INTRODUCTION

The family Rubiaceae is one among the largest families of angiosperms and includes important plant genera such as Coffea, Cinchona and Gardenia. The genus Gardenia belongs to the family Rubiaceae and has a number of species distributed in tropical and subtropical regions of the world. Several species of Gardenia are known to be medicinally important and have been used in various systems of traditional medicine. Some species yield wood which can be used as substitute for boxwood. It is also a component of certain perfumes [1, 2]. Gardenia gummifera L. f. is found distributed in different states of India namely Uttar Pradesh, Tamil Nadu, Karnataka, Kerala, Bihar and Andhra Pradesh. It is called Naadi-hingu and Dikamali in Ayurveda, and gidda or Kaatu hingu in Kannada. It is a small sized, unarmed, deciduous tree. The persistent calyx [3, 4].

Dikamali is the gum resin obtained from the leaf buds of G. gummifera. This resin is claimed to possess medicinal properties such as anthelmintic, antispasmodic, carminative, diaphoretic, expectorant, and cardiotoxic [5]. The plant has been used as traditional medicine in various parts of India to treat ailments such as haemorrhoids, bone fracture, nervous disorders, diarrhea, wounds, skin diseases and stomach ulcers [6-13]. G. gummifera is shown to exhibit various bioactivities such as antioxidant [14-16], antimicrobial [8, 17], insecticidal [18], Cytotoxic [19], hepatoprotective [20], antihyperlipidemic [21], anti-atherogenic [22], antiallergic [15], cardioprotective [23], analgesic [14], anti-inflammatory [14], antipyretic [14], and anthelmintic activity [14]. The present study was carried out to investigate antiradical and insecticidal activity of extract obtained from leaf and fruit of G. gummifera.

MATERIALS AND METHODS

Chemicals and media

Chemicals viz. DPPH and ABTS were purchased from Sigma Chemical Co., USA. Chemicals namely methanol, ascorbic acid, potassium persulfate, dimethyl sulfoxide (DMSO) and chloramphenicol and media namely Nutrient agar, Nutrient broth and Potato dextrose agar were purchased from HiMedia, Mumbai, India.

Collection and identification of plant

The plant samples were collected from Kavalegudda, which is about 7 km away from Sagara, Shimogogga district, Karnataka during February 2017. The plant was identified on the basis of its characteristics by referring standard flora [4] and with the help of taxonomist Dr. Vinayaka K. S, Assistant Professor, KFGC, Shikaripura, Shimogoga district, Karnataka, India. A voucher specimen

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Original Article

ANTIMICROBIAL, ANTIRADICAL AND INSECTICIDAL ACTIVITY OF GARDENIA GUMMIFERA L. F. (RUBIACEAE)

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ABSTRACT

Objective: The present study was carried out to investigate antimicrobial, antiradical and insecticidal potential of leaf and fruit of Gardenia gummifera L. f. (Rubiaceae).

Methods: The leaf and fruits were shade dried, powdered and extracted by maceration process using methanol. Antibacterial activity was evaluated against Gram positive and Gram negative bacteria by Agar well diffusion assay. Antifungal activity was determined against six seed-borne fungi by Poisoned food technique. Antiradical activity of leaf and fruit extracts was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate (ABTS) radical scavenging assays. Insecticidal activity of leaf and fruit extracts, in terms of larvicidal and pupicidal activity, was assessed against larvae and pupae of Aedes aegypti.

Results: Both the extracts inhibited all test bacteria. Marked antibacterial activity was displayed by fruit extract when compared to leaf extract. S. epidermidis and E. coli were inhibited to highest and least extent by both extracts respectively. Fruit extract was found to exhibit higher antifungal effect when compared to leaf extract. Leaf extract and fruit extract exhibited highest inhibitory activity against A. niger and A. flavus respectively. Leaf and fruit extracts scavenged DPPH radical’s dose dependently with an IC_{50} value of 49.01µg/ml and 2.53µg/ml respectively. The scavenging of ABTS by leaf and fruit extracts was dose dependent and the IC_{50} value for leaf and fruit extract was 2.58µg/ml and 2.31µg/ml respectively. Fruit extract was shown to exhibit marked antiradical activity when compared to leaf extract. Leaf and fruit extracts exhibited dose dependent insecticidal activity in terms of larvicidal and pupicidal activity and the susceptibility of larvae and pupae to extracts was in the order II instar larvae>IV instar larvae>pupae. Fruit extract displayed marked insecticidal potential when compared to leaf extract.

