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Identification of medicinally active flavonoids, phenolic compounds and terpenoids from traditional healing plant *barleria strigosa* and its antioxidant activity

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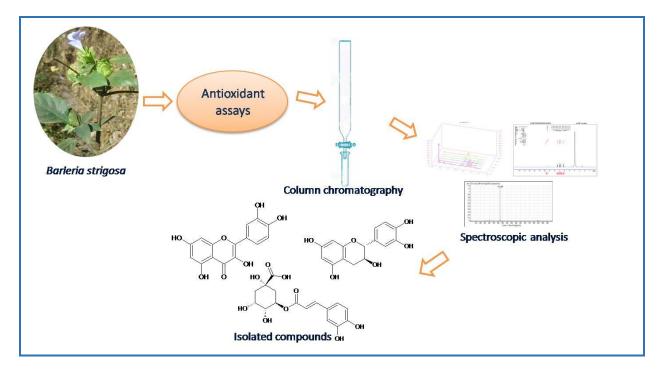
KEYWORDS

Barleria strigosa Chemical constituents Antioxidant activity ¹H NMR ¹³C NMR

ABSTRACT

Barleria strigosa willd, a popular medicinal plant used mainly in Asian countries as a natural medicine. The root and leaves of this plant has been used in various traditional medicine systems in Asia. The present study intends to explore the medicinal activities and to detect the phytochemical constituents responsible for the therapeutic activities. Major antioxidant assays such as ABTS (2,2'azinobis (3-ethylbenzothiazoline-6-sulphonic acid)) scavenging, nitric oxide quenching, ferric reducing and DPPH (1,1diphenyl-2-picrylhydrazyl) scavenging were done on different extracts to identify the effective extraction method. An activity based isolation of major compounds was conducted on the hydroalcohol extract and characterizations of the isolated compounds were done using ¹H, ¹³C NMR and mass spectrometry. Among the tested samples, hydroalcohol extract revealed higher phenolics and flavonoids contentsand also exhibited predominant results in various antioxidant assays. Significance p<0.05 was obtained for hydroalcohol extract in different antioxidant assays. Exploration for major constituents led to isolation of seven compounds including 3β -hydroxy-20(29)-lupene, lup-20(29)-ene- 3β , 28-diol, 3-beta-hydroxyolean-12-en-28oic acid, 22,23-dihydrostigmasterol, 3,3',4',5,7-pentahydroxyflavone, (2S,3R)-2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol and 3-caffeoylquinic acid. *B. strigosa* is a rich source of medicinally active terpenoid, phenolic and flavonoid compounds and possess potent antioxidant activities which are beneficial for human health as a green pharmaceutical medicine. © 2021 by SPC (Sami Publishing Company), Asian Journal of Green Chemistry, Reproduction is permitted for noncommercial purposes.

Graphical Abstract



Introduction

Barleria strigosa willd belongs to the family *Acanthaceae* is a tall shrub with blue flowers. *B. strigosa* is native to tropical regions of Asia and distributed throughout the upper gangentic plain and southern parts of India [1]. The root of this plant is being used intraditional Indian system of medicine ayurveda and the leaves in thai traditional medicine [2]. The whole plant decoction is used as a restorative, antipyretic, and antidote for poison detoxification. The leaves found to contain verbascoside, isoverbascoside, decaffeoylverbascoside, strigoside (4-hydroxyphenylethyl-4-*O*- β -*D*-glucopyranosyl-(1 \rightarrow 3)-*O*- α -*L*-rhamnopyranoside), 10-*trans*-coumaryl-eranthemoside, (+)-lyoniresinol- 3α -*O*- β -*D*-glucoside, apigenin-7-*O*- α -*L*-rhamnosyl-(1 \rightarrow 6)-*O*- β -*D*-glucoside, 7-*O*-acetyl-8-epi-loganic acid, and (3*R*)-1-octen-3-ol-3-*O*- β -*D*-xylosyl-(1 \rightarrow 6)- β -*D*-glucoside [3]. The whole plant was reported to contain β - and γ -sitosterol [4, 5]. Apigenin, vanillic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid [4] two phenylethanoid glycosides parvifloroside A and B were also reported from leaves [6].

