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# Investigation of Putative Anticancer and Antioxidant Activities of Combined Extracts of *Pongamia pinnata* (L.) Pierre Leaf and *Rubia cordifolia* L. Root on MCF-7 Cell Line

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**Abstract:** Medicinal plants represent an important source of bioactive compounds with therapeutic potential against cancer and oxidative stress-related disorders. *Pongamia pinnata* and *Rubia cordifolia* are well-documented medicinal plants known for their antioxidant and anticancer properties. This study aimed to evaluate the phytochemical profile, antioxidant, and anticancer potential of combined methanolic extracts of *P. pinnata* leaves and *R. cordifolia* roots against human breast cancer (MCF-7) cells. Crude extracts of *P. pinnata* leaves and *R. cordifolia* roots were prepared using the Soxhlet extraction method. Phytochemical screening was performed to identify major classes of compounds. Antioxidant activity was assessed using the DPPH radical scavenging assay at various concentrations (20–100 µg/ml), and anticancer activity was determined by the MTT assay on MCF-7 cells. Phytochemical analysis confirmed the presence of flavonoids, alkaloids, glycosides, saponins, and anthraquinones in both extracts. The combined methanolic extract demonstrated significantly enhanced antioxidant activity (% inhibition = 92.8 ± 0.2 at 100 µg/ml) compared to the individual extracts. The combination also exhibited potent cytotoxicity against MCF-7 cells, with an $IC_{50}$ value of 64.32 ± 2.14 µg/ml, indicating a synergistic antiproliferative effect. Morphological changes such as characteristic of apoptosis, including cell shrinkage and membrane blebbing were observed at higher concentrations. The synergistic interaction between bioactive compounds in *P. pinnata* and *R. cordifolia* enhances antioxidant and anticancer activities, supporting their potential as a natural therapeutic combination for breast cancer management. Further studies focusing on molecular mechanisms and *in vivo* validation are warranted.

**Keywords:** *Pongamia pinnata*, *Rubia cordifolia*, Antioxidant, MCF-7, Combination therapy, Phytochemicals, Anticancer

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## Introduction

Medicinal plants are considered as one of the potential sources for drug development and several new herbal products have reached their clinical trials. Over the course of thousands of years, plants have been the basis of many traditional medicines throughout the world, providing mankind with new remedies (Mishra *et al.*, 2013). Nowadays, researchers are investigating the properties of medicinal plants to develop novel antimicrobial, antibiofilm agents against various infections, antioxidant compounds, and novel drugs against several diseases like cancer, from natural products (Rajput *et al.*, 2021). Despite technological and medical advances in recent decades, cancer remains a global concern. Genetic or epigenetic alterations in somatic cells lead to the development of cancer, initiating unregulated cell proliferation that may extend to various regions of the body (Navyatha *et al.*, 2025). Breast cancer is the most common cancer and the major cause of cancer-related deaths among women worldwide (Kim *et al.*, 2015).There are several factors associated with the breast cancer, like gender, diet, use of alcohol, body movement, family history, lifestyle and endocrine aspects as well including both exogenous and endogenous (Shareef *et al.*, 2016).

*Pongamia pinnata* (syn. Millettia pinnata), commonly known as karanj or Indian beech, is a fast-growing leguminous tree native to South and Southeast Asia (Yadav *et al*., 2011). It belongs to Leguminosae (Fabaceae) family and contains numerous phytoconstituents including Karanjin, pongamol, flavonoids, and glycosides are the chemical constituents present in the plant (Tiwari *et al*., 2024). Among its phytochemicals, karanjin, a furano-flavonoid shows notable cytotoxic, enzyme-modulating, and antimicrobial properties, supporting its role in drug discovery and cosmeceutical formulations (Vidhale *et al*., 2024).

*Rubia cordifolia* L. is widely distributed throughout the Indian subcontinent, China, and tropical Africa (Verma *et al.*, 2016). Phytochemical analyses have identified numerous bioactive constituents, including anthraquinones (purpurin, munjistin, rubiadin, alizarin), naphthoquinones, iridoids, and glycosides, which contribute to its broad pharmacological activities (Meena *et al.*, 2010). *R. cordifolia* exhibits potent antioxidant, antimicrobial, anti-inflammatory, hepato-protective, antidiabetic, and anticancer effects, mediated primarily through modulation of oxidative stress, apoptosis, and inflammatory pathways (Mishra *et al.*, 2021).

