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Metabolite profile analysis of methanol extract of belulang grass (*Eleusine indica* (L.) GAERTN) leaves by gas chromatography-mass spectrophotometry (GC-MS) method

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Abstract

Leaf of belulang grass (*Eleusine indica* (L.) Gaertn) is one of the wild plants that is used as herbal medicine by the community because of its many health benefits for the treatment of certain diseases such as diabetes, high blood pressure, and cholesterol. A materil is said to have potential as a medicine because the composition of the chemical compounds contained in a plant is not always the same and requires further testing. Differences in chemical compound content can be influenced by environmental factors and plant metabolism. The aim of this research is to analyze the metabolite profile and abundance of metabolite compounds using the GC-MS instrument analysis approach. Sampling was conducted in Sidomulyo Village, Air Kumbang District, Banyuasin Regency, South Sumatra. Samples of belulang grass leaves obtained were dried, pulverized, and extracted with methanol solvent. The methanol extract was analyzed using GC-MS. The results showed that the highest 20 compound peaks were obtained based on the relative area percent of the 69 compounds obtained with an abundance of compounds amounting to 80.86%. At the highest peak, the compounds n-hexadecanoic acid, I-(+)-ascorbic acid 2, 6- dihexadecanoate, and pentadecanoic acid were found with a percent area of 31.25%.

Keyword: Leaf of Belulang Grass (*Eleusine indica* (L) Gaertn); GC-MS; Metabolite Profile; Sidomulyo Village; Herbal Medicine

1. Introduction

Degenerative diseases, or non-communicable diseases (NCDs), are one of the public health problems that continue to increase day by day. Degenerative diseases are diseases caused by the decline in the function of body tissues and cells over time, which can naturally affect the function of body organs due to the aging process In addition, lack of physical activity and an unhealthy diet can also cause degenerative diseases (Hanum, G. R., & Ardiansyah, 2018) [1].

The World Health Organization in 2011 wrote that degenerative diseases are one of the diseases caused by free radical activity that can cause death in humans. Free radicals are molecules that can cause various diseases, including heart disease, stroke, hypertension, chronic kidney disease, diabetes, purpura disease, and neurodegenerative diseases (Sutomo., Arnida., Mintowati, E., 2016) [2]. Data from the Basic Health Research (Riskesdas) of the Indonesian Ministry of Health (2018) shows that the prevalence of degenerative diseases in Indonesia continues to increase. For example, asthma (2.4%), cancer (1.8%), stroke (10.9%), chronic kidney (3.8%), joints (7.3%), diabetes (2%), heart (1.5%), hypertension (34.1%), and obesity (21.8%) continue to increase (Ministry of Health, 2022) [3]

Spine grass (*Eleusine indica* (L.) Gaertn) is a member of the grass family. The morphological characteristics of this plant include fibrous roots, hollow stems, and round, flat, hairy, and shrub forms. The leaves of this plant are generally ribbon-

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like and pointed at both ends. While the flowers are included in the type of compound flowers consisting of three to five grains and shaped like jagged teeth (Gunarti, 2023) [4]

Grass (*Eleusine indica* (L.) Gaertn) is widely used as a medicine because it contains secondary metabolites. Secondary metabolites are organic compounds that usually consist of phenylpropanoid compounds, phenols, terpenoids and flavonoids that have medicinal properties (Saifudin, 2016) [5]. Secondary metabolites have the ability to treat various diseases. One of them can be used to prevent degenerative diseases. This is due to the synergistic effect, where the increased performance of natural components can outperform the mechanism of action of synthetic drugs, due to the presence of a single compound that works (Bone, K., & Mills, 2013) [6].

Grass (*Eleusine indica* (L.) Gaertn) is used to treat various diseases, including inflammation and immune disorders. The results of phytochemical screening showed that belulang grass (*Eleusine indica* (L.) Gaertn) has compounds that act as drugs in the prevention of certain diseases. Chemical compounds found in belulang grass are flavonoids, steroids, essential oils, coumarins, fatty acids, triterpene tannins and alkaloids. In addition, belulang grass (*Eleusine indica* (L.) Gaertn) has antioxidant, antimicrobial, cytotoxic, antidiabetic, anti transformation (antihypertensive anti-inflammatory and anti trypanosomal properties (Sukor, Zahari, Rahim, Yusoff, & Salim, 2022) [7].

