

## Phytochemical screening and GC- MS analysis of *Musa ornata* pseudo stem

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### Abstract

The present investigation focuses on the phytochemistry of the *Musa ornata* pseudo-stem using advanced analytical techniques. The ethanolic extract obtained from Soxhlet extraction at 50°C for 48 h, is subjected to the preliminary phytochemical screening which revealed the presence of carbohydrates, proteins, steroids, saponins, flavonoids, and tannins. Thin Layer Chromatography (TLC) performed using four different solvent systems: ethyl acetate (1:9 v/v), ethyl acetate (2:8 v/v), toluene acetate acid (4.5:3.5:0.2 v/v/v), and ethyl acetate acid (100:11:11:27 v/v/v/v). This clearly pictured varying degrees of compound separation, with the best resolution achieved using the most complex solvent system (Plate D). Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted using an AOC-20i+S auto-injector and GC-2010 system. GC-MS analysis identified a diverse range of compounds including (1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene, 1,2,4-Trimethylbenzene, 2-(2-Ethylhexyloxy) ethanol, Nonanal, Dodec-1-ene, Decanal, and 2,6-Di-tert-butylphenol. These compounds are of significant interest in botany and phytochemistry due to their potential bioactivity and role in the plant's physiological processes. Notable plasticizers such as Dibutyl phthalate and Bis(2-ethylhexyl) phthalate were also detected. The presence of phenolic and quinone compounds suggests potential antioxidant properties, supporting the traditional medicinal use of *Musa ornata* and indicating its therapeutic potential. However, further studies are required to explore the biological activities of these compounds, underlining the importance of this research.

**Keywords:** *Musa ornata*, phytochemical analysis, thin layer chromatography, GC-MS

### Introduction

*Musa ornata*, globally known as the flowering banana, is one among 50 species of the genus *Musa* belongs to Musaceae family. *Musa ornata* is native to Southeast Asia, commonly found in Bangladesh, Burma, and India. (Royal Horticultural Society, 2008) [13]. True *Musa ornata* is native to India, but has become naturalized in Central and South America since the early 19th century. (Plummer J; Allen R 2020) [11]. *Musa ornata* possess green, slender pseudo stems 3 meters tall, early leaves are green with waxy coating and black notch at top. The medium dark green leaves with reddish midribs 2 meters long, 0.35 meters wide, petioles with 0.6 meters in length.

The plant produces suckers close to the mother plant and has an erect inflorescence with pink bracts. Compactly bunched fruits, up to 10 cm long. Young fruits with purple-pink colour and ripen ones turn to greenish-yellow, they possess limited number of viable black seeds. (Drupal, M., de Carvalho, E. B., Rouard, M., et al. 2019) [4]. *Musa ornata*, earned the Royal Horticultural Society's Award of Garden Merit for its ornamental value. It is widely cultivated for its ornamental foliage in mixed tropical plantations (Pothavorn, P., Kitdamrongsont, K., Swangpol, S., Wongniam, S., Atawongsa, K., Savasti, J., & Somana, J. 2010) [12]. Ideal as a focal plant or for mass planting, *Musa ornata* also grows well in containers. Propagation is best achieved through seeds, suckers, or division (Sulistyaningsih, L. D. 2016) [18]. Besides its ornamental use, *Musa ornata* has diverse practical applications. The male buds are used in salads, and leaves are used to make dressings. In northeastern India, the plant root is used in Ayurvedic medicine. The ash from the pseudo stem, corm, fruiting stalk, and fruit peel serves as an anti-scorbutic and aids digestion or acts as a tonic (Molina-Salinas, G. M., Uc-Cachón, A. H., Peña-Rodríguez, L. M.,

Dzul-Beh, A. D. J., & Graça-Medrano, R. M. E. 2019) [9]. Iron nanoparticles obtained from *Musa ornata* flower sheath extract under optimal conditions. The nanoparticles exhibit potent antibacterial activity against *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli*, and *Salmonella enterica* (Saranya, S., Vijayarani, K., & Pavithra, S. 2017) [15].

The current study aimed to elucidate the phytochemical profile of the ethanolic extract of *Musa ornata* pseudo-stem and evaluate its potential bioactive compounds using advanced analytical techniques. This investigation sought to validate traditional medicinal claims and explore the therapeutic potential of plant constituents.