Conclusion: Overall, fruit extract of G. gummifera exhibited marked antimicrobial, antiradical and insecticidal activity when compared to leaf extract. The plant can be used for developing agents/formulations effective against infectious microorganisms, oxidative stress and insect vectors that transmit dreadful diseases. The observed bioactivities could be ascribed to the presence of active principles which are to be isolated and characterized.

Keywords: Gardenia gummifera L. f., Agar well diffusion, Poisoned food technique, DPPH, ABTS, Insecticidal, Aedes aegypti
The leaves and fruits were separated, washed well using clean water, dried under shade and powdered. Extraction of powdered leaf and fruit (10 g) was carried out by maceration process using methanol (100 ml) in separate stoppered containers. The powders were left in methanol in stoppered containers for 48 h and the containers were stirred frequently. The contents were filtered through Whatman No. 1 filter paper and the filtrates were evaporated to dryness to get crude leaf and fruit extracts. The extracts were stored in refrigerator until use [24].

Test bacteria
Gram positive bacteria (Staphylococcus aureus NCIM 5345, Staphylococcus epidermidis NCIM 2493, Bacillus subtilis NCIM 2063 and Bacillus cereus NCIM 2016) and Gram negative bacteria (Escherichia coli NCIM 2065, Pseudomonas aeruginosa NCIM 2200 and Salmonella typhiunum NGM 2501) were used to assess their susceptibility to leaf and fruit extract of G. gummifera. The test bacteria were procured from National Chemical Laboratory (NCL), Pune, India.

Antibacterial activity of leaf and fruit extracts
The potential of leaf and fruit of G. gummifera to inhibit bacteria was determined by agar well diffusion method. 24 h old nutrient broth cultures of test bacteria were swab inoculated on sterile nutrient agar plates. Using a sterile cork borer, wells of 8 mm diameter were punched in the inoculated plates. Respective wells were filled with 100 µl of leaf and fruit extracts (20 mg/ml of DMSO), standard antibiotic (Chloramphenicol; 1 mg/ml of sterile distilled water) and DMSO. The plates were incubated in upright position for 24 h at 37 °C and zones of inhibition formed around wells were measured [25].

Test fungi
Six seed-borne fungi (isolated previously from moldy grains of sorghum) namely Aspergillus niger, A. flavus, A. fumigatus, Curvularia sp., Alternaria sp. and Fusarium sp. were screened for their susceptibility to leaf and fruit extract of G. gummifera.

Antifungal activity of leaf and fruit extracts
Poisoned food technique was employed to evaluate antifungal potential of leaf and fruit extracts. In brief, well sporulated cultures of test fungi were inoculated on control plates (without extracts) and poisoned potato dextrose agar (1 mg extract/ml of medium) plates aseptically. The plates were incubated for 96 h at room temperature in upright position and the diameter of fungal colonies in mutual perpendicular directions was measured. The inhibition of mycelial growth of test fungi (%) by leaf and fruit extracts was determined using the formula:

\[
\text{Inhibition of Mycelial Growth} = \left( \frac{\text{Control} - \text{Test}}{\text{Control}} \right) \times 100
\]

Where control and test denotes the colony diameter of test fungi on control and poisoned plates respectively [25].

Antiradical activity of leaf and fruit extracts
We screened antiradical potential of leaf and fruit extracts of G. gummifera by two in vitro assays namely DPPH radical scavenging activity and ABTS radical scavenging activity.

DPPH radical scavenging activity
DPPH radical solution and different concentrations (12.5 to 200 µg/ml) of leaf and fruit extracts and ascorbic acid (reference standard) were prepared in methanol. To each of the tubes containing 1 ml of different concentrations of leaf and fruit extracts and ascorbic acid, 3 ml of DPPH radical solution was added and the tubes were incubated in dark for 30 min. The absorbance was measured in a spectrophotometer at 517 nm. Extract replaced by methanol served as control. The radical scavenging potential of each concentration of extracts/ascorbic acid was determined using the formula:

\[
\text{Scavenging of DPPH Radicals} = \left( \frac{A - B}{A} \right) \times 100
\]

Where ‘A’ and ‘B’ represents the absorbance of DPPH control and absorbance of DPPH in presence of extract/stANDARD. The IC50 value was calculated. IC50 value represents the concentration of extract required to scavenge 50% of DPPH radicals [24].