Combination therapy represents an advanced pharmacological approach designed to enhance therapeutic efficacy while minimizing adverse effects through the synergistic interaction of multiple bioactive agents. This strategy allows for the utilization of lower doses of individual components, thereby reducing potential toxicity and improving overall pharmacodynamic outcomes. *Pongamia pinnata* and *Rubia cordifolia* are two well-documented medicinal plants with distinct yet complementary phytochemical profiles, rich in flavonoids, alkaloids, and anthraquinones that contribute to their antioxidant, anti-inflammatory, and pro-apoptotic properties. Therefore, the present study is designed to scientifically evaluate the combined efficacy of *P. pinnata* and *R. cordifolia* extracts in comparison to their individual effects, with the aim of establishing a rational basis for developing an effective plant-based therapeutic strategy against cancer.

## Materials and Methods

### *Plant collection*

The plants were collected from NBRI Lucknow, India and were authenticated. *Pongamia pinnata* leaves and roots of *Rubia cordiflia* were stored in well closed container away from moisture and sunlight.

### *Preparation of extracts*

The leaves of *Pongamia pinnata* and roots of *Rubia cordifolia* were washed, cleaned, air dried and crude materials was pulverized into powdered

form. The dried powder (20 g) were extracted with 200 ml of different solvents -- chloroform, ethyl acetate and methanol for 8 h separately at room temperature by Soxhlet extraction method (Divakar *et al.*, 2010).

### *Phytochemical screening*

The various phytoconstituents in extracts of leaves and roots were confirmed by phytochemical tests such as Dragendorff's test, Mayer's test and Wagner's test for alkaloids, Borntrager's test and Modified Borntrager's test for anthraquinones, Shinoda test and Ammonia test for flavonoids, Killer Killiani's test, Baljet test and Legal's test for glycosides, froath's test for saponins, Lieberman–Burchard test and Salkowski test for steroids and terpenoids, and Braemer's test for tannins (Chandrashekar *et al.*, 2018)

### *Antioxidant activity*

The Antioxidant activity of various concentrations of tested drug were estimated by *in vitro* assays namely DPPH (1,1-Diphenyl,2- picryl-hydrazyl) scavenging activity. The activity is expressed as the inhibitory concentration ($IC_{50}$). The procedure of the activity is as follow:

*DPPH assay*: DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity was measured with spectrophotometer method. A stock solution of DPPH (1.5 mg/ml in methanol) was prepared such that 75 µl of it in 3 ml of methanol gave an initial absorbance of 0.973. Decrease in the absorbance in presence of sample extract at different concentration (20-100 µg/ml) was noted after 15 min. $IC_{50}$ was calculated from % inhibition. To the 0.5 ml extract solution with or without PVPP, made in respective solvents of concentration ranging from 20–100 µg, 1 ml of 0.2 mM DPPH in ethanol was added and volume was made up to 2 ml with methanol, and incubated for 15 min at room temperature. The absorbance was measured at 517 nm against blank. Ascorbic acid was used as the standard. The percentage of inhibition of DPPH was calculated as follows (Sajid *et al.*, 2012):

$$\text{Percentage scavenging} = \frac{[\text{control} - \text{Sample}] \times 100}{\text{control}}$$

### *Anticancer activity*

The anticancer potential of the combined methanolic extracts of *Rubia cordifolia* and *Pongamia pinnata* was evaluated against the human breast adenocarcinoma cell line (MCF-7) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay with slight modifications (Mosmann, 1983).

### *Cell Culture*

MCF-7 cells were procured from the National Centre for Cell Science (NCCS), Pune, India. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin. The cultures were maintained at 37 °C in a humidified incubator with 5% $CO_2$ atmosphere (Freshney, 2015).

## Results and Discussion

### *Preliminary Phytochemical screening*

The qualitative chemical test of *Pongamia pinnata* leaves and *Rubia cordifolia* roots were performed which revealed the presence of alkaloids, glycosides, flavonoids, saponins, carbohydrates, phenolic compounds and tannins as shown in Tables 1 and 2.