### Materials and methods

#### Plant material

*Musa ornata* was collected from the Forest areas of Khammam, Telangana, in April 2024 and authenticated by Dr. Vijaya Bhaskar Reddy, Department of Botany, Osmania University.



**Fig 1:** *Musa ornata*

### Extraction of plant material

The pseudo stem of *Musa ornata* was collected, washed, and dried in the shade. Phytochemicals from the dried powder were extracted via Soxhlet extraction using ethanol at 50 °C for 48 h. Soxhlet extraction is a widely used method in phytochemical studies to efficiently extract compounds from plant materials. The crude extract was collected and filtered, and the solvent was evaporated using a rotary evaporator at 50 °C.

### Parentage yield

The percentage of yield was calculated using the following formula: -

$$\text{Yield (g/100 g)} = (W_1 \times 100)/W_2$$

$W_1$  = weight of the crude extract residue obtained after solvent removal

$W_2$  = weight of pseudo stem powder of *Musa ornata* packed in the Soxhlet (Dokuparthi S. K., & Reddy, T. R. M. 2021) [2, 3]

### Preliminary phytochemical analysis

The ethanolic extract of the pseudo stem of *Musa ornata* was subjected to a preliminary phytochemical analysis. This analysis is a crucial step in phytochemical studies as it helps in the identification of primary and secondary metabolites, providing a foundation for further investigation into the plant's bioactive compounds.

### Thin layer chromatography

Thin Layer Chromatography (TLC) was utilized to analyze the ethanolic extract of the pseudo stem of *Musa ornata*. A TLC plate coated with silica gel was used as the stationary phase. A faint pencil line was drawn approximately one centimeter from the bottom edge of the plate, marking the origin line where the samples were applied. Small amounts of the plant extract solution were carefully spotted onto the origin line using a capillary tube or a micropipette. The sample spots were applied evenly and spaced apart to prevent overlapping during development (Kowalska, T., & Sajewicz M 2022) [7].

TLC analysis of the *Musa ornata* pseudo-stem extract was conducted using four different solvent systems to determine the most effective separation of the compounds. Plate A utilized ethyl acetate and n-hexane solvent system at a 1:9 v/v ratio. Plate B employed a slightly different ratio of ethyl acetate to n-hexane, specifically 2:8 (v/v). For Plate C, a mixture of toluene, ethyl acetate, and formic acid was used in the proportions of 4.5:3.5:0.2 v/v/v. Finally, Plate D used a more complex solvent system of ethyl acetate, formic acid, acetic acid, and water in a 100:11:11:27 v/v/v/v ratio. Each solvent system was chosen to optimize the separation of compounds within the plant extract. The developing chamber was prepared by adding 2 ml of mobile phase. The TLC plate was placed in the chamber at a solvent level below the origin line, and the chamber was covered to prevent solvent evaporation. The solvent was allowed to ascend the plate by capillary action, carrying the sample components. Once the solvent front approached the top of the plate, the plate was removed from the chamber, and the solvent front was marked (Payne, T. D., Dixon, L. R., Schmidt, F. C., Blakeslee, J. J., Bennett, A. E., & Schultz, Z. D. 2024) [10].

The TLC plate was then allowed to air-dry. The separated compounds were visualized using UV light and spraying the plate with a detecting reagent such as Liebermann (Burchard). Different plant extract components appeared as spots on the TLC plate at varying distances from the origin. The distance traveled by each spot and solvent front was measured to calculate the R<sub>f</sub> values, representing the ratio of the distance traveled by the compound to the distance traveled by the solvent front. These R<sub>f</sub> values were used to identify the compounds by comparing them with the R<sub>f</sub> values of known standards or references (Singh, B., Kavita, Aarti, Yadav, N., Chauhan, J., Jena, N., & Kumar, S. 2024) [17].

### Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was conducted using an AOC-20i+S auto-injector and GC-2010 system. The auto-injector parameters were set to ensure high efficiency and precision: two rinses with pre-solvent, no rinses with solvent or sample post-injection, and a high plunger speed for suction and injection. The viscosity compensation time was 0.2 seconds with normal injection mode and a plunger speed of three times. The washing volume was maintained at 8 µL, with the syringe suction position at 0.0 mm and the washing injection position at -3.0 mm, using only solvent A (Chikowe, I., Bwaila, K. D., Ugbaja, S. C., *et al.* 2024) [1].