ABTS radical scavenging activity
ABTS radical was generated by mixing ABTS salt (7 mmol) with Potassium persulphate (2.45 mmol) and incubating for 16 h. The resulting radical solution was diluted with distilled water to an absorbance 0.7 in spectrophotometer. 1 ml of different concentrations (12.5 to 200 µg/ml) of leaf and fruit extracts and ascorbic acid (reference standard) was mixed with 3 ml of ABTS radical solution and the tubes were incubated in dark for 30 min at room temperature. The absorbance of reaction mixture of each tube was measured in a spectrophotometer at 730 nm. Extract replaced by methanol served as control. The radical scavenging potential of each concentration of extracts/ascorbic acid was determined using the formula:

\[
\text{Scavenging of ABTS Radicals} = \left( \frac{A - B}{A} \right) \times 100
\]

Where ‘A’ and ‘B’ represents the absorbance of ABTS control and absorbance of ABTS in presence of extract/stANDARD. The IC50 value was calculated. IC50 value represents the concentration of extract required to scavenge 50% of ABTS radicals [24].

Insecticidal activity of leaf and fruit extracts
The insecticidal potential of leaf and fruit extracts in terms of larvicidal and pupicidal activity was assessed against A. aegypti. In brief, 20 larvae (II and IV instar) and pupae were transferred separately into conical flasks containing 50 ml of water (with different concentrations of extracts namely 0.0 to 2.0 mg/ml). The mortality of larvae and pupae (%) was assessed after 24 h and was calculated using the formula:

\[
\text{Mortality} = \left( \frac{\text{Number of Dead Larvae or Pupae}}{\text{Number of Total Larvae or Pupae}} \right) \times 100
\]

LC50 value was calculated [26-27].

Statistical analysis
The experiments were conducted in triplicates and the results are presented as mean ± Standard deviation (S. D). The IC50 and LC50 values were calculated by linear regression analysis using Origin (Data Analysis and Graphing) Software version 7.0 for windows.

RESULTS AND DISCUSSION
Antibacterial activity of leaf and fruit extract of G. gummifera
Emergence of antibiotic resistant strains of bacteria is of potential threat in hospital as well as community settings. Antibiotic resistant strains of bacteria such as Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Mycobacterium tuberculosis are the main cause of death by infectious agents worldwide. Indiscriminate use of antibiotics and the ability of resistant strains of pathogens to transmit the resistance trait to susceptible strains by genetic means have made the situation even worst. Natural products are known to be an important alternate for the disease therapy. They are safer, cheaper and are not associated with side effects. Interest in botanicals with antibacterial activity has been triggered in recent years due to the drawbacks of antibiotics. Several studies have shown the potential of higher plants and their metabolites to inhibit pathogenic bacteria including antibiotic resistant strains [28-34]. In the present study, we determined the antibacterial potential of leaf and fruit extract of G. gummifera by Agar well diffusion method. This method has been extensively used to evaluate antibacterial activity of several plants and the presence of zone of inhibition around well is taken positive for antibacterial activity. The result of antibacterial activity of leaf and fruit extract is shown in table 1. Both extracts inhibited test bacteria as revealed by the absence of growth around the wells. Among extracts, marked activity was displayed by fruit extract of G. gummifera (SRNMN/PK/Gg-01) was deposited in the department herbaria for future reference.
extract when compared to leaf extract. *S. epidermidis* and *E. coli* were inhibited to highest and least extent by both extracts respectively. Inhibitory activity of antibiotic was higher when compared to leaf and fruit extracts. Overall, Gram positive bacteria were inhibited to higher extent than Gram negative bacteria by both extracts and antibiotic. DMSO did not cause inhibition of test bacteria. In an earlier study, Tambekar and Kante [8] found inhibitory activity of various solvent extracts of resin obtained from leaf buds and shoots of *G. gummifera* against *S. aureus*, *Klebsiella pneumoniae* and *Enterobacter aerogenes*. In another study, Narware et al. [17] showed the antibacterial potential of solvent extracts of gum of *G. gummifera* against *S. aureus* and *E. coli*. Other species of *Gardenia* such as *G. volkensii* [35], *G. aqualla* [36] and *G. resinifera* [37] have also shown antibacterial activity.

