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Research Article

Phytochemical evaluation and *in vitro* antioxidant studies of selected species of *Gomphostemma* Wall. ex Benth. from Western Ghats

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ABSTRACT

Plants are a rich source of bioactive compounds and have played a vital role in traditional medicine. Plant derived compounds are proved to have less side effects as compared to synthetic compounds. However, a truncated history on the contribution of plants to medicine together with lack of validation of plant derived components have led to massive research in phytochemistry and allied fields. Present study was intended to identify as well as to assess and compare different phytochemicals and antioxidant potential of selected taxa of *Gomphostemma* endemic to Western Ghats. Methanolic extracts of aerial parts of *Gomphostemma heyneanum* var. *heyneanum*, *Gomphostemma heyneanum* var. *rotteri* and *Gomphostemma eriocarpum* were subjected to preliminary phytochemical screening. Quantitative estimation of phenols and flavonoids was done with Folin-Ciocalteu method and modified Aluminium chloride method respectively. Antioxidant potential was examined by DPPH assay, Hydroxyl radical scavenging assay and Reducing power activity. Preliminary screening indicated the presence of many secondary metabolites like phenols, flavonoids, alkaloids, terpenoids and steroids. Results of quantitative estimation revealed that *G. heyneanum* var. *heyneanum* has the highest concentration of phenols and flavonoids. Antioxidant potential determined in terms of inhibition percentage also displayed that *Gomphostemma* species have significant radical scavenging activity. The antioxidant potential exhibited by the plant species may be attributed to their phenol and flavonoid content.

Keywords: Phytochemical constituents, *Gomphostemma*, Quantitative estimation, Total phenolics, Flavonoids, Antioxidant activity, DPPH assay, Hydroxyl radical scavenging assay, Reducing power activity.

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INTRODUCTION

Plants are nature's pharmaceutical treasure chest as they form one of the major bio resources of compounds having miraculous effect in preventing and treating many diseases along with improving the general health of living organisms. Herbal plants thus form the backbone of traditional system of treatments throughout the globe and contribute compounds to pharmaceutical industry for drug development. Secondary metabolites like phenols, terpenes, flavonoids, alkaloids, phytosterols *etc.* which are produced naturally by the plants are held responsible for the therapeutic potential exhibited by them.

Free radicals are molecules and atoms with unpaired electrons in their outer shell. They are highly reactive and are formed in processes that involve oxygen. Free radicals that originate from oxygen are called reactive oxygen species (ROS), whereas free radicals that originate from the reaction of oxygen with nitrogen are considered as a subclass of free radicals and are called reactive

nitrogen species (RNS) ¹. Free radicals often called as ROS (Reactive Oxygen Species) are produced in the body of all organisms as a result of innumerable enzymatic reactions and is balanced by body's endogenous antioxidant system. But increase in ROS or decrease in the cellular antioxidant capacity can cause cellular oxidative stress. In either case it results in direct or indirect ROS-mediated damage of nucleic acids, proteins and lipids ². There is increasing evidence of ROS involvement in implicating carcinogenesis, ageing, neurodegeneration, renal and hepatic disorders ³. Plant derived antioxidants are proved to be with less side effects and good radical scavenging activity. It is because of this that antioxidants of plant origin can be a safe replacement for harmful and synthetic antioxidants ⁴.

Lamiaceae, a prominent medicinal aromatic family is very popular in folk medicine to treat various health problems such as throat infections, stomach disorders, ulcer, spasm, cold, haemorrhages and skin problems. Species of this botanical family contain a wide range of bioactive constituents that make them very important from

pharmacological point of view. *Gomphostemma* Wall. ex Benth. is an under exploited taxa of Lamiaceae. It is reported to have ethnomedicinal uses in treating diarrhoea, dysentery, arthritis and in curing inflammation by insect stings ^{5, 6}. Recently, there are reports on the antimalarial property of terpenes isolated from *Gomphostemma* ⁷. Members of *Gomphostemma* reported from South India are endemic to Western Ghats and to this date not many reports are available regarding their phytochemical constituents and bioactivity. Present study is intended to screen the selected taxa of *Gomphostemma* endemic to Western Ghats viz., *Gomphostemma heyneanum* Wall. ex Benth. var. *heyneanum*, *Gomphostemma heyneanum* var. *rottleri* Prain and *Gomphostemma eriocarpum* Benth. for their phytochemicals and antioxidative potential.