Metabolomics is an emerging field of research that uses analytical techniques to determine the location, type and amount of metabolite content in an organism (Irwan, A., & Junaidi, 2020) [8]. Metabolomics plays an important role in plant research studies, covering all classes of metabolites more thoroughly (theowidavitya, muttaqin, miftahudin, & tjahjoleksono, 2019) [9]. Metabolomic methods include synchronised and integrated systems to determine metabolites that occur under certain conditions and times (Irwan, A., & Junaidi, 2020) [8]. One of the methods used to analyse metabolites is metabolite profiling. Metabolite profiling is a form of analysis that has a function intended to describe the profile of metabolite chemical compounds present in plants (Krastanov, 2010) [10].

The method to identify metabolite compounds in samples that can be used is the GC-MS (gas chromatography-mass spectrophotometry) method. The GC-MS instrument method was chosen because it can be used for the analysis of low molecular weight and volatile compounds for a wide variety of plants, both targeted and non-targeted compounds, so it is considered to be used to explain the metabolite fingerprint of a plant in finding initial information for a study with scientific objectives (Irwan, A., & Junaidi, 2020)[8]. The advantage of the GC-MS method is that there is a fairly high sensitivity, so it is able to analyse compounds in small concentrations (Sparkman, D., Penton, Z. E., & Kitson, 2011)[11].

Information on the metabolite profile of belulang grass leaves is thought to affect the content and quality of metabolite concentrations produced. Metabolite profile information can also be used as a reference source of information on raw materials for natural medicines in the future. Therefore, there is a need for research related to the analysis of the metabolite profile content of belulang grass leaves (*Eleusine indica* (L.) Gaertn).

2. Material and Method

2.1. Location and time of research

This research was conducted from April 2024 to June 2024. Rumput Belulang leaves used in this study were obtained from Sidomulyo Village, Air Kumbang Subdistrict, Banyuasin Regency, South Sumatra. Sample processing such as drying and extraction was carried out at the Genetics and Biotechnology Laboratory, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Indralaya. Analysis of metabolite content of methanol extract of belulang grass leaves by GC-MS was carried out at the Integrated Research and Testing Laboratory, Gadjah Mada University, Yogyakarta.

2.2. Tools and materials

The tools used in this research are altimeter, glassware, aluminium foil, blender, glass funnel, cooling box, camera, flannel cloth, cardboard, filter paper, label paper, digital balance, oven, hair dryer, sifter, transparent plastic, vacuum rotary evaporator, soil meter, thermohygrometer, and GC-MS. The test material used in this study was the leaves of belulang grass (*Eleusine indica* (L.) Gaertn).

2.3. Sampling, preparation and making simplisia

Sampling of belulang grass leaves is done by picking the leaves with a total weight of approximately 3 kg and the sample is put into transparent plastic. Then wet sorted from impurities. Furthermore, the leaves were washed thoroughly with running water until clean and drained. Then dried by aerating with direct sunlight for 2 days and dry sorting. The dried

leaves were weighed as much as 500 grams and mashed in a blender until it became a simplisia powder of belulang grass leaves.

2.4. Extraction

The process of determining the activity of antioxidant compounds is carried out by maceration method. The stages of the extraction process by means of powdered simplisia of belulang grass leaves weighed as much as 500 grams and extracted for 2 x 24 hours using methanol solvent as much as 2 litres. The filtrate produced from the 2 litre maceration and remaceration process was then evaporated using a rotary evaporator with a temperature of 50˚C.

2.5. Metabolite profile analysis using GC-MS

GC-MS instrument is a method through spectra reading on GC and MS (Gani et al., 2017). Sample preparation was carried out using methanol solvent. Thick extracts of belulang grass leaves were taken as much as 1 gram to analyse the metabolite content using GC-MS (Gas Chromatography- Mass Spectrometry) at the Integrated Research and Testing Laboratory of Gadjah Mada University, Yogyakarta.

