

## Analysis of Chemical Components and Antibacterial Activity in Essential Oil of Lantana Flowers (*Lantana Camara* L)

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### ABSTRACT

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The purpose of this research was to evaluate the essential oil composition and antibacterial activity of *Lantana camara* L flowers essential oil against four bacterial strains. *Lantana camara* L flowers essential oil of is obtained by hydrodistillation method using Sthal and analyzed by GC-MS. The antibacterial activity of essential oil against four bacterial strains was tested by the disk diffusion method. The results showed 72 compounds for *Lantana camara* flowers essential oil, of which caryophyllene (10,87%), davanone (9.84%),  $\alpha$ -humulene (7.59%),  $\alpha$ -curcumene (3.35%), germacrene D (3.09%), calarene (2.42%),  $\alpha$ -muurolene (2.27%), p-cymene (1.79%), 1,8 cineole (1.59%) ,  $\delta$ -cadinene (1.59%),  $\alpha$ -copaene (1.12%), nerolidol B (1%) and  $\beta$ -ocimene (0.54%) were detected as major components. The antibacterial activity assay of *Lantana camara* L flowers essential oil examined four bacterial strains including gram positive and negative bacterial strain by using disk diffusion agar method. All the tested gram positive and negative bacterial strains displayed slight to moderate antibacterial activity (7 – 10 mm) against all concentrations of essential oil. The essential oil of *Lantana camara* L flowers showed remarkable antibacterial activity with inhibition in concentrations of 10% and 20% against the phatogen bacterias *Eschechia coli* ( 8.4 mm ; 9.6 mm) *Basillus subtilis* (9.6 mm; 10.8 mm), *Pseudomonas aeruginosa* ( 8.3 mm ; 9.3 mm), and *Propionibacterium acnes* ( 7.6 mm; 8.7 mm) showed from zone of inhibition that was formed, zone of inhibition in concentration 20% was bigger than concentration 10%.

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## INTRODUCTION

Indonesia is a country that has enough potential in the production of essential oils. Essential oils are a complex mixtures of volatile compounds produced as secondary metabolites in aromatic plants (Verma & Balasubramanian, 2014). Essential oils, including monoterpenes, sesquiterpenes, and phenylpropanoids are among the major bioactive secondary metabolites of plants. It is found naturally in plants with strong scents, as a complex mixture of volatile compounds (Buchanan et al., 2007; J. Wang et al., 2018).

Natural ingredients essential oil of have been widely used in food and beverage products, soaps, perfumes, cosmetics, etc. (Huang et al., 2021; Guliani et al., 2021). The essential oil are commonly used in aromatherapy, pharmaceutical industries, and have shown antibacterial and antioxidant activities against microorganisms (Crusell et al., 2018; Hazrati et al., 2020).

*Lantana camara* L is an evergreen aromatic shrub that can grow up 2 - 4 m which belonging to to Verbenaceae family. *Lantana camara* is well known tropical folk medicine and traditional. It is a strong, aromatic flowering with distinct fragrance; its ripe fruits are blackish and its flowers are small, tubular and are found in various colors like white, pink, yellow, violet, and red (Dougnon & Ito, 2020; Kazmi et al., 2013 ; Nea et al., 2020; Ayalew, 2020). The essential oil of *Lantana camara* L and its extracts of the plant contains a high amount of sesquiterpenes, and is used for treatment of skin itches, chicken pox, asthma, as an antiseptic for wounds, tumor, cancer, high blood pressure, ulcers, tetanus, rheumatism, and externally for scabies and leprosy (Seth et al., 2012; Janardhan & Vijayan, 2012; Ajitha et al., 2015; (Barros et al., 2016).

*Lantana camara* L essential oil exhibits a wide spectrum of antimicrobial, antibacterial, and antifungal activities (Zoubiri & Baaliouamer, 2011). The main components of essential oil are  $\beta$ -caryophyllene, geranyl acetate, terpenyl, bornyl acetate,  $\delta$ -limonene, and acetate showed antibacterial and antifungal activity against *Aspergillus niger*, *Candida albicans*, *Fusarium solani*, and *Pseudomonas aeruginosa*. (Bevilacqua et al., 2011). In

general, essential oils are used as one of the functional ingredients in cosmetics, perfumes, and various pharmaceutical products due to their aroma and antibacterial activity (Septiana et al., 2020).