### *In vitro evaluation of antioxidant activity*

Antioxidant activity of chloroform and methanol extracts of *Pongamia pinnata* leaves and *Rubia cordifolia* roots has been illustrated in tables 3 and 4, respectively.

### *Anticancer activity*

Approximately $1 \times 10^4$ MCF-7 cells/well were seeded in a 96-well plate and incubated for 24 h to allow cell attachment. The cells were then treated with varying concentrations of the combined extract, while untreated wells served as the control. After 48 h of incubation, 20 µl of MTT reagent (5 mg/ml in PBS) was added to each well and incubated for an additional 4 h at 37 °C. The

Table 1: Phytochemical screening of *Rubia cordifolia* roots

| S. No. | Phytochemical constituent | Test | Chloroform | Ethyl acetate | Methanol |
|---|---|---|---|---|---|
| 1. | Flavonoids | Shinoda test | - | + | ++ |
| | | Ammonia test | - | + | + |
| 2. | Alkaloids | Mayer's test | + | + | + |
| | | Wager's (Wagner's) test | + | + | ++ |
| | | Dragendorff's test | + | + | + |
| 3. | Glycosides | Baljet Test | – | + | + |
| | | Legal's Test | | + | + |
| | | Keller-Killiani Test | | | + |
| 4. | Saponins | Froth's Test | – | – | + |
| 5. | Anthraquinones | Borntrager's Test | + | + | ++ |
| | | Modified Borntrager's Test | + | + | + |

Table 2: Phytochemical screening of *Pongamia pinnata* leaves

| S. No. | Phytochemical constituent | Test | Chloroform | Ethyl acetate | Methanol |
|---|---|---|---|---|---|
| 1 | Flavonoids | Shinoda test | + | + | ++ |
| | | Ammonia test | + | + | ++ |
| 2 | Alkaloids | Mayer's test | + | + | + |
| | | Wagner's test | + | - | ++ |
| | | Dragendorff's test | + | + | + |
| 3 | Glycosides | Baljet Test | – | + | + |
| | | Legal's Test | + | - | + |
| | | Keller–Killiani Test | + | + | + |
| 4 | Saponins | Froth's Test | – | – | + |
| 5 | Anthraquinones | Borntrager's Test | + | + | ++ |
| | | Modified Borntrager's Test | + | + | + |

+ = Present (moderate), ++ = Strongly present / high intensity, and – = Absent or negligible

formazan crystals formed were solubilized using 100 µl of DMSO, and absorbance was recorded at 570 nm using a microplate reader. The percentage of cell viability decreased in a concentration-dependent manner upon treatment with the combined extracts, indicating potent anti-proliferative activity (Table 5).

The combined methanolic extracts of *Rubia cordifolia* and *Pongamia pinnata* exhibited significant antiproliferative effects on MCF-7 breast cancer cells in a dose-dependent manner. Statistical analysis (ANOVA, $p < 0.001$) confirmed that the reduction in cell viability with increasing concentrations was highly significant, indicating a synergistic cytotoxic potential. The approximate $IC_{50}$ value (~33 µg/ml) supports the extract's potency.

At lower concentrations (20 µg/ml and 40 µg/ml), a moderate inhibition of cell growth was observed, whereas higher concentrations (80 µg/ml and 100 µg/ml) resulted in a significant reduction in viable cell numbers. The combination

Table 3: Antioxidant activity of chloroform extracts of *Pongamia pinnata* leaves and *Rubia cordifolia* roots