In the present scenario, green chemistry methods have wide applications in pharmaceutical field such as green synthesis of nanoparticles using plant extracts were found very promising [7]. Isolation of medicinally active phytochemical from plants will help in recognizing it for future medicinal chemistry research [8]. Identification of structure of compounds present in plant extracts may lead to their usage in pharmaceutical industry [10]. Identification of chemical constituents will also help

to justifies the pharmacological usage of traditional medicinal plants in modern pharmaceutical industry [11].

A survey of literature revealed that, the medicinal aspects of root of *Barleria* have not been evaluated yet and no systematic investigation has been carried out on chemical constituents. The present study deals with important *in-vitro* antioxidant assays and isolation of major chemical constituents by column chromatography and characterization of the compounds using various techniques including ¹H NMR, ¹³C NMR and mass spectrometry. Identification of therapeutic activities and chemical constituents of the raw materials used in herbal medicine is very essential for the worldwide acceptance green pharmaceutical from herbal medicines.

Experimental

Materils and methods

NMR spectra were recorded on a Bruker DRX 500 NMR instrument operating at 400 MHz for ¹H and 100 MHz for ¹³C at room temperature. Signals were referred to as the internal standard tetramethylsilane (TMS). CDCl₃ and DMSO were used for recording the spectra. Mass spectroscopic analysis was carried out on agilent 6520 accurate mass quadrupole-time of flight LC/MS coupled with agilent LC 1200 and gradient elution was performed at a constant flow rate of 0.6 mL/min. Data were given as mean \pm standard deviation (SD) of three values. The EC₅₀ values for all activities were calculated by linear-regression analysis method. Results were calculated by employing the statistical software (COSTAT, Monterey, U.S.A). Chemicals used in the study are quercetin, 2,2'azinobis (3-ethylbenzothiazoline-6-sulphonic acid), 1,1-diphenyl-2-picrylhydrazyl, ascorbic acid, greiss reagent, ferric chloride, potassium persulfate, Folin-Ciocalteu reagent, gallic acid were procured from sigma chemicals (India). Methanol, formic acid and acetonitrile (LC/MS grade) were obtained from Merck India.

Preparation of extract

Fresh plant materials were collected and authenticated by plant systematic and genetic resources division, centre for medicinal plants researchand a voucher specimen was deposited in herbarium. Materials were shade dried and powdered. Root material 3 g each was extracted with ethyl acetate, methanol and hydroalcohol (50:50) for 5 h using soxhlet method. After extraction excess solvents were removed by rotary evaporator and were made up to 10 mL.

Total phenolics and flavonoid assay

Total phenolics content was determined by using the Folin-Ciocalteu assay [12] and expressed as mg gallic acid equivalents (GAE). Total flavonoid content was measured by the aluminum chloride colorimetric assay [13] and expressed as mg quercetin equivalents (QE).

ABTS free radical-scavenging assay

ABTS assay was done using a slightly modified previous method [14]. ABTS radical cation was prepared by reacting ABTS solution with potassium persulfate solution. The absorbance was measured after addition of extracts and standard to ABTS solution. Ascorbic acid was used as the standard for study.

Nitric oxide quenching assay

The nitric oxide scavenging activity was measured by spectrophotometrically using greiss reagent [15]. Sodium nitroprusside and phosphate buffer was added to the various concentrations of the plant extracts and standard solutions. The reaction mixture was treated with griess reagent and absorbance was measured. Ascorbic acid was used as standard.

Ferric reducing assay

The reducing power of samples was determined according to modified previous method [16]. Different concentrations of plant extracts and standard solutions were mixed with phosphate buffer, potassium ferricyanide, and trichloro acetic acid. The solution was mixed with distilled water and ferric chloride and absorbance was measured. Ascorbic acid was used as standard.

DPPH radical scavenging assay

The DPPH radical scavenging assay was done using a previous method with minor modifications [17]. DPPH solution was mixed with equal volume of extracts and standard solution with different concentrations and absorbance was measured. Quercetin was used as the standard.

Isolation chemical constituents

Preparation of extract for isolation

Powdered root (1 kg) was extracted with hydroalcohol by soxhlet technique for 24 h. Then the mixture was filtered and solvent was removed by rotary evaporator method under the reduced pressure. The crude hydroalcohol extract was dissolved in ethyl acetate to separate the low polar compounds. Hydroalcoholic extract after ethyl acetate separation was further dissolved in methanol.