Although the chemical composition of *Lantana camara* L leaf essential oils from North Sumatera has been reported before, but the composition and the antibacterial activity of essential oils from *Lantana camara* L flower from North Sumatera have not been studied. In this research, we investigated the chemical composition of *Lantana camara* L flowers essential oils and its antibacterial activity assay against *Escherichia coli* (*E.coli*), *Bacillus subtilis* (*B.subtilis*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Propionibacterium acnes* (*P. acnes*).

## MATERIALS AND METHODS

### Time and Place

This research was carried out in the Organic Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara (USU) for the aim of isolating the essential oil of *Lantana camara* L flowers. The antibacterial analysis was carried out in the Laboratory of Microbiology, Pharmacy Faculty of Universitas Sumatera Utara. Gas chromatographic - mass spectrometry was performed in Organic Chemistry Laboratory of Faculty of Mathematics and Natural Sciences, Gadjah Mada University.

### Plant material and reagents

*Lantana camara* L (yellow-orange flowers) flowers of were collected from Kabanjahe regency, North Sumatera Province in the Republic of Indonesia in October 2013. Identification was confirmed by Medanense Herbarium (MEDA), Universitas Sumatera Utara (sample number 202/MEDA/2013). Dimethylsulfoxide (DMSO, purify 99%), ethanol (96%), anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ). Mueller-Hinton Agar (MHA), Nutrient Agar (NA), Nutrient Broth (NB) were purchased from Oxoid, Ireland.

### Extraction of *Lantana camara* L essential oil

Fresh *Lantana camara* L flowers (200 g) were weighed and subjected to hydrodistilled in 150 mL distilled water in a graduated Clevenger type apparatus for 5 h, the experiment was carried out three times. Distillation is a way of isolating compounds as essential oils from *Lantana camara* L is distillation. During distillation, *Lantana camara* L, which has a fragrant aroma is exposed to boiling water or steam, releasing their essential oils. The collected essential oil of *Lantana camara* L flowers was subsequently dried over anhydrous sodium sulfate anhydrous (Na<sub>2</sub>SO<sub>4</sub>), and stored at 4 °C until the GC-MS and antibacterial analysis were carried out.

### GC/MS analysis

GC-MS analysis of *Lantana camara* L essential oil was performed on GCMS : GC2010 MSQP 2010S Shimadzu, a gas chromatograph equipped with splitless sampler data management system. The column was Agilent HP 5MS capillary column. Helium (He) was the carrier gas with a flow rate of 0.5 ml/min. The temperatures of the injector, interface, and ion source were maintained at 310 °C, 305 °C, and 250 °C, respectively. Identify individual component by matching the recorded mass spectrum with the library (NIST ; Wiley) provided by the instrument software, and comparing their calculated values. The relative area percentage of each components is obtained from GC-FID analysis.

### Bacterial strains

Total four bacterial strains were used in the experiment. Out of them, 2 types gram negative bacteria strains namely *Escherichia coli* (*E.coli*), and *Pseudomonas aeruginosa* (*P. aeruginosa*) and 2 types gram positive bacteria strains namely *Bacillus subtilis* (*B.subtilis*), *Propionibacterium acnes* (*P. acnes*). These strains were obtained from culture collection of the Laboratory of Microbiology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan.

### Preparation of culture media

Three types of media was prepared namely Nutrient Agar, Mueller Hinton Agar, and Nutrient

Broth. Each medium was weighed according to its own weight 7 gr, 9.5 gr and 3.25 gr then added 250 mL distilled water into each conical flask. Each solution was thoroughly mixed and warmed until completely dissolved each medium in the conical flask. All solutions were autoclaved for 15 minutes at 121 °C and cooled at room temperature and stored 4 °C for further experiment. If the solution were used the same day, the agar media was poured into sterile petri dishes and allowed to solidify. After dried for about 30 min before covering the plates to prevent contamination from microorganism and the formation of water on the agar surface then stored for further use.