| S. No. | Groups | 20 µg/ml | 40 µg/ml | 80 µg/ml | 100 µg/ml |
|---|---|---|---|---|---|
| 1 | Control | 0 | 0 | 0 | 0 |
| 2 | Standard (Ascorbic acid) | 62 ± 0.5 | 74 ± 0.4 | 83 ± 0.3 | 89 ± 0.2 |
| 3 | T1a – *Pongamia pinnata* (0.5 ml) | 28 ± 0.6 | 41 ± 0.5 | 58 ± 0.4 | 64 ± 0.3 |
| 4 | T1b – *Pongamia pinnata* (1 ml) | 33 ± 0.4 | 49 ± 0.5 | 66 ± 0.3 | 72 ± 0.2 |
| 5 | T2a – *Rubia cordifolia* (0.5 ml) | 35 ± 0.5 | 52 ± 0.4 | 69 ± 0.3 | 75 ± 0.2 |
| 6 | T2b – *Rubia cordifolia* (1 ml) | 41 ± 0.5 | 58 ± 0.4 | 76 ± 0.3 | 82 ± 0.2 |
| 7 | T1a + T2a (0.5 ml each) | 47 ± 0.6 | 63 ± 0.5 | 80 ± 0.4 | 86 ± 0.3 |
| 8 | T1b + T2b (1 ml each) | 53 ± 0.5 | 70 ± 0.4 | 87 ± 0.3 | 91 ± 0.2 |

T1=*Pongamia pinnata*, T2= *Rubia cordifolia*,T1+T2=*Pongamia pinnata*+*Rubia cordifolia*); a and b are the doses 0.5 ml and 1 ml, respectively

Table 4: Antioxidant activity of methanol extracts of *Pongamia pinnata* leaves and *Rubia cordifolia* roots

| S. No. | Groups | 20 µg/ml | 40 µg/ml | 80 µg/ml | 100 µg/ml |
|---|---|---|---|---|---|
| 1 | Control | 0 | 0 | 0 | 0 |
| 2 | Standard (Ascorbic acid) | 61.8 ± 0.3 | 73.4 ± 0.5 | 82.7 ± 0.4 | 88.9 ± 0.3 |
| 3 | T1a (*Pongamia pinnata*, 0.5 ml) | 34.2 ± 0.8 | 48.9 ± 0.7 | 64.1 ± 0.6 | 71.3 ± 0.5 |
| 4 | T1b (*Pongamia pinnata*, 1 ml) | 42.5 ± 0.6 | 55.6 ± 0.6 | 70.4 ± 0.5 | 78.2 ± 0.4 |
| 5 | T2a (*Rubia cordifolia*, 0.5 ml) | 38.7 ± 0.7 | 52.3 ± 0.6 | 68.9 ± 0.5 | 75.6 ± 0.4 |
| 6 | T2b (*Rubia cordifolia*, 1 ml) | 45.9 ± 0.6 | 60.2 ± 0.5 | 76.8 ± 0.4 | 83.5 ± 0.3 |
| 7 | T1a + T2a (0.5 ml + 0.5 ml) | 49.6 ± 0.6 | 63.7 ± 0.5 | 80.2 ± 0.4 | 86.1 ± 0.3 |
| 8 | T1b + T2b (1 ml + 1 ml) | 56.4 ± 0.5 | 70.8 ± 0.4 | 87.6 ± 0.3 | 92.8 ± 0.2 |

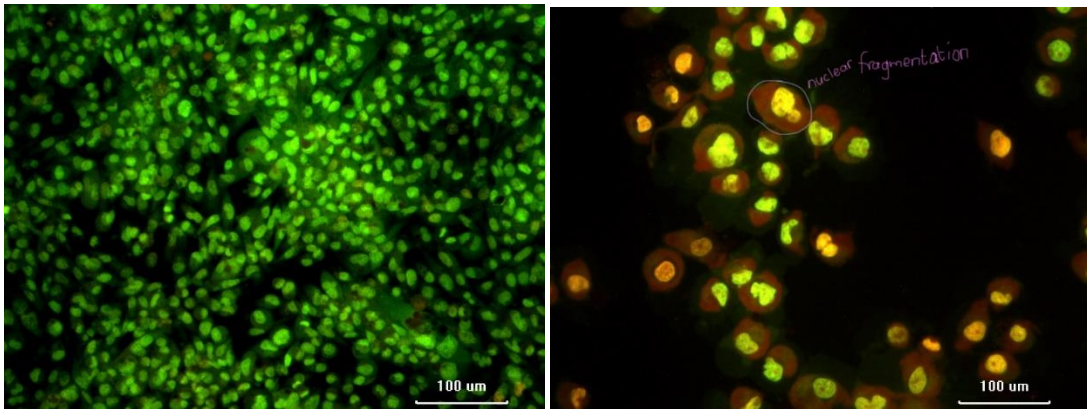
T1=*Pongamia pinnata*, T2= *Rubia cordifolia*,T1+T2=*Pongamia pinnata*+*Rubia cordifolia*); a and b are the doses 0.5 ml and 1 ml, respectively