**Table 1: Antibacterial activity of leaf and fruit extract of *G. gummifera* and antibiotic**

| Test bacteria       | Zone of inhibition in mm (mean±SD n=3) | Leaf extract | Fruit extract | Antibiotic | DMSO       |
|---------------------|----------------------------------------|--------------|---------------|------------|------------|
|                     |                                        |              |               |            |            |
| *S. aureus*         |                                        | 12.67±0.58   | 14.33±0.58    | 33.00±1.00 | 0.00±0.00  |
| *S. epidermidis*    |                                        | 17.33±0.58   | 19.33±0.58    | 37.33±0.58 | 0.00±0.00  |
| *B. cereus*         |                                        | 14.00±0.00   | 15.33±0.58    | 36.00±0.00 | 0.00±0.00  |
| *B. subtilis*       |                                        | 15.00±0.00   | 15.67±0.58    | 33.00±1.00 | 0.00±0.00  |
| *P. aeruginosa*     |                                        | 11.67±0.58   | 13.00±0.00    | 27.67±0.58 | 0.00±0.00  |
| *E. coli*           |                                        | 11.00±0.00   | 11.00±0.00    | 25.33±0.58 | 0.00±0.00  |
| *S. typhimurium*    |                                        | 12.67±0.58   | 13.67±0.58    | 21.33±1.53 | 0.00±0.00  |

**Antifungal activity of leaf and fruit extract of *G. gummifera***

Management of fungal pathogens by chemical agents is not much beneficial as they are costly, not easily degraded and implicated in environmental pollution. Besides, the development of resistance in fungal pathogens against the synthetic fungicides is another serious problem associated with their indiscriminate use. Plants and plant based formulations appear to be promising alternatives for synthetic agents. Fungicides from botanical origin are safer, cheaper, easily degraded and do not cause environmental pollution. Several studies have shown the potential of plants and plant based formulations to inhibit a wide range of phytopathogenic fungi [38-44]. In the present study, we evaluated antifungal potential of leaf and fruit extract of *G. gummifera* by Poisoned food technique. This technique is one among the widely used antifungal assays in which reduction in the mycelial growth of test fungi on poisoned plates is taken positive for antifungal activity. Fruit extract of *G. gummifera* was found to exhibit higher antifungal effect when compared to leaf extract. Leaf extract exhibited highest and least inhibitory activity against *A. niger* and *Fusarium* sp. respectively. Fruit extract displayed highest and least inhibitory activity against *A. flavus* and *Fusarium* sp. respectively. Among test fungi, least susceptibility to extracts was shown by *Fusarium* sp. none of the fungi were inhibited to >50% by leaf extract while >50% inhibition by fruit extract was recorded against *A. niger* and *A. flavus* (Table 2 and fig. 1). In an earlier study, Kafua et al. [45] showed the potential of leaf extract of *G. brighamii* to inhibit fumonisin producing *Fusarium* species. Genipin and geniposide isolated from *G. jasminoides* were shown to exhibit inhibitory activity against phytopathogenic fungi [46].

**Table 2: Antifungal activity of leaf and fruit extract of *G. gummifera***

| Treatment          | Colony diameter in mm (mean±SD n=3) | A. niger     | A. flavus    | A. fumigatus | Curvularia sp. | Alternaria sp. | Fusarium sp.  |
|--------------------|-------------------------------------|--------------|--------------|--------------|----------------|----------------|--------------|
|                    |                                     | 56.67±0.58   | 43.00±0.00   | 38.67±1.53   | 45.33±0.58     | 52.00±1.00     | 42.67±0.58   |
| Control            |                                     |              |              |              |                |                |              |
| Leaf extract       |                                     | 29.00±0.00   | 29.67±0.50   | 26.00±0.00   | 32.00±1.00     | 38.00±0.00     | 32.00±0.00   |
| Fruit extract      |                                     | 27.67±0.58   | 20.00±0.00   | 23.67±0.58   | 22.67±0.58     | 35.33±1.15     | 31.00±0.00   |