### Antibacterial activity assay

The essential oil of flowers of *Lantana camara* L was tested for its antibacterial activity against 2 types gram negative bacteria strains and 2 types gram positive bacteria strains by using by the disc diffusion method. Antibacterial activity tested was carried out by using two distinctive concentrations (10% and 20%) of *Lantana camara* L essential oil in DMSO as a solvent. Each bacterial strain as much 0,1 mL, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Propionibacterium acnes* were entered into petri dish and added 15 mL Mueller Hinton Agar (MHA) solution in temperature 45 – 50°C until homogeneous and allowed to solidify, respectively. The disc paper with a size of 5 mm in size was immersed in each concentration of essential oil and placed on the surface of media in each agar petri dish. All petri dishes were stored in an incubator for 24 hours at a constant of temperature 37 °C. The antibacterial activity of two different concentrations were determined by calculating the inhibition zone diameter manually.

## RESULTS AND DISCUSSION

### Chemical composition of *Lantana camara* L flowers essential oil by GC-MS analysis

The hydrodistillation method is one of most frequently used and best techniques devoted to extracting essential oil. The general hydrodistillation was executed in Clevenger

apparatur . Clevenger apparatur is a classical laboratory equipment for collecting essential oil based on the cyclic distillation method. It should be noted that all the distillate (hydrolyzate and oil) is not solvent extracted (Y. H. Wang & Zhang, 2020). The essential oil of *Lantana camara* L flowers (yellow-orange) was obtained three times

hydrodistillation (Table 1). The *Lantana camara* L flowers produced the 0.05% (w/w) of essential oil and pale yellow oil in colour.

**Table 1.** The *Lantana camara* L flowers essential oil was obtained by hydrodistillation method

Sample weight (g)	Results (mL)			Total average	Essential oil weight (g)
	I	II	III		
200	0.13	0.12	0.10	0.11	0.093

GC-MS chromatography-assisted analysis of chemical constituents of *Lantana camara* L flowers essential oil (Figure 1). Identify essential oil compounds by comparing retention time and molecular weight with reference compounds in Wiley and NIST libraries. *Lantana camara* L flowers essential oil were found to contain 72 different compounds representing 96.7 % of total oil were identified (Figure 1). All compounds belonged to four chemical classes. The major classes were sesquiterpene hydrocarbons 67.87 %, and monoterpene hydrocarbons 16.4 % while the oxygenated sesquiterpenes 7.03 % and oxygenated monoterpenes 5.4 % were the minor classes.

According to the results, Table 2 shows the 13 major components of *Lantana camara* L essential oil compounds. Among all major components of *Lantana camara* L essential oil the main components was caryophyllene (10.87%), followed by davanone (9.84%),  $\alpha$ -humulene (7.59%),  $\alpha$ -curcumene 3.53%, germacrene D (3.09%), calarene (2.42%),  $\alpha$ -muurolene (2.27%), p-cymene (1.79%) ,  $\delta$ - cadinene (1.59%), 1,8 cineole (1.59%),  $\alpha$ -copaene (1.12%), nerolidol-B (1%), and  $\beta$ - ocimene (0.54%).

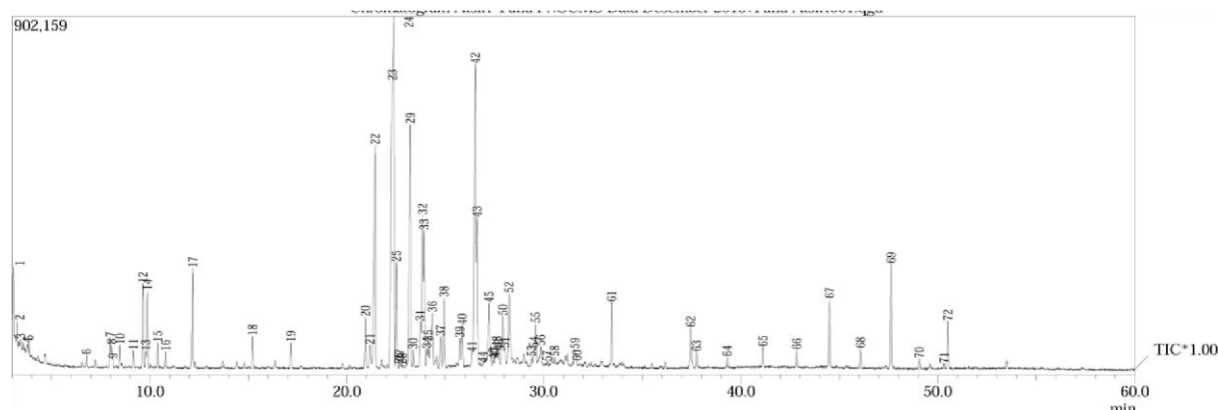
The components with concentration higher than or close to 1% are two monoterpenes, eight sesquiterpenes and three oxygenated derivatives. Among the monoterpenes, p-cymene is the major one, followed by  $\beta$ - ocimene. Among the sesquiterpenes components, caryophyllene is the main, followed by minor amount of