2.6. Data analysis

The results of metabolite identification were analysed using quantitative analysis applied descriptively in the form of data tables. Information on metabolic profiles was presented in the form of tabular statistics. Furthermore, the dominant metabolites were identified based on the most abundant regions and biosynthetic pathways were performed on the dominant metabolites using *PubChem, KEGG, ChEBI, Greenmolbd, PlantCyc, and Spectrabase websites.*

3. Result and Discussion

3.1. Metabolite Profile of Belulang Grass (*Eleusine indica* (L.) Gaertn) Leaves

The results of the interpretation of mass spectrometry (MS) chromatograms of the leaf extract of belulang grass (*Eleusine indica* (L.) Gaertn) showed a diversity of metabolite compounds. The metabolite profiles identified from GC-MS analysis of the leaf extracts of belulang grass belonged to the classes of fatty acids, thiosulfinates, vitamins, phytosterols, carboxylic acids, terpenoids, antibiotics, and steroids. Class groupings were analysed based on data from CheBI, KEGG, PlantCye, PubChem, Spectrabase, and Greenmolb websites.

The compound components present in the leaf samples were examined using gas chromatography, which was presented in the form of chromatograms and compared with an MS instrument to provide information on the relative molecular weight of each chemical compound present in the leaf samples. According to (Sheren et al., 2015) [12], the identification of each peak on the chromatogram is done by matching the MS spectrum with the results of the compounds detected in each peak, using the Wiley database, which helps identify the type of compound.

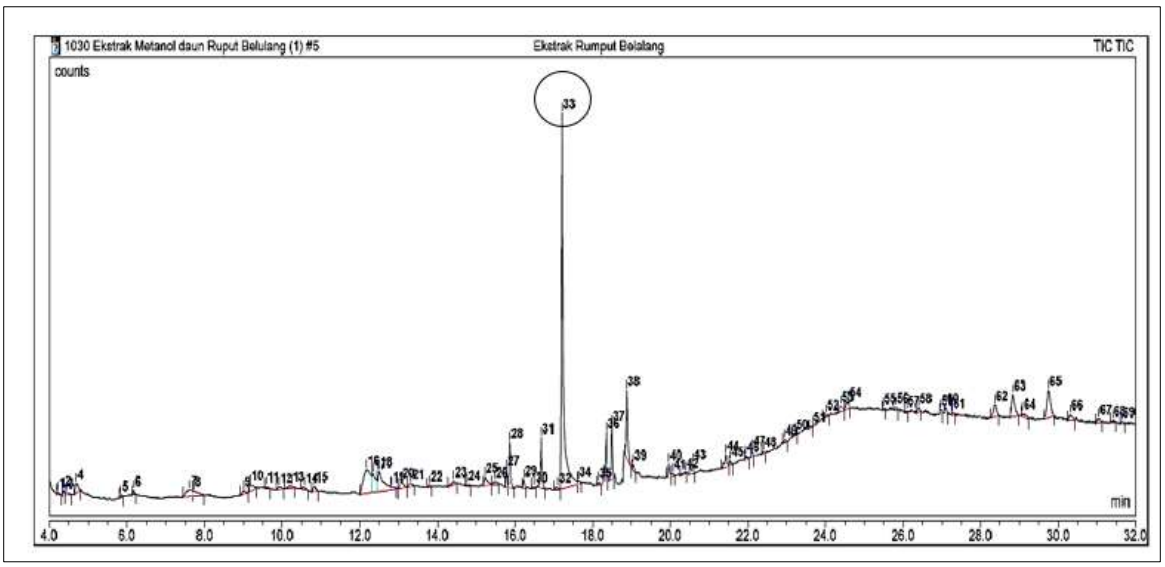

Figure 1 Chromatogram of Belulang Grass (*Eleusine indica* (L.) Gaertn.) Leaves

The chromatogram in (Figure 1) shows that the first 69 peaks were detected for each sample analysed, indicating that each peak is a unique compound with a different retention time. One peak can detect one molecular ion of a different compound type due to the process of phosphorus compound formation during the stationary phase. The black line in (Figure 1) connects the peak with the outermost area of the other peaks. The perpendicular area shows the detected compound abundance. (Fitriana, 2017) [13], states that each detected compound has a relative indicator based on the relative size of the peak area to the entire peak.