For the GC-2010 system, the column oven temperature program started at 50°C, with an initial hold time of 2 min, followed by temperature ramps: 50°C to 180°C at a rate of 5.00°C/min with a 2-minute hold, 180°C to 250°C at 5.00°C/min with a 2-minute hold, and 250°C to 260°C at 5.00°C/min with a final hold time of 2 min. The injection port temperature was set to 250°C in splitless mode, with a sampling time of 1.00 minutes. The linear velocity mode was employed for flow control at 36.5 cm/sec, with a total flow of 54.4 mL/min and column flow of 1.01 mL/min. The high-pressure injection was turned off, and the carrier gas saver was also off (Konappa, N., Udayashankar, A. C., Krishnamurthy, S., *et al.* 2020) [6].

MS analysis was performed using a GCMS-TQ8050 system with an ion source temperature of 200.0°C and an interface temperature of 270.0°C. The solvent cut time was set to 6.00 minutes, and the detector gain mode was relative to the tuning result, with a detector gain of 1.15 kV ± 0.20 kV and a threshold of 500. The MS table parameters included a start time of 6.00 minutes, an end time of 50.00 minutes, an acquisition mode of Q3 scan, and a scan speed of 5000 with a start m/z of 45.00 and an end m/z of 500.00. The sample inlet unit was configured for the GC. The relative percentage of each component was calculated by comparing the average peak area to the total area. The supplier provided the MS solution software to control the system and acquire data (Shettar, P. S., & Hiremath, M. B. 2024) [16].

### Identification of compounds

The compounds in the GC-MS analysis were identified by matching the retention times and mass spectral library searches. The sample was injected into the GC-MS system, vaporized, and separated using a capillary column. Each compound was eluted at a specific retention time, providing an initial indication for identification. As the compounds entered the mass spectrometer, they were ionized, and the resulting ions were detected to generate the mass spectra.

These spectra were compared with known spectra in the NIST library, generating a list of potential matches ranked by similarity. The identity of each compound was confirmed by comparing the retention times and mass spectra with NIST library spectra, ensuring accurate and reliable identification that integrated the chromatographic peaks and matched the spectra against the NIST library, providing a list of detected compounds with their corresponding similarity scores (Maity, S., Padhy, G. K., Kanthal, L. K., & Pattanayak, S. 2024) [8].

## Results & discussion

### Percentage yield

The percentage yield was calculated using the above formula as 3.9%.

### Phytochemical screening

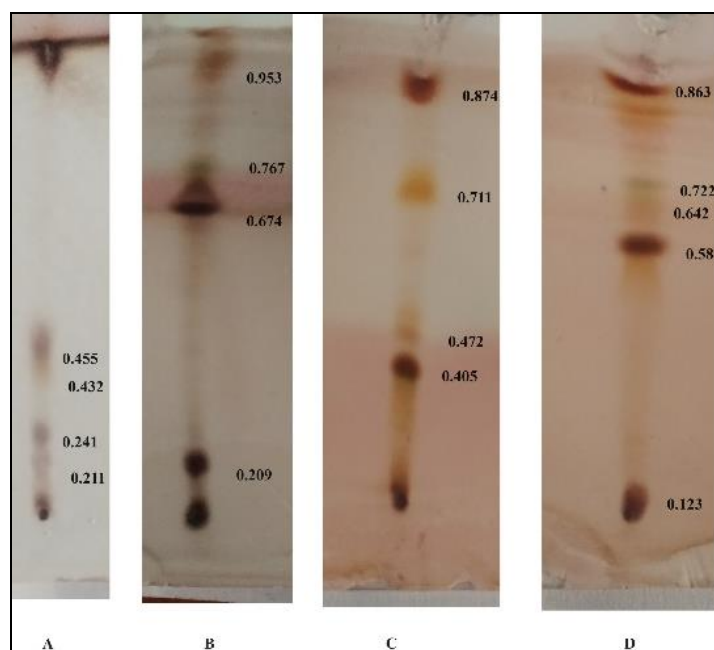
Phytochemical analysis of *Musa ornata* pseudo-stem extract revealed the presence of several bioactive compounds (Table 1).