**Fig. 1: Extent of inhibition of test fungi (%) by leaf and fruit extract**
Antiradical activity of leaf and fruit extract of *G. gummifera*

Free radicals are produced during normal metabolism of oxygen and some cell mediated immune functions of the body. There exists a dynamic balance between free radical generation and antioxidant defense (enzymatic and non-enzymatic) of the body. However, when the excessive generation of free radicals occur it result in oxidative stress which is implicated in several diseases or conditions such as Diabetes, Atherosclerosis, Alzheimer’s disease, Parkinsonism, Cardiovascular diseases, Inflammatory conditions, Neonatal diseases, Cancer and Aging. Free radicals are compounds with one or more unpaired electrons and are known to damage biomolecules such as nucleic acid, proteins and lipids. Antioxidants are substances capable of inhibiting or delaying oxidative damage when present in small quantities compared to an oxidizable substrate.

Antioxidants act by effective quenching free radicals or inhibiting damage caused by them. In pathophysiological conditions, there is an extra need for antioxidants from exogenous sources. Interest in botanicals as antioxidants has emerged due to suspected negative effects that are associated with the use of synthetic antioxidants such BHA, BHT etc. many plant species have been shown to possess marked antioxidant activity which is attributed to the presence of phytochemicals mainly phenolic compounds [31, 47-55].

Among various *in vitro* radical scavenging assays, the assay involving scavenging of DPPH radicals is one of the widely used assays. The method is simple, rapid, does not generate of radicals and the results can be reproducible. DPPH radical is a stable, organic nitrogen centered free radical having purple color and an absorption maximum at 517 nm in alcoholic solution. On accepting a proton from a donor (an antioxidant), the DPPH loses the free radical nature and gets converted into a corresponding yellow colored hydrazine i.e., DPPHH and the extent of bleaching of purple color depends on proton donating ability of sample. The assay has been employed by various researchers to evaluate radical scavenging potential of various kinds of samples including plant extracts and plant metabolites [24, 33, 53, 56-63]. In this study, we evaluated the potential of leaf and fruit extract of *G. gummifera* by DPPH assay and the result is shown in fig 2. Both extracts and ascorbic acid exhibited concentration dependent scavenging of DPPH radicals. Among extracts, the fruit extract scavenged DPPH radicals to higher extent with an IC_{50} value of 2.53µg/ml when compared to leaf extract (IC_{50} 49.01µg/ml). At concentration 25µg/ml, fruit extract exhibited a scavenging activity of >50% while leaf extract displayed>50% scavenging of radicals at 50µg/ml concentration. A scavenging activity of >90% was shown by both leaf and fruit extracts at concentration 200µg/ml. Ascorbic acid scavenged radicals with an IC_{50} value of 10.19µg/ml. In an earlier study, Vindhya and Leelavathi [16] showed DPPH radical scavenging activity of various solvent extracts of leaf of *G. gummifera*. Ethanol extract exhibited marked scavenging of DPPH radicals with an IC_{50} value of 48.33µg/ml.

The study of Uddin et al. [53] showed significant scavenging of DPPH radicals by leaf extract of *G. jasminoides*. Debnath et al. [52] showed the DPPH radical scavenging efficacy of aqueous and ethanolic extract of fruits of *G. jasminoides*. Leaf extracts of *G. latifolia* have shown scavenging potential against DPPH radicals [64].