MATERIALS AND METHODS

Aerial parts of selected *Gomphostemma* spp. were collected from their natural populations in Nelliampathy forest area of Palakkad district and Ranni forest area of Pathanamthitta district, Kerala. Plants were identified and voucher specimens (CALI 123772 for *G. heyneanum* var. *heyneanum*, CALI 123773 for *G. heyneanum* var. *rottleri*, CALI 123774 for *G. eriocarpum*) were deposited in Calicut University Herbarium.

Preparation of plant extract

Twenty grams of dry powder of each plant material was subjected to soxhlet extraction using methanol as solvent. All methanolic extracts were concentrated in a rotary evaporator at 40°C. The crude extracts thus obtained were stored in refrigerator at 4°C and used for further analysis.

Preliminary phytochemical screening

Methanolic extracts of selected taxa were subjected to qualitative phytochemical analysis to identify the presence of various secondary phytoconstituents as per standard procedures ^{8,9}.

Test for phenols: A fraction of the extract was treated with 5% ferric chloride solution. Appearance of deep blue or black colour indicates the presence of phenols.

Tests for flavonoids:

Alkaline reagent test: To 2mL of the extract, a few drops of 20% sodium hydroxide were added. Appearance of yellow coloration which disappears on adding dilute hydrochloric acid infers the presence of flavonoids.

Test for alkaloids:

Wagner's Test: To sample solution Wagner's reagent was added and formation of reddish precipitate is taken as evidence for the presence of alkaloids.

Tests for terpenoids:

Salkowski test: To a fraction of extract, 2mL of chloroform was added followed by the addition of conc. H₂SO₄ along the sides of the test tube to form a layer. Formation of reddish brown coloration at the interface of the solution infers the presence of terpenoids.

Test for steroids:

Liebermann -Burchard test: To 2mL of methanolic extract, few drops of chloroform, acetic anhydride and conc. H₂SO₄ were added and formation of dark pink or red colour is an indication of the presence of steroids.

Test for tannins:

Braymer's test: About 2mL of extract was treated with 10% ferric chloride solution and appearance of blue or greenish coloration indicates the presence of tannins.

Test for saponins: In a test tube 1mL of extract was taken and 20mL of water was added. It was then shaken vigorously and observed for the formation of foam layer stable for 15min which is taken as an indication for the presence of saponins.

Estimation of total phenolic content

The total phenolic content of the plant extracts was determined using a modified Folin- Ciocalteu method ¹⁰. Gallic acid was used as the standard. An aliquot of sample/standard was mixed with 0.5mL of distilled water followed by the addition of 1.25mL of Folin - Ciocalteu reagent. The mixture was allowed to stand for 6 min before adding 1.25 mL of 7% Na₂CO₃ and the final volume was made to 3mL with distilled water. The whole reaction mixture was incubated in dark at room temperature for 90 min. The absorbance was read at 760nm against blank (distilled water) in a spectrophotometer (Elico SL 218, India). All values were expressed as mean in mg of gallic acid equivalents per gram of dry weight (mg GAE/g DW) ± standard error of mean (SEM) for three replications.

Estimation of total flavonoid content

The total flavonoid content was determined with the aluminium chloride colorimetric method with slight modification ¹¹. Quercetin was used as the standard. An aliquot of standard/sample added to 75µl of 7% NaNO₂ solution and mixed for 6 min. before adding 0.15mL of AlCl₃ (10%). This was allowed to stand for 5min. followed by the addition of 1M NaOH and the final volume was made to 2.5mL. The absorbance of the final mixture was determined at 510nm. The total flavonoid content was expressed as mg quercetin equivalent per gram dry weight (mg QE/g DW) ± standard error of mean (SEM) for three replications.