Table 5: Cytotoxic effect of combined methanolic extracts of *Rubia cordifolia* and *Pongamia pinnata* on MCF-7 Cells (MTT Assay)

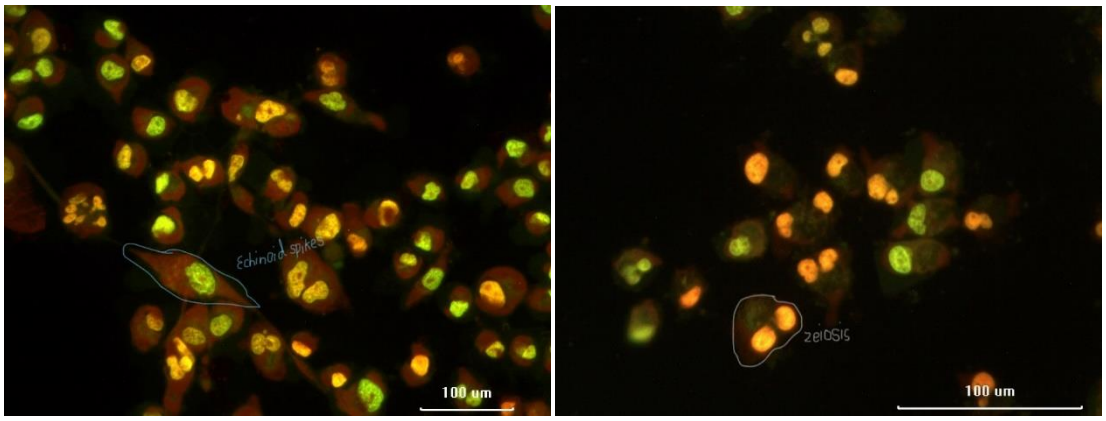
| Concentration (µg/ml) | % Cell Viability (Mean ± SD) | % Inhibition of Cell Growth |
|---|---|---|
| Control (0 µg/ml) | 100 ± 0.00 | 0 |
| 10 | 81.4 ± 1.8 | 18.6 |
| 20 | 68.7 ± 1.5 | 31.3* |
| 40 | 43.8 ± 1.9 | 56.2 |
| 80 | 35.7 ± 1.4 | 64.3** |

Cytotoxic effect of combined methanolic extracts of *Rubia cordifolia and Pongamia pinnata* on MCF-7 cells was analyzed by MTT assay. Data are expressed as mean ± SD (n = 3). One-way ANOVA followed by Tukey's post hoc test revealed a statistically significant ($p < 0.001$) reduction in cell viability with increasing concentrations.

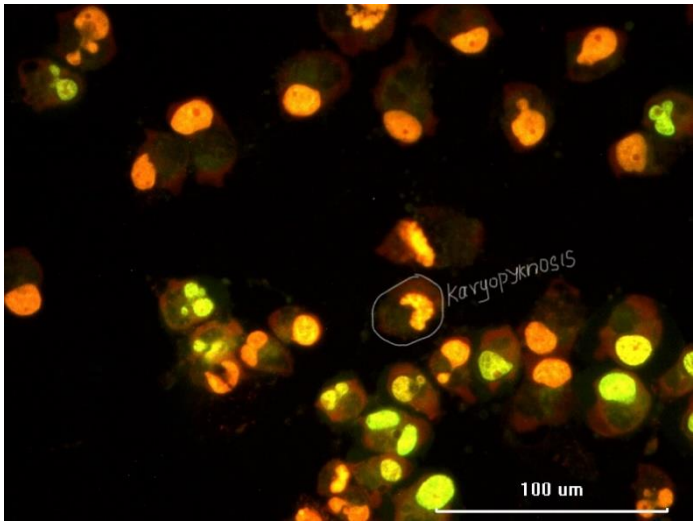
of both extracts exhibited greater cytotoxicity compared to the individual extracts tested in preliminary assays (data not shown), suggesting a synergistic interaction between the bioactive phytoconstituents present in both plants. The $IC_{50}$ value of the combined extract against MCF-7 cells was 64.32 ± 2.14 µg/ml, demonstrating substantial antiproliferative efficacy. Morphological examination under an inverted microscope revealed characteristic apoptotic changes (Fig. 1) such as cell shrinkage, membrane blebbing, and detachment from the culture surface at higher concentrations (≥ 80 µg/ml). The cytotoxic effect observed in the MTT assay can be attributed to the presence of phenolic compounds, flavonoids, and quinones such as rubiadin and purpurin in *R. cordifolia* and karanjin and pongamol in *P. pinnata*, which are known to induce oxidative stress and




