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Review article

Cycadaceae: An Important Source for Biflavonoids and Various Pharmacological Effects of Different *Cycas* Species

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ABSTRACT

Cycadaceae is regarded as one of the largest cycad families. It is made up of 120 species of the single genus *Cycas*. Genus *Cycas* is found only in tropical and subtropical climates. They are dioecious plants with male and female reproductive cones. Members of Cycadaceae family are excellent suppliers of a wide range of beneficial phytochemicals however, only a few numbers of species had undergone phytochemical and pharmacological research. A wide battery of metabolites, including flavonoids, biflavonoids, phenolic acids, sterols, amino acids, lignans, and fatty acids, have been isolated and structurally identified from various *Cycas* species. Since biflavonoids are a defining trait of all cycads, we focused in this review on the various biflavonoids nuclei found in Cycadaceae and their distribution in distinct *Cycas* species among other isolated secondary metabolites. Therefore, we outlined the various biflavonoids nuclei found in Cycadaceae and their distribution in distinct *Cycas* species in this review. Additionally, we highlighted the diverse pharmacological effects of different *Cycas* species that their biflavonoid content can be credited with.

Keywords: Amentoflavone, Biapigenin di-C-glucoside, Cytotoxicity, Hinokiflavone, Toxoplasmocidal.

1. INTRODUCTION

Family Cycadaceae belongs to order Cycadales, which is also known as the Cycads. Cycads are among the largest living groups of gymnosperms and the oldest ancient ones still alive, with a fossil record that dates back more than 200 million years [1]. The only recognized genus in the family Cycadaceae is *Cycas* [2]. According to the most recent classification proposed by Christenhusz *et al.*, about 120 *Cycas* species are included under this family [3]. They are distributed in Africa, Asia, Australia, India & southwestern Pacific countries [3]. They thrive in tropical woodlands and forests, mainly near coastlines, where summer rains are common [4]. *Cycas* species are palm-like, they get their name from the Greek word kykas, which means "palm" [1]. *Cycas* plants feature a columnar aerial trunk and pinnately complex leaf crowns. Usually, the stem is unbranched. A crown of leaves arranged in a spiral at the summit of young plants' underground tuberous stems [5]. *Cycas* produce cones, spirally aggregated reproductive organs comprised of sporophylls, which are highly modified leaves. Each male sporophyll carries many sporangia (pollen capsules), frequently on its bottom surface. While each female sporophyll holds ovules, typically two [6,7]. *Cycas* species are considered rich sources of biflavonoids in addition, they are proven to exert a wide variety of significant pharmacological effects. Therefore, in this review, we summarized all biflavonoids isolated from various *Cycas* species and the most valuable pharmacological

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effects of different *Cycas* members from the beginning of phytochemical research on *Cycas* species to the present.

2. BIFLAVONOIDS DISTRIBUTION IN DIFFERENT *CYCAS* SPECIES

There are many different types of biflavonoids according to the structure of the flavonoid monomer and the connection mode of biflavonoids [8]. Amongst these different types of biflavonoids, *Cycas* species contain three types of biflavonoids series 3'-8'' (compounds **1**-**15**), 4'-O-6'' (compounds **16**-**19**), and 4'-O-4''' (compound **20**). These biflavonoids nuclei are considered the distinguishing features of the family Cycadaceae (Figure 1, Table 1).

3. PHARMACOLOGICAL ACTIVITIES OF VARIOUS *CYCAS* SPECIES

Numerous *Cycas* species feature a variety of biological traits, including larvicidal, antiprotozoal antiviral, antibacterial, antioxidant, cytotoxic, and antispasmodic ones.

3.1. Antimicrobial activity

Cycas circinalis leaf and stem petroleum ether extract had shown effective anti-bacterial action against *Escherichia coli*, *Salmonella typhii*, *Klebsiella pneumonia*, and *Enterobacter aerogenes*. Additionally, the methanol extract of *C. circinalis* seeds showed antibacterial efficacy against *Bacillus cereus*, *Staphylococcus aureus*, and *Xanthomanas axonapodis pv. malvacearum* when compared to a control medication (Vancomycin) [16].