Assessment of antioxidant activity

DPPH radical scavenging assay

The scavenging activity of DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical by plant extracts was measured using accepted method with slight modification ¹². Ascorbic acid was used as reference standard for comparison. Extracts/ reference standard (20µl) of different concentrations (12.5 - 200µg/ml) was mixed with 1.48mL of DPPH (0.1mM) solution. A control without test compound but with equivalent amount (20 µL) of distilled water and DPPH solution was also prepared. The reaction mixture was incubated in room temperature for 20 min and absorbance was read at 517nm with methanol as blank. The inhibition percent was calculated from the following equation:

$$\% \text{ inhibition} = \frac{A_{\text{control}} - A_{\text{test sample}}}{A_{\text{control}}} \times 100$$

Where, A_{control} = Absorbance of control,

A_{test sample} = Absorbance of sample/standard.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging was assayed with slight modification ¹³. Gallic acid was used as reference standard. Different concentration of samples/ reference standard (125-2000µg/mL) were mixed with 500µL reaction mixture (2 deoxy 2 ribose (2.8mM), FeCl₃ (100µM), EDTA

(100µM), H₂O₂ (1.0mM), ascorbic acid (100µM) in KH₂PO₄ - KOH buffer (20 mM pH 7.4) and made up to a final volume of 1 mL. A control without the test compound, but with equivalent amount of distilled water was taken. After incubation for 1 hour at 37°C, 1ml of 2.8% TCA and then 1mL 1% aqueous TBA was added and the mixture was incubated at 90°C for 15 minutes to develop the colour. After cooling, absorbance was measured at 532nm against a blank solution. The inhibition percentage was calculated from the following equation:

$$\% \text{ inhibition} = \frac{A_{\text{control}} - A_{\text{test sample}}}{A_{\text{control}}} \times 100$$

Where, A_{control} = Absorbance of control,

A_{test sample} = Absorbance of sample/standard.

Reducing power assay

The reducing power of extracts was determined by the method of Yen and Duh ¹⁴. Quercetin was the reference standard. Different concentration of extracts/reference standard (125-2000µg/mL) was mixed with 2.5ml of phosphate buffer (200mM) (pH 6.6) and 2.5ml of 1%

potassium ferric cyanide was added. The reaction mixture was boiled for 20 minutes at 50°C. A control without the test compound was also taken. After incubation, 2.5 ml of 10% TCA was added to the mixtures followed by centrifugation for 10 minutes. The upper layer was mixed with 5mL of distilled water and 1mL of 0.1% ferric chloride was added. The absorbance was read at 700nm.

Statistical analysis

All data are given as the mean ± standard error of mean (SEM) for three replication. IC₅₀ values were calculated with GraphPad prism Version 7 for Windows (GraphPad Software Inc).

RESULTS

The preliminary phytochemical screening of aerial parts of selected taxa of *Gomphostemma* revealed the presence of phytoconstituents like phenols, flavonoids, alkaloids, terpenoids and steroids. Whereas tannins and saponins were found to be absent. The results of qualitative phytochemical screening are shown in Table 1.

Table 1: Results of qualitative phytochemical analysis of selected taxa of *Gomphostemma* from Western Ghats.

Phytochemical components	Tests	GHH	GHR	GE
Phenols	Ferric chloride test	+	+	+
Flavonoids	Alkaline reagent test	+	+	+
Alkaloids	Wagner's test	+	+	+
Terpenoids	Salkowski test	+	+	+
Steroids	Liebermann –Burchard test	+	+	+
Tannins	Braymer's test	-	-	-
Saponins	Foam test	-	-	-

(+) / (-) indicates presence/absence. GHH- *G. heyneanum* var. *heyneanum*, GHR- *G. heyneanum* var. *rottleri*, GE - *G. eriocarpum*.

Total phenolic content

Phenolic compounds from natural source are well identified as potent antioxidants. Total phenols in plant extracts were estimated with Folin – Ciocalteu reagent in terms of Gallic acid standard. The result obtained shows that *G. heyneanum* var. *heyneanum* has a higher amount of phenolic content (260.31 mg GAE/g DW) as compared to other two taxa (Table 2).