Based on the results in (Figure 1) shows the abundance of the highest metabolite compounds based on the percentage of relative area, which is referred to as major compounds. The dominant compound found in belulang grass leaves is a class of fatty acids. Major compounds of n-hexadecanoic acid, I-(+)- ascorbic acid 2,6-dihydroxy-decanoic acid and pentadecanoic acid (percentage area 31.25%), samples with desulfosinigrin compounds, L-glucose and d-glycero-di-ido-heptose (percentage 7.64%), 2-myristinolipanthene, ethyl isoallocholate and octahydrobenzo[b]pyran, 4a-acetoxy-5,5,8a-trimethyl (5.17%). According to (Peni Pindan, Saleh, Rahayu Magdaleni, & Kerayan Kampus Gunung Kelua, 2021) [14], hexadecanoic acid is one type of compound commonly found in the ethyl acetate fraction of plant extracts. This is supported by research (Sogandi, Darma, & Jannah, 2019)[15], in GC-MS analysis of licorice plants found n-hexadecanoic acid content in the extract of 14.14% in ethyl acetate solvent. Another study conducted (Amalia, 2022) [16], at the level of development of sungkai leaves also found n-hexadecanoic acid of 10.95%.

This palmitic acid or n-hexadecanoic acid is the most common type of saturated fat found in animals and plants. It is usually observed in the form of esters (glycerides) in milk and dairy products such as vegetable and animal. Palmitic acid or n-hexadecanoic acid is a fatty acid with antibacterial properties that enhances the antibacterial effect by destroying the cell wall and cell membrane structure through the mechanism of combining various active compounds into new molecules (Simple molecules into complex molecules) due to the combined reaction of acetyl-CoA carboxylase (ACC) and fatty acid enzymes (He, Qin, Wang, & Ding, 2020)[17]. Some studies say that hexadecanoic acid can act as an antioxidant that can prevent oxidation, can play a role in preventing cell damage caused by oxidative stress and has antibacterial properties. This makes it a compound that has anti-inflammatory activity because it absorbs nutrients that can interfere with cell function which is proven by inhibiting bacterial growth, can inhibit phospholipase A2 activity (Aparna et al., 2012) [18], and inhibit the diffusion of enzymes and water (Sogandi et al., 2019) [15].

3.2. Identification of metabolite compounds from the leaves of belulang grass

Based on the results of the analysis of the metabolite profile of belulang grass leaves using GC-MS, 20 metabolite compounds were found that have dominant compounds based on the highest peak and percent area. The identification results of these metabolite compounds can be seen in (Table 1).

Tabel 1 Compound Identification, Molecular Formula, Class, Total Abundance and Pharmacological Activity

No	Compound name	Molecular Formula	Class	Total Relative Area Percent (%)	Pharmacological Activity
1	*n-Hexadecanoic acid*	$C_{16}H_{32}O_2$	Fatty acid	31.25	Antioksidan, Antibakteri and Antiinflamasi
2	*Desulphosinigrin*	$C_{10}H_{17}NO_6S$	Tiosulfinat	7.64	Antioksidan and Antibakteri
3	*2-Myristynoyl pantetheine*	$C_{25}H_{44}N_2O_5S$	Pantotenat (Vitamin B5)	5.17	Sintesis asam lemak and sintesis kolesterol
4	*Sitosterol*	$C_{29}H_{50}O$	Fitosterol	4.48	Antiinflamasi
5	*9,12,15- Octadecatrienoic acid*	$C_{18}H_{30}O_2$	Fatty acid	4.62	Antioksidan, Antikanker, Antiinflamasi and Hipokolesterolemia
6	*3-Pyridinecarboxylic acid*	$C_{32}H_{39}NO_{10}$	Carboxylic acid	3.07	Antidiabetes
7	*Paromomycin*	$C_{23}H_{45}N_5O_{14}$	Antibiotik	3.05	Antibakteri