$\alpha$ -humulene,  $\alpha$ -curcumene, germacrene D, calarene ,  $\alpha$ -muurolene,  $\delta$ - cadinene, and

$\alpha$ -copaene. The oxygenated components were davanone as the main components, followed by 1,8 cineole, and nerolidol-B (Table 2).

The *Lantana camara* L essential oil is reportedly rich in monoterpenes and sesquiterpenes, which many included trans/  $\beta$  caryophyllene, E-citral, bicyclogermacrene sabinene, germacrene,  $\alpha$ -curcumene 1,8-cineole,  $\alpha$ -hemulene, and limonene, (Patil & Kumbhar, 2018). *Lantana camara* L leaf and flower essential oil contain oxygenated sesquiterpenes as the main compound category. In flowers oil, the content of sesquiterpene hydrocarbons is 2 to 3 times aliphatic hydrocarbons, and the content of leaf oil is 5 to 6 times higher than that in flower oil (Khan *et al*, 2016).

The components that characterize the chemical essential oil of *Lantana camara* L (yellow-orange flowers) are  $\alpha$ - humulene, sabinene, 1,8-cineole,  $\beta$ -caryophyllene, linalool, caryophyllene oxide, davanone bisabolene, cadinene, ar-curcume and the main component was  $\beta$ -caryophyllene (Khalid, 2019; Randrianalijaona *et al*, 2005).



**Figure 1.** Gas chromatography - mass spectrometry chromatogram of *Lantana camara* L flowers essential oil

**Table 2.** Major chemical compounds of *Lantana camara* L flowers essential oil indentified by GC-MS

Retention time (minutes)	Compound	Peak Area	Area %	Molecular formula
7.974	$\beta$ -Ocymen	228252	0.54	C <sub>10</sub> H <sub>16</sub>
9.645	p-Cymene	753134	1.79	C <sub>10</sub> H <sub>14</sub>
9.872	1,8- Cineole	667927	1.59	C <sub>10</sub> H <sub>18</sub> O
20.944	$\alpha$ -Copaen	472727	1.12	C <sub>15</sub> H <sub>24</sub>
22.317	Caryophyllene	457377	10.87	C <sub>15</sub> H <sub>24</sub>
22.521	Calarene	1017919	2.42	C <sub>15</sub> H <sub>24</sub>
23.226	$\alpha$ -Humulene	3196169	7.59	C <sub>15</sub> H <sub>24</sub>
23.852	$\alpha$ -Curcumene	1483747	3.53	C <sub>15</sub> H <sub>24</sub>
23.933	Germacene-D	1302080	3.09	C <sub>15</sub> H <sub>24</sub>
24.346	$\alpha$ -Murolene	954826	2.27	C <sub>15</sub> H <sub>24</sub>
24.935	$\delta$ -Cadinene	669831	1.59	C <sub>15</sub> H <sub>24</sub>
25.861	Nerolidol	419502	1.00	C <sub>15</sub> H <sub>26</sub> O
26.527	Davanone	4139361	9.84	C <sub>15</sub> H <sub>24</sub> O <sub>2</sub>