Total flavonoid content

Flavonoids are phytoconstituents with a wide range of biological and pharmacological activities especially

antioxidant property. Total favonoids was determined by spectrometric method with aluminium chloride method as described above. The content of flavonoids was expressed in terms of quercetin equivalents. The summary of quantities of flavonoid estimated in the tested plant extracts is shown in Table 2. Highest concentration of flavonoid was measured in *G. heyneanum* var. *heyneanum* (129.22 mg QE/g DW) followed by *G. heyneanum* var. *rottleri*, whereas lowest flavonoid content was measured in *G. eriocarpum*.

Table 2: Total phenolic content and total flavonoid content of selected species of *Gomphostemma* from Western Ghats.

Plant	Total phenolic content (mg GAE/g DW)	Total flavonoid content (mg QE/g DW)
GHH	260.31 ± 11.62	129.22 ± 10.658
GHR	178.78 ± 9.32	88.96 ± 0.550
GE	32.56 ± 3.18	33.69 ± 0.528

Total phenolic content analysed as GAE mg/g of dry weight of sample; Total flavonoid content analysed as QE mg/g of dry weight of sample; GAE is gallic acid equivalents, QE is quercetin equivalents; GHH- *G. heyneanum* var. *heyneanum*, GHR- *G. heyneanum* var. *rottleri*, GE - *G. eriocarpum*. Data expressed as Mean ± SE of three replicates.

DPPH assay

DPPH assay is an easy, rapid and sensitive method to survey the free radical scavenging ability of a compound or plant extract. All samples showed a dose dependent

inhibition of DPPH radicals as shown in Fig. 1. Among the samples, *G.heyneanum* var. *heyneanum* showed significant antioxidant activity with IC₅₀ of 46.63 ± 2.26 µg/mL, comparable to that of standard ascorbic acid with IC₅₀ value of 48.93 ± 1.75 µg/mL as shown in Table 3.

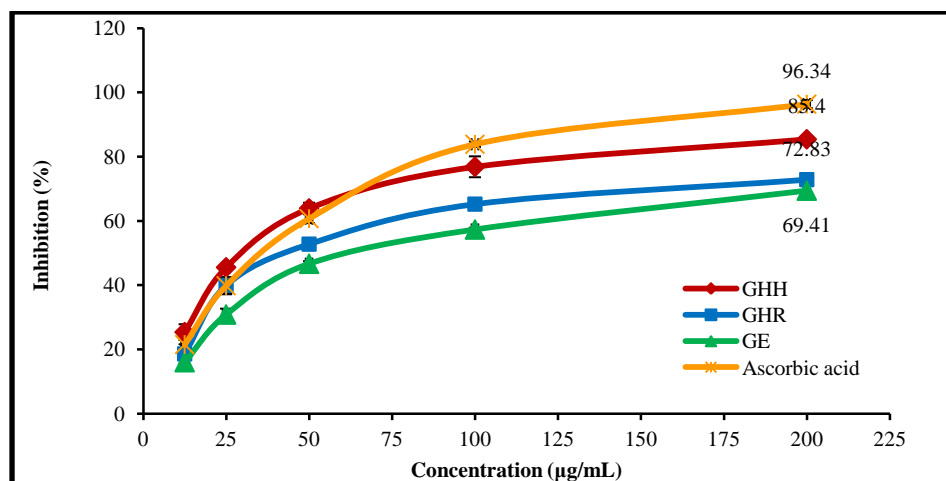


Figure 1: DPPH radical scavenging activity

GHH- *G. heyneanum* var. *heyneanum*, GHR- *G. heyneanum* var. *rottleri*, GE - *G. eriocarpum*.
Data expressed as Mean \pm SE of three replicates.

Hydroxyl radical scavenging activity

Hydroxyl radical is the most reactive oxygen species that can attack and damage bio molecules. In the present study all the three extracts showed good scavenging ability. In scavenging hydroxyl radical, both *G. heyneanum* var. *heyneanum* and *G. eriocarpum* showed significant activity

with higher inhibition percentage and lower IC_{50} values as compared to standard gallic acid (Table 3). Maximum inhibition for scavenging hydroxyl radical was 78.12 ± 3.68 for *G. eriocarpum* and 76.87 ± 3.26 for *G. heyneanum* var. *heyneanum* at a concentration of $2000 \mu\text{g/mL}$ (Fig. 2), which is a clear indication of strong antioxidant property of *Gomphostemma* spp.