Comparing the hydro-alcoholic leaf extract of *C. revoluta* to the antibacterial medication chloramphenicol, *Cycas* extract showed significant antibiotic action against *K. pneumonia*, *E. coli*, and *Saccharomyces cerevisae* [17]. Comparing *C. revoluta* female cone chloroform extract to erythromycin and fluconazole conventional medications, it was found that the female cone's chloroform extract was more efficient against (MRSA) Methicillin-resistant *Staphylococcus aureus* strains, *Candida albicans*, *Aspergillus niger*, *Micrococcus luteus*, and *Salmonella abony* [18].

E. coli and *K. pneumonia* were found to respond better to the methanol extract of *C. revoluta* leaves due to the presence of a high level of dihydro bilobetin (**8**) [19], whereas the ethanol extract was more effective against *Pseudomonas aeruginosa* [20]. The high antibacterial activity of *C. revoluta* and *C. circinalis* can be attributed to their high content of 2,3 dihydro amentoflavone (**7**), 2,3,2'',3'' tetrahydro bilobetin (**14**) and 2,3,2'',3'' tetrahydro isoginkgetin (**15**). These compounds displayed moderate antibacterial activity against MRSA with IC_{50} of 11.5, 12.5, and 5.9 µM, respectively, and *S. aureus* with IC_{50} of 8.2, 9.6, and 3.8 µM, respectively [21]. Additionally, limited activity was shown by the ethanol extract of *C. revoluta* against two distinct strains of *Helicobacter pylori* [22]. *C. beddomei's* leaf and bark extracts had excellent antibacterial action against *S. aureus*, *Bacillus subtilis*, and *E. coli* when compared to the antibiotic gentamycin [23]. Additionally, moderate anticandidal activity was seen in the *C. beddomei* ethanol and ethyl acetate extracts [24]. Gram +ve *Scaphirhynchus albus* and Gram -ve *Shigella boydii* were significantly inhibited by *C. rumphii* ethyl acetate fraction [15].

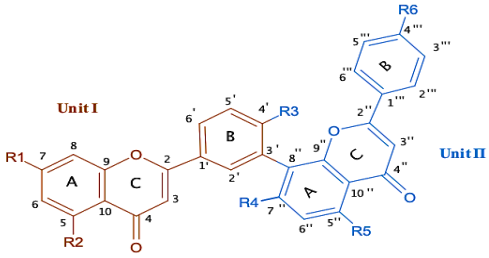
3.2. Antioxidant activity

Using in vitro 2,2-diphenyl-1-picryl-hydrazyl (DPPH), superoxide, and azino-bis (3 ethyl benzo-thiazoline-6-sulfonic acid (ABTS) scavenging assay techniques, *C. beddomei* male cone methanol extract shown a substantial antioxidant activity when compared to the standard ascorbic acid [25].

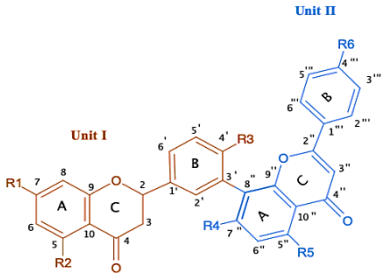
Using the DPPH test method with ascorbic acid as the standard, the chloroform, hydroalcoholic extracts of the female cone of the plant *C. revoluta* demonstrated greater antioxidant activity than the extract of the plant's leaves, with IC_{50} values of 12 µg/mL for the female cone and 15 µg/mL for the leaves. In addition, *C. revoluta* hydro alcoholic extract demonstrated significant antioxidant activity using the superoxide anion radicle scavenging assay technique [26]. This significant antioxidant power of *C. revoluta* is due to the presence of high levels of amentoflavone (**1**) and amentoflavone-4'-*O*-α-D-glucoside (**6**) which showed antioxidant activity nearly two to four folds higher than that of quercetin using DPPH assay method [27].