### Antibacterial activities of *Lantana camara* L flowers essential oil

The antibacterial activity test of *Lantana camara* L flower essential oil against gram-positive and negative bacterial strains screened by the disc diffusion method was studied. According to our research results (Table 2), the inhibitory diameters of the strains studied in *Lantana camara* L flowers essential oil from 7 to 10 mm. The highest inhibitory diameter of *Lantana camara* L flowers essential oil was obtained for *Bacillus subtilis* (10.8 ; 9.6 mm), *Escheria coli* (9.6 ; 8.4 mm), *Pseudomonas aeruginosa* (9.3 ; 8.3), while the

lowest inhibitory diameter was *Propionibacterium acnes* (8.7 ; 7.6 mm) (Figure 2). Compared with other concentrations, the highest concentration of essential oil has the highest potential. This means that the concentration of essential oils is increasing and the antibacterial potential is increasing. It was measured by inhibition zone (IZ) was increased (Ashmawy *et al.*, 2018).

The result of this research showed the essential oil of *Lantana camara* L flowers have a reasonable antibacterial influence on four bacteria strains ( gram-positive and gram-negative). In general, gram-positive bacteria are more sensitive to essential oil than the gram-negative bacteria (Hazrati *et al.*, 2020).

It causes gram-negative bacteria has extra lipopolysaccharide and protein cell wall (double membranes) providing a permeable barrier for gram-positive bacteria that have a single-layer cell wall for antibacterial agent (Salem *et al.*, 2013). It can be speculated that the antibacterial activity of *Lantana camara* L essential oil is related to one or more bioactive compounds, especially sesquiterpenes, which have many components and also contain oxygen-containing monoterpenes too. Both the minor and major compounds should significantly affect the activity of the oil (Zoubiri & Baaliouamer, 2012). It can be said that the main components has an antibacterial activity, but the secondary components also contributes to a synergistic action (Al-Dhahli *et al.*, 2020).

The factor that determines of essential oil activity is the functional groups of the composition present in active components. Oxygenated sesquiterpenes exhibit shows better antibacterial activity than hydrocarbons, which can occur by inhibiting the basic microbial rejuvenation process in lipid membrane function (Cecchini *et al.*, 2021; Babarinde *et al.*, 2021). High percentage of sesquiterpenes in *Lantana camara* L was primary responsible for the antibacterial activities (Zhu *et al.*, 2013).

Nerolidol (3,7,11-trimethyl-1,6,10-dodecatrien-3-ol) is a major sesquiterpene found in the essential oils of many plants. Nerolidol has many medical benefits, such as antibacterial, antitumor, antiulcer, and anti-schistosomiasis properties (Xia *et al.*, 2018).

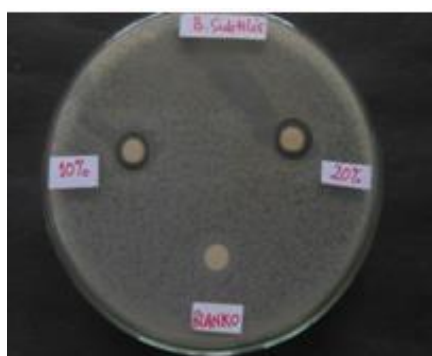
The 1,8 cineole (1,3,3-trimethyl-2-oxabicyclo [2.2.2.] octane) is a monoterpene oxide, which is a colorless or pale yellow liquid at room temperature and has a “cool”, “fresh” and “medical” smell (Yin *et al.*, 2021). It is mainly derived from essential oil of Eucalyptus. The 1,8-cineole has various biological activities including antibacterial, anti-oxidative, analgesic, and anticancer properties (Rodenak-Kladniew *et al.*, 2020). Bajalan *et al.*, 2017 reported the antibacterial activity of 1,8 cineole against *S.aureus*, *P. aeruginosa*, *E.coli* and *C. albicans*. These result were in agreement with other repots about 1,8 cineole.