Isolation of chemical constituents

Ethyl acetate and methanolic fractions were separately adsorbed on the activated silica gel. Column of 1000 mm height and 50 mm diameter was packed with silica gel 100-200 mesh with *n*-hexane as solvent in wet packing method. Silica gel with extract was loaded to the column and eluted with solvents. Eluting solvents for ethyl acetate fraction were *n*-hexane with ethyl acetate in increasing polarity order. Solvents used for methanolic fraction were ethyl acetate with methanol in increasing polarity order. The fractions obtained from the column were analyzed using thin layer chromatography.

Results and discussion

Phenolics and flavonoids content

Among the samples extracts, hydroalcohol extract demonstrated the highest phenolics content (115 mg GAE) and followed by methanol extract (103 mg GAE) and ethyl acetate extract (53 mg GAE) showed the least. Like phenolics, hydroalcohol extract revealed maximum flavonoid content (43.1 mg QE) then methanolic extract (35.3 mg QE) and least shown by ethyl acetate extract (23.1mg QE). Detailed results are presented in Table 1.

	1	5				
Extract	Phenolics	Flavonoids	Ferric	Nitric	ABTS	DPPH
	content (mg	Content (mg	reducing	oxide	assay (IC ₅₀	assay
	eqgallic	eqquerctin)	assay	assay (IC ₅₀	μg/mL)	(IC ₅₀
	acid)		(100 µg/mL)	µg/mL)		μg/mL)
Ethyl acetate	53±1.3	23.1±0.5	0.11±0.5	391 ±1.1	401±1.3	153.1±1
Methanol	103±1.5	35.3±1	0.33±1	353 ±1	343±1.1	51±0.3
Hydroalcohol	115±1.1	43.1±1.3	0.51±1.3	345 ± 0.5	315±1.5	33±0.5
Ascorbic acid	-	-	0.57±0.3	343±0.3	313±0.3	11±0.3
Quercetin	-	-	-	-	-	5.3±0.1

Table 1. Results of quantitative assays and antioxidant activities

ABTS activity

The result showed that different concentrations of extracts showed varying degree of scavenging potential on ABTS⁺⁺ radicals in a concentration dependent way. Hydroalcohol extract exhibited the lowest IC₅₀ value 315 μ g/mL, which means the higher scavenging activity. The IC₅₀ value for methanolic extract was 343 μ g/mL and for ethyl acetate 401 μ g/mL.

Nitric oxide activity

All the extracts showed a moderate dose-dependent inhibition of nitric oxide radical. The IC_{50} values of extracts were hydroalcohol 345 µg/mL, methanol 353 µg/mL and ethyl acetate 391 µg/mL.

Reducing power

The reducing power of extracts increased with increasing concentrations. The concentration of extracts used were 100, 500, 1000 μ g/mL and the values showed 0.51, 0.53 and 0.63 for hydroalcohol extract, 0.33, 0.35 and 0.43 for methanol extract, 0.11, 0.13 and 0.17 for ethyl acetate extract.

DPPH activity

The radical scavenging assay follows the same manner of phenolics as the highest activity was shown by hydroalcohol extract with IC_{50} value 33 µg/mL. Methanolic extract also showed a better activity with IC_{50} value 51 µg/mL. Ethyl acetate extract showed low activity with IC_{50} value 153 µg/mL.

Lup-20(29)-en-3 β -ol (Compound 1)

¹H NMR (CDCl₃, 400 MHz): *δ* -4.68, 4.56 (2H, *s*, H-29 a, 29 b), 3.16 (1H, *dd*, *J* = 4.76, 11.00 Hz, H-3), 0.75, 0.78, 0.82, 0.93, 0.95, 1.02, 1.25 (each 3H, *s*, 7-CH₃). On LC MS Q TOF analysis it showed M-H molecular mass ion at 413.7.