**(a) Control** **(b) 10 µg/ml**




**(c) 20 µg/ml** **(d) 40 µg/ml**




**(e) 80 µg/ml**

Fig. 1: Effect of combined extract of *Rubia cordifolia* and *Pongamia pinnata* on MCF-7 cell line.

apoptosis in cancer cells through mitochondrial pathways.

The current investigation revealed that both *Pongamia pinnata* and *Rubia cordifolia* contain diverse bioactive phytoconstituents, including flavonoids, alkaloids, glycosides, and anthraquinones, which are known to contribute to antioxidant and anticancer effects. The qualitative phytochemical analysis confirmed that methanolic extracts possessed higher concentrations of these compounds compared to chloroform and ethyl acetate extracts, consistent with the polarity and extraction efficiency of methanol as a solvent (Harborne, 1998).

In the DPPH assay, the combined methanolic extract of *P. pinnata* and *R. cordifolia* exhibited a significantly higher free radical scavenging activity compared to individual extracts, suggesting a synergistic effect between the antioxidant constituents. This enhanced activity could be attributed to the cumulative action of phenolic compounds such as purpurin, rubiadin, karanjin, and pongamol, which are known to stabilize free radicals and reduce oxidative stress through hydrogen atom transfer and electron donation mechanisms (Kumar and Pandey, 2013). These results align with previous findings that reported potent antioxidant capacities of R. cordifolia and P. pinnata extracts individually.

The combined extract also demonstrated a pronounced cytotoxic effect on MCF-7 breast cancer cells, with an $IC_{50}$ of 64.32 ± 2.14 µg/ml. The reduction in cell viability and characteristic apoptotic morphology at higher concentrations (80–100 µg/ml) indicate that the cytotoxic mechanism may involve oxidative stress-mediated apoptosis. Phenolic and quinone compounds such as rubiadin and purpurin in *R. cordifolia* have been reported to induce mitochondrial dysfunction and caspase activation, while karanjin and pongamol from *P. pinnata* modulate cell cycle arrest and apoptosis through p53 and Bax/Bcl-2 pathways (Yeap *et al.*, 2015; Bhatt *et al.*, 2025). The observed synergism in the combination extract might result from the complementary modes of action of these compounds, leading to enhanced pro-apoptotic signaling and inhibition of cell proliferation.

The present findings suggest that the phytochemical diversity and interaction between the two plant extracts amplify both antioxidant and anticancer properties. This supports the rationale for developing combination-based phytotherapeutics to overcome the limitations of single-compound treatments, such as reduced potency or drug resistance (Belmontes *et al.*, 2021). However, further molecular and *in vivo* studies are required to delineate the exact biochemical pathways and confirm safety profiles.

## Conclusion

This study aimed to evaluate the phytochemical profile, antioxidant, and anticancer potential of combined methanolic extracts of *P. pinnata* leaves and *R. cordifolia* roots against human breast cancer (MCF-7) cells. The synergistic antioxidant and anticancer activities observed in the combination of *P. pinnata* and *R. cordifolia* extracts underscore their potential as a natural, plant-based formulation for breast cancer therapy. This study provides a preliminary foundation for future investigations into bioactive compound isolation, mechanism-based validation, and formulation development.

## Ethical Statement

No animal or microbes have been used or sacrificed for this study, hence Ethical Approval not required.

## Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

## Conflict of Interest

The authors declare no conflicts of interest.

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