Davanone ([2S-[2 $\alpha$ (R),5 $\alpha$ ]] (5ethenyltetrahydro-5-methyl furanyl)-6-methyl-5-hepten-3-one) is the most representative compound in the total oil (Taylor *et al.*, 2011). Davanone is a sesquiterpene lactone found in a variety of Artemisia plants and has a chemical taxonomic value. Davanone has antibacterial, antispasmodic, and antifungal properties. In addition for healing, hypoglycemic, antihelminthic, and antidepressant activities also. It is also used as a food flavoring agent (Goudjil *et al.*, 2015). Davanone is effective against *Candida albicans* (Obistioiu *et al.*, 2014). Davanone is isolated from natural source widely used in the medical field and in the food industry. Furthermore, davanone is reported to be odorless, so the odor component of the davanone containing oil is attributed to the presence of davanone ether (Aati *et al.*, 2020).

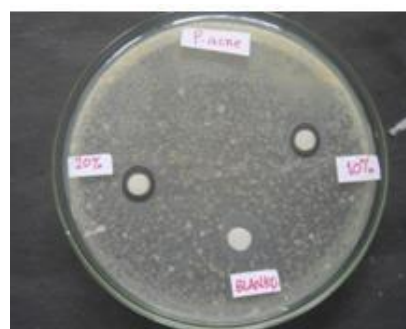
The combined antimicrobial activity of may be explained by taking into account the difference in their chemical structures which act on several cellular targets through different mechanisms. Therefore, in our research, the synergistic effect of different concentration combinations of *Lantana camara* L essential oil can be explained to a large extent as the synergistic effect of its main components. However, other minor components of essential oils may also have potential and contribute to the observed antibacterial activity through synergistic interactions with the main compounds (Al Zuhairi *et al.*, 2020; Mulyaningsih *et al.*, 2010; Soulaïmani *et al.*, 2021).

**Table 3.** Antibacterial activity assay of *Lantana camara* L essential oil against *Bacillus subtilis*, *Propionibacterium acnes*, *Escherichia coli*, *Pseudomonas aeruginosa* (diameter of inhibition zones in mm)

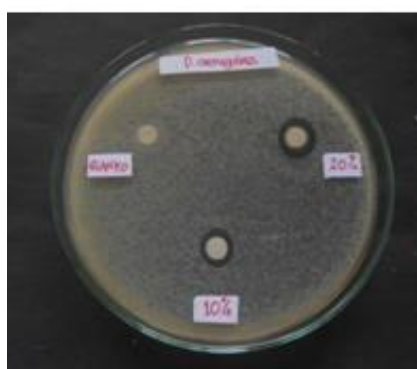
Concentration (%)	Diameter of inhibition zones (mm)			
	<i>Bacillus subtilis</i>	<i>Propionibacterium acnes</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
10	9.6	7.6	8.4	8.3
20	10.8	8.7	9.6	9.3



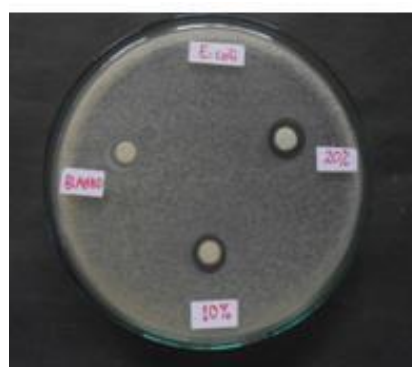
a. *Bacillus subtilis*



b. *Propionibacterium acnes*



c. *Escherichia coli*



d. *Pseudomonas aeruginosa*

**Figure 2.** The diameter of inhibition zone of *Lantana camara* L essential oil (a) *Bacillus subtilis*, (b) *Propionibacterium acnes*, (c) *Escherichia coli*, (d) *Pseudomonas aeruginosa*

## CONCLUSION

The present study that the monoterpenes and sesquiterpenes hydrocarbon followed by oxygenated monoterpenoids were the predominant parts of the *Lantana camara* L flowers essential oil. The essential oil of *Lantana camara* L flowers contained terpenoid compounds which inhibited all tested microbial strains, *Bacillus subtilis*,

*Propionibacterium acnes*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Therefore, the most bioactive compounds of *Lantana camara* L flowers essential oil had antibacterial activity, specially 1,8cinoele, nerolidol-b, and davanone which have terpenoid compounds. The antibacterial activity of *Lantana camara* L flowers essential oil is mainly attributed to major components and also due to

synergistic effect of other minor components of its essential oil.

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### CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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