22,23-dihydrostigmasterol (Compound 1)

¹H NMR (400 MHz, CDCl₃): δ 3.2 (1H, m, H-3), 5.26 (1H, m, H-6), 5.19 (1H, m, H-23), 4.68 (1H, m, H-22), 3.63 (1H, m, H-3), 2.38 (1H, m, H-20), 1.8-2 (5H, m) ppm. δ 0.76-0.89 (m, 9H), 0.91-1.05 (m, 5H), 1.35-1.42 (m, 4H), 0.69-0.73 (m, 3H), 1.8-2 (m, 5H), 1.07-1.13 (m, 3H), 1.35-1.6 (m, 9H). ¹³C NMR (100 MHz, CDCl₃): δ 150.9, 145.2 (C-5), 139.8 (C-22), 121.7, 118.8 (C-6), 79.0 (C-3), 55.3 (C-14), 55.1 (C-17), 50.45 (C-9), 48.3 (C-9), 40.8 (C-20), 40.1 (C-12), 39.2 (C-13), 38.9 (C-4), 38.6 (C-12), 37.1 (C-1), 37.1 (C-10), 36.3 (C-8), 35.5 (C-20), 34.2 (C-22), 34.2 (C-7), 32.6 (C-8), 29.8 (C-25), 29.7 (C-16), 28.4 (C-2), 28.1 (C-15), 27.4 (C-28), 26.1 (C-11, 26), 21.6 (C-27), 19.32 (C-19), 17.71 (C-21), 15.6 (C-18, 29). On LC MS Q TOF analysis it showed M-H molecular mass ion at 455.3.

Lup-20(29)-ene-3 β ,28-diol (Compound 3)

¹H NMR (400 MHz, CDCl₃): δ 4.6, (1H, s, Ha-29), 4.5 (1H, s, Hb-29), 3.71 (1H, d, Ha-28), 3.34 (1H, m, Hb-28), 3.1 (1H, *dd*, H-3), 2.3 (1H, m, H-19), 1.61 (3H, m, H-30), 1.07 (3H, s, H-23), 0.98 (3H, s, H-27) 0.96 (3H, s, H-26), 0.82 (3H, s, H-25), 0.76 (3H, s, H-24), 0.69 (3H, s, H-21). ¹³C NMR (100 MHz,

CDCl₃): *δ* 149.7 (C-20), 108.9 (C-29), 78.2 (C-3), 59.8 (C-28), 54.5 (C-5), 49.6 (C-9), 48.0 (C-19), 47.0 (C-17), 47.9 (C-18), 41.9 (C-14), 40.1 (C-8), 38.1 (C-1), 37.9 (C-4), 36.5 (C-10), 36.4 (C-13), 33.5 (C-7), 33.2 (C-22), 29.0 (C-21), 28.4 (C-16), 27.2 (C-23), 26.6 (C-2), 26.3 (C-15), 24.4 (C-12), 20.1 (C-11), 18.3 (C-30), 17.5 (C-6), 15.3 (C-25), 15.2 (C-26), 14.6 (C-24), 14.1 (C-27). On LC MS Q TOF analysis it showed M-H molecular mass ion at 441.3.

3-β-hydroxyolean-12-en-28-oic acid (Compound 4)

¹H NMR (400 MHz, CDCl₃): δ -5.29 (1H, s, H-12), 3.23 (1H, t, H-3), 3.30 (1H, m, H-18), 1.13 (3H, s, CH3-27), 0.96 (3H, s, CH₃-30), 0.91 (3H, s, CH₃-25), 0.89 (3H, s, CH3-23), 0.87 (3H, s, CH3-24), 0.75 (3H, s, CH3-26). ¹³C NMR (100 MHz, CDCl₃): δ -180.2 (C-28), 144.8 (C-13), 122.5 (C-12), 78.0 (C-3), 55.7 (C-5), 48.0 (C-9), 46.6 (C-8, 17), 42.1 (C-14), 39.7 (C-4), 39.4 (C-1), 37.3 (C-10), 33.2 (C-7), 32.9 (C-29), 32.4 (C-21), 30.9 (C-20), 28.7 (C-23), 27.2 (C-2), 26.9 (C-15), 26.1 (C-30), 23.7 (C-11), 23.6 (C-16), 18.7 (C-6), 17.4 (C-26), 16.5 (C-24), 15.5 (C-25). On LC MS Q TOF analysis it showed M-H molecular mass ion at 455.3.