3.3. Cytotoxic activity

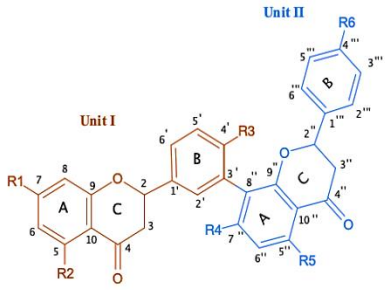
Negm *et al.* found that MCF-7 breast cancer cell and HePG-2 liver cancer cell lines using SRB (Sulforhodamine B) assay were highly sensitive to the ethyl acetate (EtOAc), *n*-butanol (*n*-But) fractions of *C. revoluta*, respectively [27]. The high cytotoxic potential of *C. revoluta* is due to their content of amentoflavone (**1**) and amentoflavone-4'-*O*-*α*-D-glucoside (**6**) which showed significant cytotoxic activity against MCF-7 breast cancer cell line with IC_{50} values of 18.70 and 6.12 µg/mL, respectively compared to doxorubicin as a standard (IC_{50}= 4.13 µg/mL) [27]. Another study demonstrated that *C. revoluta* methanol and EtOAc extracts exhibit large percentages of inhibition against the activity of the human aromatase enzyme, but only *C. rumphii* methanol extract displayed high activity against the same enzyme [28].



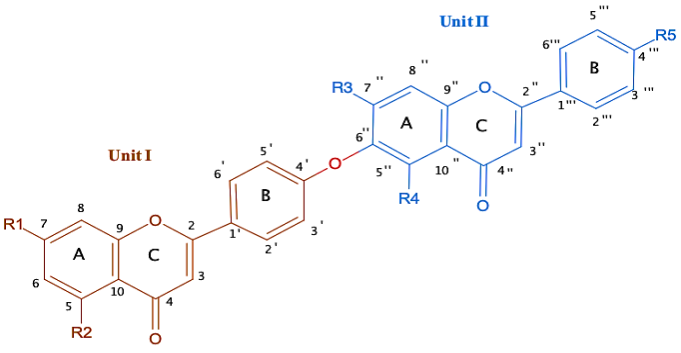
1- Amentoflavone: R1, R2, R3, R4, R5, R6= OH
2- Bilobetin: R1, R2, R4, R5, R6= OH, R3= OCH_3
3- Sotetsuflavone: R1, R2, R3, R5, R6= OH, R4= OCH_3
4- Podocarpusflavone-A: R1, R2, R3, R4, R5= OH, R6= OCH_3
5- Isoginkgetin: R1, R2, R4, R5= OH, R3, R6= OCH_3
6- Amentoflavone-4'-*O*-α-D-glucoside: R1, R2, R4, R5, R6= OH, R3= O-Glu



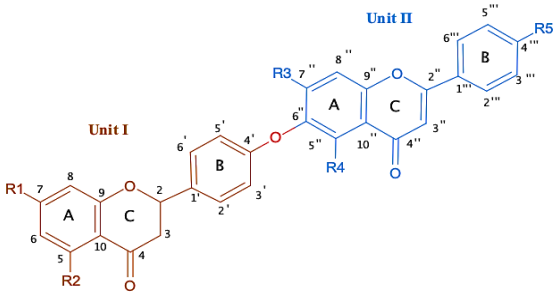
7- 2,3 Dihydro amentoflavone: R1, R2, R3, R4, R5, R6= OH
8- 2,3 Dihydro-4'-*O*-methyl amentoflavone: R1, R2, R4, R5, R6= OH, R3= OCH
9- 2,3 Dihydro-4'''-*O*-methyl amentoflavone: R1, R2, R3, R4, R5= OH, R6= OCH
10- 2,3 Dihydro-4',4'''-*O*-methyl amentoflavone: R1, R2, R4, R5= OH, R3, R6= OCH
11- 2,3 Dihydro-7-*O*-β-D-glucopyranosyl amentoflavone: R2, R3, R4, R5, R6= OH, R1= O-G
12- 2,3 Dihydro-7,7''-*O*-β-D-glucopyranosyl amentoflavone: R2, R3, R5, R6= OH, R1, R4= O-G



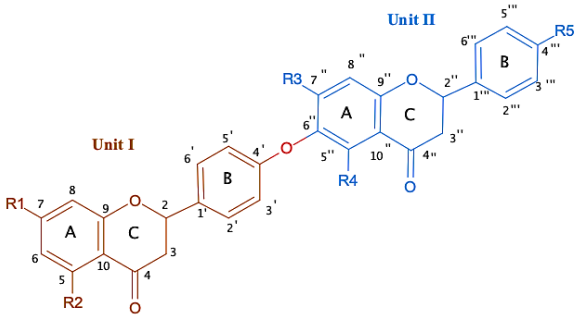
13- 2,3,2'',3''' Tetrahydro amentoflavone: R1, R2, R3, R4, R5, R6= OH
14- 2,3,2'',3''' Tetrahydro-4'-*O*-methyl amentoflavone: R1, R2, R4, R5, R6= OH, R3= OCH
15- 2,3,2'',3''' Tetrahydro-4',4'''-O-methyl amentoflavone: R1, R2, R4, R5= OH, R3, R6= OCH