ABTS radical scavenging assay is another widely used *in vitro* radical assay. It differs from DPPH assay in that it needs the generation of radicals prior to assay. The generation of ABTS radicals can be carried out by mixing ABTS salt with an oxidizing agent such as potassium persulphate or potassium permanganate. Substances with electronic donating potential (antioxidant species) will reduce the blue-green colored radical solution to colorless neutral form which is shown by the suppression of characteristic long wavelength absorption spectrum. The method of scavenging of ABTS radicals has been extensively used to evaluate radical scavenging nature of plant extracts [24, 52, 58, 61, 62, 65, 66]. In the present study, we evaluated radical scavenging nature of leaf and fruit extract of *G. gummifera* by ABTS assay and the result is shown in fig. 3. Both the extracts and ascorbic acid displayed concentration dependent scavenging of ABTS radicals. All concentrations of extracts displayed>75% scavenging of radicals. At 100µg/ml, only fruit extract showed>90% scavenging of radicals. Among extracts, fruit extract exhibited marked scavenging of ABTS radicals with (an IC_{50} value of 2.31µg/ml) when compared to leaf extract (IC_{50} value of 2.58µg/ml). Ascorbic acid displayed marked scavenging of ABTS radicals (IC_{50} value of 1.64µg/ml) when compared to leaf and fruit extracts. In an earlier study, aqueous and ethanolic extract of *G. jasminoides* fruit exhibited marked scavenging potential against ABTS radicals [52]. A water soluble polysaccharide isolated from *G. jasminoides* was shown to exhibit dose dependent scavenging of ABTS radicals [67].
Insecticidal activity of leaf and fruit extract of G. gummifera

Mosquitoes are considered as the major public health problem worldwide as the mosquitoes are well known as vectors of transmission of various human diseases such as malaria, filariasis, dengue, chikungunya, Japanese encephalitis and yellow fever. Species of Culex, Aedes and Anopheles are more important mosquito genera as they transmit dreadful human diseases. Filariasis is transmitted by Culex quinquefasciatus, malaria is transmitted by female Anopheles mosquito and diseases such as chikungunya and dengue are transmitted by Aedes aegypti. It is very important to prevent and control mosquitoes in order to achieve control of mosquito-borne diseases. Several stages in the life cycle of mosquitoes are targeted in order to prevent mosquito-borne diseases. Strategies such as prevention of egg hatching, killing of larvae, pupae and adult mosquitoes and use of mosquito repellents have been used for controlling mosquitoes. Synthetic insecticides are being used extensively, however, their use is associated with several drawbacks. The use of botanicals offers a safer and cheaper strategy for mosquito control. It is shown that plants, plant based formulations and plant metabolites exhibit insecticidal activity against several mosquitoes such as species of Aedes, Culex and Anopheles [26, 27, 68-75].

In the present study we evaluated insecticidal activity of various concentrations of leaf and fruit extract of G. gummifera against larvae and pupae of A. aegypti. The extracts were effective in killing larvae and pupae in a concentration dependent manner (fig. 4 and 5). The susceptibility of larvae and pupae to extracts was in the order: II instar larvae>IV instar larvae>pupae. Fruit extract exhibited marked insecticidal activity when compared to leaf extract. The LC50 of leaf extract against II instar larvae, IV instar larvae and pupae was found to be 0.5, 1.5 and 2.5 mg/ml respectively. The LC50 of fruit extract was 0.42, 1.0 and 1.94 mg/ml for II instar larvae, IV instar larvae and pupae respectively. In an earlier study, the ethanolic extract of dried exudates from G. gummifera was shown to exhibit dose dependent larvicidal activity against Culex quinquefasciatus [18].

Fig. 3: Scavenging of ABTS radicals by leaf and fruit extract of G. gummifera

Fig. 4: Mortality of larvae and pupae at different concentrations of leaf extract
CONCLUSION
The present study has shown promising antimicrobial, antiradical and insecticidal activity of leaf and fruit of *G. gummifera*. Overall, fruit extract was found to exhibit higher antimicrobial, antiradical and insecticidal activity when compared to leaf extract. The observed bioactivities could be ascribed to the presence of phytochemicals in extracts. In suitable form, the plant can be used to treat infectious diseases, oxidative stress and to control phytopathogenic fungi and insect vectors.

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AUTHORS CONTRIBUTION
The author Prashith was involved in the collection of plant material and all the bioactivities conducted in the study. Prashith and Raghavendra have planned and framed the objectives. Shilpa, Pushpavathi, Petkar and Siddigha contributed equally for carrying out extraction and antimicrobial and antiradical activity. Insecticidal activity and statistical analysis was carried out by Prashith and Raghavendra. Furthermore, Prashith and Raghavendra wrote the draft paper and the final paper was corrected and approved by all authors.

CONFLICT OF INTERESTS
Authors declared that there are no potential conflicts of interest.

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