Table 3: IC_{50} values of antioxidant assays

Plant	$IC_{50} \pm SE (\mu\text{g/mL})$	
	DPPH radical scavenging	Hydroxyl radical scavenging
GHH	46.63 ± 2.26	745.17 ± 2.13
GHR	80.41 ± 1.87	1108.56 ± 2.12
GE	101.61 ± 1.22	587.17 ± 2.97
Standard	48.93 ± 1.75	1347.51 ± 1.94

GHH- *G. heyneanum* var. *heyneanum*, GHR- *G. heyneanum* var. *rottleri*, GE - *G. eriocarpum*.
Data expressed as Mean \pm SE of three replicates.

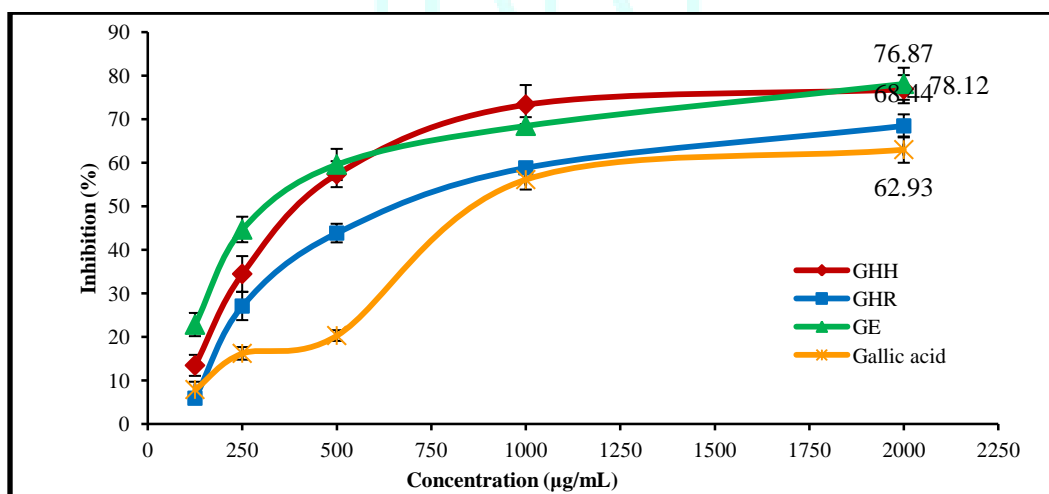


Figure 2: Hydroxyl radical scavenging activity

GHH- *G. heyneanum* var. *heyneanum*, GHR- *G. heyneanum* var. *rottleri*, GE - *G. eriocarpum*.
Data expressed as Mean \pm SE of three replicates.

Reducing power assay

Reducing power of samples analysed using potassium ferricyanide method indicated that the reducing ability of extracts increased with increase in concentration as shown

by increasing optical density at 700 nm (Fig. 3). Among the samples tested, *G. heyneanum* var. *heyneanum* showed maximum reducing potential but less compared to the standard quercetin.

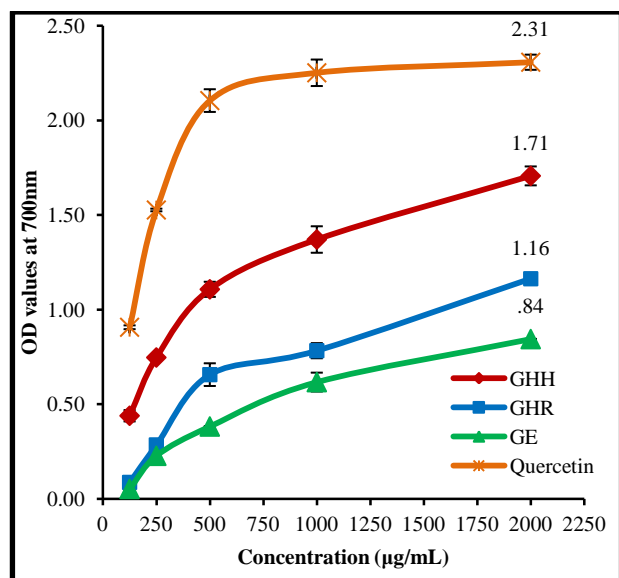


Figure 3: Reducing power activity

GHH- *G. heyneanum* var. *heyneanum*, GHR- *G. heyneanum* var. *rottleri*, GE - *G. eriocarpum*.