3,3',4',5,7-pentahydroxyflavone (Compound 5)

¹H NMR (400 MHz, DMSO-*d*₆): δ -9.62 (1H, s, OH-3), 12.48 (1H, s, OH-5), 6.19 (1H, d, H-6), 10.76 (1H, s, OH-7), 6.41 (1H, d, H-8), 7.67 (1H, d, H-2'), 9.62 (1H, s, OH-3'), 9.35 (1H, s, OH-4'), 6.90 (1H, d, H-5'), 7.53 (1H, *dd*, H-6'). ¹³C NMR (100 MHz, DMSO): δ -147.5 (C-2), 135.9 (C-3), 176.0 (C-4), 160.4 (C-5), 98.1 (C-6), 163.7 (C-7), 93.3 (C-8), 156.1 (C-9), 102.9 (C-10), 121.9 (C-1'), 115.5 (C-2'), 144.9 (C-3'), 146.7 (C-4'), 115.5 (C-5'), 119.9 (C-6'). On LC MS Q TOF analysis it showed M-H molecular mass ion at 301.1.

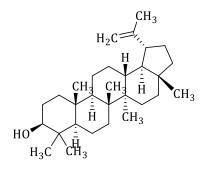
(2S,3R)-2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol (Compound 6)

¹H NMR (400 MHz, DMSO-*d*₆): δ -9.41 (1H, s, OH-3), 9.20 (1H, s, OH-3'), 8.99 (1H, s, OH-4'), 12.43 (1H, s, OH-5), 10.71 (1H, s, OH-7), 7.61 (1H, d, H-2'), 6.51 (1H, d, H-8), 6.7 (1H, d, H-5'), 7.53 (1H, *dd*, H-6'), 4.50 (1H, d, H-2), 2.6 (2H, m, H-3), 2.3 (2H, m, H-4). ¹³C NMR (100 MHz, DMSO-*d*₆): δ -147.5 (C-2), 135.9 (C-3), 176.0 (C-4), 156.4 (C-5), 98.1 (C-6), 156.7 (C-7), 93.3 (C-8), 156.1 (C-9), 102.9 (C-10), 118.9 (C-1'), 115.5 (C-2'), 144.9 (C-3'), 146.7 (C-4'), 115.5 (C-5'), 119.9 (C-6'), 93 (C-2), 66.1 (C-3), 27 (C-4). On LC MS Q TOF analysis it showed M-H molecular mass ion at 289.1.

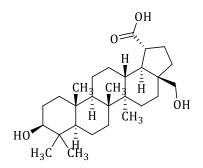
3-caffeoylquinic acid (Compound 7)

¹H NMR (400 MHz, DMSO-*d*₆): δ -9.72 (s, OH-3'), 9.40 (s, OH-4'), 7.42 (H-7'), 7.03 (H-2'), 6.98 (H-6'), 6.76 (H-5'), 6.15 (H-8'), 5.06 (H-5), 3.91 (H-3), 3.56 (H-4), 2.06 (H-2), 1.983, 1.89 (H-6), 1.77 (H-6'), 1.77 (H-6'

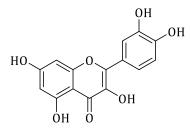
2). ¹³C NMR (100 MHz, DMSO-*d*₆): δ -174.9 (C-7), 165.9 (C-9'), 148.2 (C-4'), 145.4 (C-3'), 145.0 (C-7'), 125.5 (C-1'), 121.6 (C-6'), 115.7 (C-5'), 114.3 (C-8'), 114.1 (C-2'), 73.4 (C-1), 70.7 (C-5), 71.2 (C-4), 68.0 (C-3), 37.3 (C-6), 36.4 (C-2). On LC MS Q TOF analysis it showed M-H molecular mass ion at 353.1.

