through fractionation. The n-hexane fraction was tested explicitly against six fungi strains: *Aspergillus niger*, *Aspergillus ustus*, *Aspergillus ochraceus*, *Candida albicans*, *Trichophyton rubrum*, and *Rizopus oryzae*, and was found to be biologically active. It also showed antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Bacillus cereus*, *Bacillus subtilis*, and *Bacillus anthracis*), though lower than Kanamycin, the positive control. The fraction was inactive against the tested gram-negative bacteria (*Escherichia coli*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Shigella flexneri*, and *Pseudomonas aeruginosa*). The GC-MS analysis of the n-hexane fraction revealed the presence of Eicosane; Phenol, 2,4-bis(1,1-dimethylethyl); 6-isopropyl-3-methylcyclohex-2-en-1-one; (9Z,12Z,15Z)-9,12,15-Octadecatrienoic; Nonanoic acid; Oleic acid, Palmitic acid, which are known compounds having antifungal activities. Ethyl palmitate was also identified and shown to have anti-inflammatory activity. The results show that the n-hexane fraction from *Parinari kerstingii* leaves can be used to produce herbal or polyherbal skincare products for the management of topical fungal infections.

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AUTHORS CONTRIBUTIONS

All authors contributed to the studies and writing/editing of the work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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