Data expressed as Mean \pm SE of three replicates.

DISCUSSION

Plants produce a wide range of bioactive chemical compounds as a result of secondary metabolism. These phytochemicals are considered as the active ingredients attributing therapeutic potential to plant and plant derived products ¹⁵. In the present study, selected taxa of *Gomphostemma* belonging to Lamiaceae was subjected to phytochemical screening, which revealed the presence of a variety of phytoconstituents like phenols, flavonoids, alkaloids, terpenoids and steroids. Presence of such diverse class of phytochemicals can be taken as an indication of the analeptic property of these plants. Phenolic compounds of plant origin are vital in defence responses, such as anti-aging, anti-inflammatory, antioxidant and anti-proliferative activities ¹⁶. Flavonoids from natural sources are considered as an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function ¹⁷. In the present study, total phenol and flavonoid contents were estimated to be highest in *G. heyneanum* var. *heyneanum*. However, *G. eriocarpum* revealed fewer amounts of phenols and flavonoids as compared to other two taxa, but still both were measured in it in significant quantity.

Reactive oxygen species (ROS) are constantly produced in living cells as a result of metabolic reactions. Even though some basic cellular functions are apparently regulated by ROS generated in the cells, excess accumulation may lead to oxidative stress ¹⁸. ROS have been connected with the development and progression of cancer, inflammation and ageing ¹⁹. An antioxidant is any substance which even when present at low concentration significantly delays or prevents oxidation of that substrate ²⁰. Antioxidant capacity is regarded as the ability of a compound to decrease oxidation ²¹. Antioxidants may be synthetic or natural but safety concerns and limitation on the use of synthetic antioxidants have increased the interest on

obtaining natural antioxidants from edible materials, edible by products and residual sources ²². Many of the natural antioxidants of plant origin belong to phenolic and phenolic class of compounds ²³. Phenolic acids are secondary metabolites which are extensively distributed in the plant kingdom and are regarded to be natural antioxidants. Like phenolic acids, flavonoids are secondary metabolites of plants with polyphenolic structure well known for their antioxidant properties ²⁴. Therefore, determination of total phenolics and flavonoids is helpful in revealing the antioxidant capacity of the plant extracts.

In general, extracts with higher phenolic and flavonoid content shows a higher antioxidant activity because of the antioxidant potential of these class of compounds. In the present study also *G. heyneanum* var. *heyneanum* which showed a higher phenolic and flavonoid content exhibited significant antioxidant potential in all the three antioxidant assays conducted. However, it is striking to note that *G. eriocarpum* which showed a lower total phenolic and flavonoid content compared to the other two extracts, portrayed a good hydroxyl scavenging ability. This can be attributed to the varied molecular antioxidant response of individual phenolic compounds and the interference from other chemical components of the extract ²⁵. Current study thus furnishes a lead to the therapeutic potential of selected taxa of *Gomphostemma* and suggests for extensive studies on these plants.

CONCLUSION

In the present study selected taxa of *Gomphostemma* endemic to Western Ghats were screened for their phytoconstituents and antioxidative potential. Preliminary phytochemical analysis revealed the presence of flavonoids, phenols, alkaloids, terpenoids and steroids. Estimation of total phenolics and flavonoids showed a significant amount of these class of compounds which can be taken as a lead for the bioactive and therapeutic efficacy of this taxa. A measurement of antioxidant potential with three comprehensive assays like DPPH scavenging, hydroxyl radical scavenging and reducing power activity showed that *Gomphostemma* spp. like other members of Lamiaceae are eligible to be considered and can very well be exploited for their therapeutic potential. This is particularly relevant in the present scenario, as there is a constant rise in need for natural products with fewer side effects but with effective therapeutic value for treating and curing many life threatening and life style diseases like cancer, diabetes, hepatic diseases etc. However, detailed research and in depth analysis are required for identifying and isolating the specific compounds which are rendering these plants with their bioactivity potential. Present study thus provides a lead to categorise *Gomphostemma* spp. as a potent source of therapeutic ingredients.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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