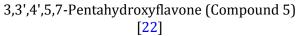


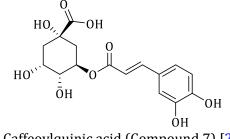
Lup-20(29)-en-3β-ol (Compound 1) [18]



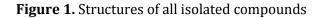
Lup-20(29)-ene-3*β*,28-diol (Compound 3) [20]

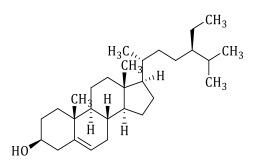




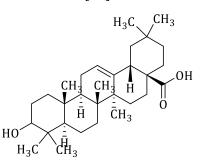


3-Caffeoylquinic acid (Compound 7) [24]

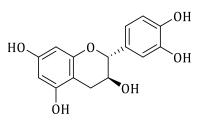




22,23-dihydrostigmasterol (Compound 2) [19]



3-β-hydroxyolean-12-en-28-oic acid (Compound 4) [21]



(2S,3R)-2-(3,4-Dihydroxyphenyl)chroman-3,5,7-triol (Compound 6) [23]

Hydroalchol extract showed the higher percentage of phenolics and flavonoids contents in quantitative assays than methanol and ethyl acetate extracts. Phenolics and flavonoids are the largest assembly of phytocompounds those are responsible for the various pharmacological activities including the antioxidant activities of plants extracts [25]. Various extracts of *B. strigosa* root found to have significant antioxidant activity inantioxidant assays. The antioxidant activities were in correlation with their phenolic/flavonoid contents and hydroalcohol extract showed predominant results in ABTS, DPPH, nitric oxide radical scavenging and ferric reducing assays. It could be estimated that the phenolics and flavonoid compounds present in the extracts act as an antioxidants through the mechanism of scavenging and also the reducing of oxidized intermediate in the chain reaction [26]. The significance p<0.05 was obtained for hydroalchol extract compared to ascorbic acid. Methanol extract also showed a comparable activity and this indicating that the extracting solvent also significantly influencing the antioxidant activity. The compound Lup-20(29)-en-3 β -olis a triterpene and a pharmacologically active compound with known anti-inflammatory, antiproliferative, anti-invasive, anti-angiogenic and cholesterol lowering activities [27]. 22,23dihydrostigmasterol is an important phytosterol with chemical structure similar to that of cholesterol. The compound showed antioxidant, anti-cancer, anti-diabetic activities [28]. The compound Lup-20(29)-ene- 3β ,28-diolis a triterpene and a reported anti-inflammatory and antitumor agent [29]. The compound 3-beta-hydroxyolean-12-en-28-oic acid is a triterpenoids a wellknown hepatoprotective agent [30]. Compound 5 was 3,3',4',5,7-pentahydroxyflavone is a wellknown antioxidant and showed neurological effects, anti-viral, anti-cancer, cardiovascular protective activities [31]. Compound 6, (2S,3R)-2-(3,4-dihydroxyphenyl) chroman-3,5,7-triol an important flavan-3-ol. This compound is an efficient anti-oxidantand effective in cancer and related diseases therapy and an ideal compound for nanoformulations to be used in antioxidant therapy [32]. Compound 7 was 3-caffeoylquinic acidand it is an ester of caffeic acid and quinic acid. It is an important biologically active dietary polyphenol, playing several important therapeutic roles such as antioxidant, neuroprotective and central nervous system stimulator [33]. The isolated compounds might have a good contribution towards the various antioxidant activities presented by the plant extract.

Conclusions

Phytochemical groups were quantitatively evaluated and various antioxidant activities were analyzed in ethyl acetate, methanol and hydro alcohol extracts of *B. strigosa* root. Hydroalcohol extract showed predominant results in all assays among the tested extracts. An activity based isolation of chemical constituents was carried out in hydroalcohol extract. Seven compounds were

isolated on the detailed column chromatography. The isolated compounds were characterized using various spectroscopic techniques. Three terpenoids such as Lup-20(29)-en-3 β -ol, lup-20(29)-ene-3 β ,28-diol, 3- β -hydroxyolean-12-en-28-oic acid and a steroid 22,23-dihydrostigmasterol were isolated from ethyl acetate fraction. A flavonol 3,3',4',5,7-pentahydroxyflavone, a flavan-3-ol (2S,3R)-2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol and a phenolic acid 3-caffeoylquinic acid were isolated from methanol fraction. 3,3',4',5,7-pentahydroxyflavone, (2S,3R)-2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol, 3-caffeoylquinic acid were well known antioxidants and that may be the reason for the good antioxidant activity exhibited by the plant extract.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

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