**Table 1:** Phytochemicals present in *Musa ornata*

Phytochemicals	<i>Musa ornata</i> pseudo stem extract
Test for carbohydrates	Detected
Test for protein	Detected
Test for steroids	Detected
Test for glycosides	Not detected
Test for alkaloids	Not detected
Test for saponins	Detected
Test for flavonoids	Detected
Test for Tannins	Detected

### Thin Layer Chromatography

As shown in the image, the TLC results for the *Musa ornata* pseudo-stem extract illustrate the separation and identification of various compounds present in the extract across four different TLC plates (A, B, C, and D) with various mobile phases. Each plate had several spots with corresponding Rf values, indicating the distance traveled by each compound relative to the solvent front. Plate A, utilizing ethyl acetate and n-hexane (1:9 v/v), revealed four closely spaced spots with Rf values of 0.211, 0.241, 0.432, and 0.455, respectively, indicating limited separation. Plate B, with increased ethyl acetate concentration (2:8 v/v), showed a slight improvement, displaying five spots with Rf values of 0.209, 0.674, 0.767, and 0.953. Plate C, using a mixture of toluene, ethyl acetate, and formic acid (4.5:3.5:0.2 v/v/v), achieved moderate separation with three spots with Rf values of 0.405, 0.472, 0.711, and 0.874. However, Plate D, which employed a more complex solvent system of ethyl acetate, formic acid, acetic acid, and water (100:11:11:27 v/v/v/v), demonstrated the best resolution. It effectively separated the compounds into five distinct spots with Rf values of 0.123, 0.581, 0.642, 0.722, and 0.863, respectively. The varied solvent composition in Plate D provided the most effective separation, highlighting its superiority in resolving the compounds present in the extract. The TLC analysis effectively separated the compounds in the *Musa ornata* pseudo stem extract, revealing a range of polarities. This separation provides insight into the chemical composition of the extract and serves as a preliminary step for further identifying and characterizing individual phytochemicals (Figure 2).



**Fig 2:** TLC of *Musa ornata* pseudo stem extract

Plate A= Ethyl acetate: n-Hexane (1:9v/v), Plate B= Ethyl acetate: n-Hexane (2:8v/v), Plate C=Toluene, Ethyl acetate and formic acid (4.5:3.5:0.2 v/v/v), Plate D= Ethyl acetate–formic acid–acetic acid–water (100:11:11:27, v/v/v/v).

### GC-MS analysis

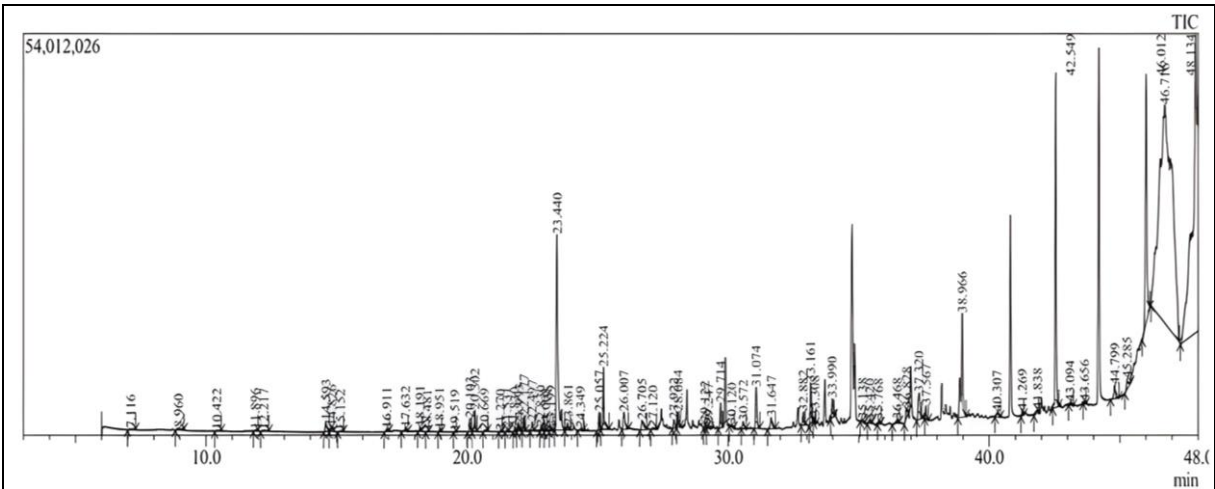
GC-MS analysis revealed a diverse range of compounds, each eluted at a different retention time, indicating their unique presence and abundance in the sample. Compound