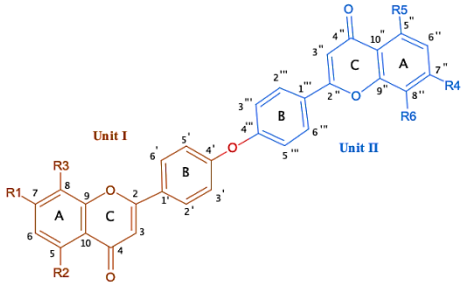
16- Hinokiflavone: R1, R2, R3, R4, R5= OH



17- 2,3 Dihydro hinokiflavone: R1, R2, R3, R4, R5= OH
18- 2,3 Dihydro isocryptomerine: R1, R2, R4, R5= OH, R3=OCH



19- 2,3,2'',3''' Tetrahydro hinokiflavone: R1, R2, R3, R4, R5= O



20- 4',4''' Biapigenin di-C-β-D-glucopyranosyide: R1, R2, R4, R5= OH, R3= C- Glu, R6= C- Glu [1''''''→3''''']glu

Figure 1: Different biflavonoids structures in the family Cycadaceae

Table 1: Distribution of different biflavonoid series in cycadaceae members

	Biflavonoid	Plant source and organ
3’ -8’ ’ Series	1- Amentoflavone	*Cycas armstrongii* leaflets [9] *C. kennedyana* leaflets [9] *C. siamensis* leaflets [9] *C. taiwaniana* leaflets [9] *C. rumphii*[10] *C. cairnsiana* leaflets [11] *C. circinalis* leaflets [11] *C. media* leaflets [11] *C. neocaladonica* leaflets [11] *C. pectinate* leaflets [11] *C. revoluta* leaflets [11] *C. thuarsii*[11] *C. beddomei* leaflets [12] *C. panzhihuaensis* leaflets [12]
	2- Bilobetin	*Cycas siamensis* leaflets [9] *C. circinalis* leaflets [12] *C. rumphii* leaflets [10]
	3- Sotetsuflavone	*Cycas cairnsiana* leaflets [11] *C. circinalis* leaflets [11] *C. media* leaflets [11] *C. neocaladonica* leaflets [11] *C. pectinate* leaflets [11] *C. revoluta* leaflets [11] *C. rumphii* leaflets [11] *C. siamensis* leaflets [11] *C. thuarsii* leaflets [11] *C. beddomei* leaflets [13]
	4- Podocarpusflavone A	*Cycas armstrongii* seed testa [14] *C. kennedyana* seed testa [14] *C. revoluta* leaflets [12] *C. panzhihuaensis* leaflets [12] *C. rumphii* leaflets [15]
	5- Isoginkgetin	*Cycas circinalis* leaflets [12] *C. armstrongii* leaflets [12] *C. rumphii* leaflets [15]
	6- Amentoflavone-4’-*O*-α-D-glucoside	*Cycas revoluta* leaflets [12]
	7- 2,3 Dihydro amentoflavone	*Cycas armstrongii* leaflets [12] *C. pectinate* fruits [12] *C. revoluta* leaflets [12] *C. circinalis* leaflets [12]
	8- 2,3 Dihydro-4’-*O*-methyl amentoflavone	*Cycas armstrongii* leaflets [12] *C. circinalis* leaflets [12] *C. revoluta* leaflets and female cones [12] *C. rumphii* leaflets [10]
	9- 2,3 Dihydro-4’’’-*O*-methyl amentoflavone	*Cycas beddomei* cones [13]
	10- 2,3 Dihydro-4’,4’’’-*O*-methyl amentoflavone	*Cycas circinalis* leaflets [12]
	11- 2,3 Dihydro-7-*O*-β-D-glucopyranosyl amentoflavone	*Cycas revoluta* leaflets [12]
	12- 2,3 Dihydro-7,7’’-*O*-β-D-glucopyranosyl amentoflavone	*Cycas revoluta* leaflets [12]
	13- 2,3,2’’,3’’Tetrahydro amentoflavone	*Cycas revoluta* leaflets [12] *C. beddomei* cones [13]
	14- 2,3,2’’,3’’Tetrahydro-4’-*O*-methyl amentoflavone	*Cycas revoluta* leaflets [12] *C. circinalis* leaflets [12]
	15- 2,3,2’’,3’’Tetrahydro-4’,4’’’-*O*-methyl amentoflavone	*Cycas circinalis* leaflets [12]
4’ -O-6’ ’ Series	16- Hinokiflavone	*Cycas cairnsiana* leaflets [11] *C. circinalis* leaflets [11] *C. thuarsii* leaflets [11] *C. siamensis* leaflets [11] *C. rumphii* leaflets [11] *C. revoluta* leaflets [12]
	17- 2,3 Dihydro hinokiflavone	*Cycas rumphii* leaflets [10] *C. cairnsiana* leaflets [11] *C. circinalis* leaflets [11] *C. media* leaflets [11] *C. neocaladonica* leaflets [11] *C. pectinate* leaflets [11] *C. revoluta* leaflets [11] *C. siamensis* leaflets [11] *C. thuarsii* leaflets [11] *C. armstrongii* leaflets [12] *C. panzhihuaensis* leaflets [12] *C. beddomei* cones [13]
	18- 2,3 Dihydro isocryptomerin	*Cycas revoluta* leaflets [12]
	19- 2,3,2’’,3’’ Tetrahydro hinokiflavone	*Cycas revoluta* leaflets [12] *C. beddomei* cones and stems [13]
4’ -O-4’ ’ Series	20- 4’,4’’’ Biapigenin di-C-β-D-glucopyranoside	*Cycas rumphii* leaflets [10]