(1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene was detected early in the analysis, with a retention time of 7.116 min, indicating that it is a relatively volatile compound with a lower boiling point. This was followed by 1,2,4-Trimethylbenzene and 1,2-Diethylbenzene, which eluted at 8.960 and 10.422 min, respectively, representing the aromatic hydrocarbons commonly found in various organic mixtures.

Furthermore, 2-(2-Ethylhexyloxy) ethanol and nonanal appeared at retention times of 11.896 and 12.217 min, respectively. These compounds indicate alcohols and aldehydes, respectively, which are known for their distinct chemical properties and applications in the fragrance and flavor industries. Dodec-1-ene and Dodecane, eluting at 14.593 and 14.826 minutes, respectively, signify the presence of alkenes and alkanes, which are fundamental components in petrochemical and synthetic organic chemistry.

The identification of compounds such as Decanal, 2,6,11-Trimethyldodecane, and 2-Butyloctan-1-ol at retention times of 15.152, 16.911, and 17.632 min, respectively, further exemplifies the complexity of the sample, including higher-molecular-weight aldehydes and alcohols. Notably, 2,2,6,7-Tetramethyl-10-oxatricyclo [4.3.0.1(1,7)] decan-5-one and 9-Methylnonadecane were detected at 18.481 and 19.519 min, respectively, representing unique cyclic and branched hydrocarbons.

As the analysis progressed, a variety of long-chain alkanes, alcohols, and esters were identified, including Pentadec-1-ene, Tetradecane, and 2-Hexyldecan-1-ol, with retention times of 20.103, 20.302, and 23.008 min, respectively. The detection of compounds like 2,6-Di-tert-butylphenol and 2,6-Di-tert-butyl-1,4-benzoquinone, appearing at 21.270 and 21.993 min, respectively, indicates the presence of phenolic and quinone compounds, which are known for their antioxidant properties. Later in the analysis, notable compounds such as dibutyl phthalate and bis (2-ethylhexyl) phthalate were identified with 29.122 and 36.828 min retention times, respectively. These plasticizers are commonly used, highlighting the potential contamination or the presence of plastic sources. Identifying high molecular weight compounds such as Tetrapentacontane and Hexacontane, with retention times of 30.120 and 35.138 min, suggests the presence of long-chain hydrocarbons (Table 2 & Figure 3).

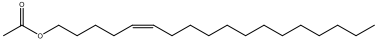
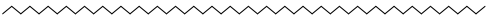

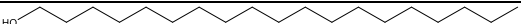
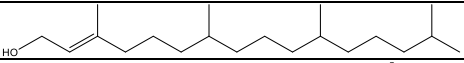
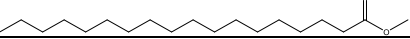
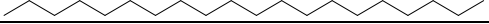
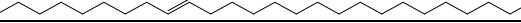
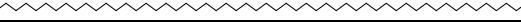


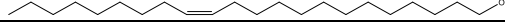
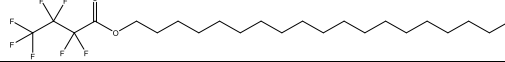
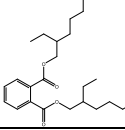
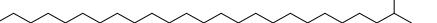
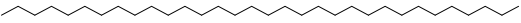
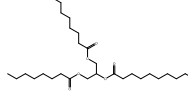
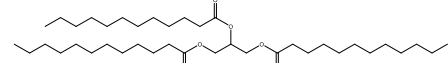
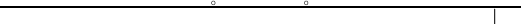
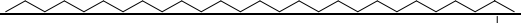



**Fig 3:** GC-MS Chromatogram of *Musa ornata* pseudo stem extract

**Table 2:** GC-MS report of *Musa ornata* pseudo stem extract

R. Time	Name	Category	Structure
7.116	(1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene	Terpene	
8.960	1,2,4-Trimethylbenzene	Aromatic Hydrocarbon	
10.422	1,2-Diethylbenzene	Aromatic Hydrocarbon	
11.896	2-(2-Ethylhexyloxy) ethanol	Alcohol	
12.217	Nonanal	Aldehyde	
14.593	Dodec-1-ene	Alkene	
14.826	Dodecane	Alkane	
15.152	Decanal	Aldehyde	
16.911	2,6,11-Trimethyldodecane	Alkane	
17.632	2-Butyloctan-1-ol	Alcohol	

18.191	4,6-Dimethyldodecane	Alkane	
18.481	3-Ethyl-3-methylheptane	Alkane	
18.951	2,2,6,7-Tetramethyl-10-oxatricyclo [4.3.0.1(1,7)] decan-5-one	Terpene	
19.519	9-Methylnonadecane	Alkane	
20.103	Pentadec-1-ene	Alkene	
20.302	Tetradecane	Alkane	
20.669	Tetradecanal	Aldehyde	
21.270	2,6-Di-tert-butylphenol	Phenol	
21.531	2-(2,2-Dimethylcyclopentyl) butanal	Aldehyde	
21.830	2,6,10-Trimethyltridecane	Alkane	
21.993	2,6-Di-tert-butyl-1,4-benzoquinone	Quinone	
22.177	2,5-Di-tert-butyl-1,4-benzoquinone	Quinone	
22.497	Pentadecane	Alkane	
22.830	2,6-Di-tert-butyl-4-methylphenol	Phenol	
23.008	2-Hexyldec-1-ol	Alcohol	
23.199	2,4-Di-tert-butylphenol	Phenol	
23.440	11-Methyltricosane	Alkane	
23.861	Heneicosane	Alkane	
24.349	Nonadec-1-ene	Alkene	
25.057	2-Methylpentyl tridecyl malonate	Ester	
25.224	Propan-2-yl tetradecanoate	Ester	
26.007	6,10,14-Trimethylpentadecan-2-one	Ketone	
26.705	Hex-3-yl 3-methylbutyl phthalate	Ester	
27.120	Trichloro(octadecyl)silane	Silane	
27.922	Methyl hexadecanoate	Ester	
28.084	3,5-Di-tert-butyl-4-hydroxybenzenepropanoic acid	Acid	
29.122	Dibutyl phthalate	Phthalate	
29.247	Nonyl hexadecyl ether	Ether	

29.714	(Z)-5-Octadecenyl acetate	Ester	
30.120	Tetrapentacontane	Alkane	
30.572	2,6,6-Trimethylbicyclo [3.1.1] heptan-2-ol	Terpene	
31.074	Nonadecan-1-ol	Alcohol	
31.647	Phytol	Alcohol	
32.882	Methyl octadecanoate	Ester	
33.161	Eicosane	Alkane	
33.308	9-Hexacosene	Alkene	
33.990	Tetrapentacontane	Alkane	
35.138	Hexacontane	Alkane	
35.420	Tetracosane	Alkane	
35.768	(Z)-Docos-13-en-1-ol	Alcohol	
36.468	Nonadecyl heptafluorobutanoate	Ester	
36.828	Bis(2-ethylhexyl) phthalate	Phthalate	
37.320	2-Methylpentacosane	Alkane	
37.567	Dotriacontane	Alkane	
38.966	2-(Decanoyloxy) propane-1,3-diyl bis(octanoate)	Ester	
40.307	Propane-1,2,3-triyl tris(dodecanoate)	Ester	
41.269	2-Methylheptacosane	Alkane	
41.838	2-Methyloctacosane	Alkane	
42.549	Hexatriacontane	Alkane	

## Conclusion

*Musa ornata* exhibits a rich phytochemical profile with various bioactive compounds that support its traditional medicinal uses, suggesting new therapeutic and industrial applications. This study demonstrates the versatility of this plant and underscores the importance of further research to explore and harness its potential benefits fully.

## Acknowledgements

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## Conflict of interest

The authors declare that there is no conflict of interest

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