Additionally, in our previous study of the cytotoxic capability of *C. rumphii* in different extracts, we found that strong cytotoxic activity is detected in the whole methanol extract of *C. rumphii* against HePG-2, HeLA, and HCT-116, with IC_{50} values of 10.09, 11.79, and 12.58 µg/mL, respectively compared to doxorubicin as a standard drug. Interestingly, doxorubicin, the positive control, had an IC_{50} of 7.79 µg/mL while the entire MeOH extract of *C. rumphii* had an IC_{50} of 53.72 µg/mL against the normal cell line (WISH) [10]. As a result, it is believed that *C. rumphii* MeOH extracts are less harmful to healthy cells and more effective against cancer cells. Also, *C. rumphii* different fractions were tested against these cell lines, the findings showed that the EtOAc fraction had the strongest cytotoxic activity when compared to the other *C. rumphii* examined fractions and doxorubicin. This fraction has IC_{50} values of 6.98, 7.94, and 8.70 µg/mL against the HePG-2, HeLA, and HCT-116 cell lines, respectively [10]. The isolated compounds from *C. rumphii* EtOAc fraction were assessed for their cytotoxicity against the same cell lines and the results indicated that novel compound 4’,4’’’ Biapigenin di-C-β-D-glucopyranoside (**20**) is the highest effective biflavonoid against the tested cell lines

with IC_{50} values of 21.47, 15.66 and 18.17 µg/mL, respectively [10]. Another study using cell viability assay, colony formation assay, ROS determination, flow cytometry, DAPI staining assay, and Tunel assay were used to assess the methanolic extract of *C. revoluta* cone for its anti-colon cancer properties. By causing apoptosis and lowering colon cancer cell (HCT-8) line proliferation, the extract demonstrated strong anti-colon cancer efficacy [12].

3.4. Aphrodisiac activity

Comparing the *C. circinalis* methanol extract to the sildenafil citrate-treated positive control group, the *C. circinalis* extract significantly increased sexual performance and overall sexual behavior in the animals [29].

3.5. Antispasmodic activity

An in vivo study indicated that the acetylcholine-induced colic and intestinal muscle spasms were inhibited by the aqueous, chloroform, and ethyl acetate extracts of *C. circinalis* leaves [30].

3.6. Antidiabetic activity

A research study investigated the antidiabetic ability of amentoflavone (**1**) and 2,3 dihydro amentoflavone (**7**) which were isolated from the EtOAc fraction of *C. pectinate* fruit against *α*-glucosidase and *α*-amylase. The results revealed that amentoflavone and 2,3 dihydro amentoflavone had a strong inhibitory effect against *α*-glucosidase with IC_{50} of 8.09 and 9.77 µM, respectively, and *α*-amylase with IC_{50} of 73.6 and 39.69 µM, respectively [31]. These findings were in line with the antidiabetic activity of the EtOAc subfraction of *C. pectinata* fruits from which these compounds were isolated against streptozotocin (STZ)-induced diabetic rats [31].

3.7. Antiviral activity

Cycas siamensis extract was found to have a moderate antiviral activity against *Sindbis* virus [32].

3.8. Larvicidal activity

The *C. circinalis* chloroform: methanol extract (1:2) demonstrated larvicidal activity against *Aedes aegypti* larvae while the *C. circinalis* hexane extract demonstrated larvicidal efficacy against *Culex quinquefasciatus* larvae. This implied a potential use of novel phytochemicals as environmentally benign natural insecticides [33].

3.9. Antiprotozoal activity

In a previous study, we investigated the toxoplasmocidal activity of *C. rumphii* MeOH extract and their different fractions against *Toxoplasma gondii* RH strain tachyzoites using trypan blue exclusion method and cotrimoxazole as the standard drug. The results indicated that *C. rumphii* MeOH extract exhibited a significant toxoplasmocidal effect with EC_{50} of 5.15 µg/mL while the standard drug, cotrimoxazole, showed an EC_{50} of 4.18 µg/mL. Additionally, the results of *C. rumphii* different fractions against *T. gondii* revealed that the EtOAc fraction was the most efficient of the studied fractions, with an EC_{50} of 3.51 µg/mL, which is lower than that of cotrimoxazole [10].

3.10. Anthelmintic activity

The effectiveness of *C. beddomei* aqueous, alcoholic, and methanolic extracts as anthelmintics against *Pheretima posthuma* was assessed. Results showed that male cone and leaf extracts were more active than bark extracts. However, pith and female cones exhibited no activity [34].

3.11. Analgesic activity

The acetic acid-induced writhing test was used to evaluate the analgesic efficacy of methanol and aqueous extracts of the male cone of *C. beddomei* using a dose range (250 to 1000 mg). Results revealed that the efficacy of *C. beddomei* methanol extract at a dose of 500 mg/kg b.wt was equal to that of diazepam at a 10 mg dosage [35].

3.12. Anti-arthritic activity

In comparison to the standard medication diclofenac, the methanol extract of *C. beddomei* male cone at dosages of 250, 500, and 1000 mg/kg b.wt, and the aqueous extract at 1000 mg/kg b.wt showed more substantial anti-arthritic action than standard drug [35].

3.13. Anti-inflammatory activity

A protein denaturation experiment was used to assess the anti-inflammatory properties of the methanol extract of *C. pectinata* leaves. In contrast to diclofenac sodium, which inhibited protein denaturation by 83.50% however, 500 µg/mL of methanol extract showed a 38.12% maximal inhibition [36].

3.14. Thrombolytic activity

Different doses of the *C. pectinata* leaf methanol extract demonstrated a moderate ability to dissolve clots. The leaf extract at 10 mg/mL concentration demonstrated 35.72% clot lysis activity compared to the positive control streptokinase which showed 74.52% clot lysis activity [36].

3.15. Neuropharmacological defects

Both the anxiolytic and locomotor effects of the methanol extract of *C. pectinate* leaves have been studied. The findings showed that the methanol extract of leaves significantly reduced locomotor activity with an effective anxiolytic effect in a dose-dependent manner when compared to diazepam as a reference treatment [36].

4. CONCLUSION

This review outlined the distribution of various biflavonoids series among members of the Cycadaceae family and highlighted the diverse pharmacological properties of various *Cycas* species that can be related to their biflavonoids content. There are many other unstudied species that may yield potential phytochemicals and other advantageous pharmacological actions, as all the biflavonoids reported in this research were isolated from a small number of *Cycas* species.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

FINANCIAL DISCLOSURE STATEMENT

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