8	*Phytol*	$C_{20}H_{40}O$	Terpenoid	2.90	Antimikroba, Antiinflamasi, Antikanker and Antijamur
9	*Hexadecanoic acid, methyl ester*	$C_{17}H_{34}O_2$	Fatty acid	2.80	Antioksidan, Antibakteri and Antiinflamasi
10	*2-Pentadecanone, 6,10,14-trimethyl*	$C_{18}H_{36}O$	Terpenoid	2.61	Antimikroba, Antiinflamasi and Antikanker
11	*9,12,15- Octadecatrienoic acid, methyl ester*	$C_{19}H_{32}O_2$	Fatty acid	1.85	Antimikroba, Antikanker and Antiinflamasi
12	*Acetamide, N-(2-acetyl-3-oxo-4-isoxazolidinyl)*	$C_7H_{10}N_2O_4$	Isoxazolidinone	1.80	-
13	*Cholesta-5,7,9(11)-trien-3-ol acetate*	$C_{29}H_{44}O_2$	Steroid	1.68	Antioksidan
14	*6ß- Hydroxyfluoxymesterone*	$C_{20}H_{29}FO_4$	Steroid anabolik	1.29	Antiinflamasi and aktivitas anabolik
15	*2- Bromotetradecanoic acid*	$C_{14}H_{27}BrO_2$	Fatty acid	1.20	Antibakteri
16	*Ethyl iso-allocholate*	$C_{26}H_{44}O_5$	Steroid	1.14	Antimikroba, Antikanker, Antiarthritik, Antiastma, diuretik and Antiinflamasi
17	*Mannosamine*	$C_6H_{13}NO_5$	Carbohydrate	1.13	Sintesis asam sialik and analisis enzim
18	*Stevioside*	$C_{38}H_{60}O_{18}$	Glikosida diterpenoid	1.10	Pemanis alami and Antihiperglikemia
19	*Deoxyspergualin*	$C_{17}H_{37}N_7O_3$	*Small molecule*	1.05	Lupus nefritis, reaksi kronik and diabetes mellitus tipe 1
20	*3-Pyridinecarboxylic acid, 2,7,10-tris (acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro*	$C_{32}H_{39}NO_{10}$	Carboxylic acid	1.03	-
Total Abundance 80,86					

Based on the data in (Table 1), the potential of the grass leaves and the diversity of chemical compound components were found. The bioactivities of the phytochemical compounds mentioned above include antioxidant, anti-inflammatory, anticancer, antibacterial, antimicrobial, antifungal, cholesterol, enzymatic and lipophilic analyses, as well as some whose functions are still unclear. According to (Diningrat, Restuati, Kusdianti, Sari, & Marwani, 2018) [19], there are generally two types of phytophagia in compounds: those with preventive properties and those with curative properties. It is known that some metabolite compounds such as n-Hexadecanoic acid, Desulphosinigrin, 9,12,15-Octadecatrienoic acid, Hexadecanoic acid, methyl ester, and Cholesta-5,7,9(11)-trien-3-ol acetate are compounds that have benefits as antioxidant agents. According to (Preethi, Devanathan, & Loganathan, 2010) [20], hexadecanoic acid, methyl ester or hexadecanoic acid compounds have antioxidant, antimicrobial and anticancer power. According to research (Suharti, 2010) [21], hexadecanoic acid has a mechanism of action in inhibiting bacterial growth by absorbing nutrients in bacteria and has the capacity to inhibit water and block the enzyme system of some bacteria.

Grass leaf samples obtained from Sidomulyo Village showed the presence of organic compounds included in the class of fatty acids, thiosulfinates, vitamins, terpenoids, steroids, small molecules, filosterols, carboxylic acids and antibiotics (Table 1) which are examples of primary and secondary organic substances. The organic matter in question functions as a plant defence against the environment, and its bioavailability can be used to improve the health of the body by acting as anti-inflammatory, antibacterial, antioxidant, and anti-cancer substances. (Dixon, 2001) [22] states that organic matter loss in plants is a by-product of the evolutionary process of plants to protect the environment so that it

must always be conserved. (Prabowo, Teti, & Indria, 2014) [23], stated that various studies on bioactive compounds were carried out with the aim of human health, whether it was used as a supplement, medicine for humans and also used for the food and beverage industry and agriculture.

According to the biosynthetic pathways of the identified metabolites, there were 11 different types of metabolites in the grass clippings samples. The metabolite types with more abundant spectra can be seen as follows (Figure 4.9).

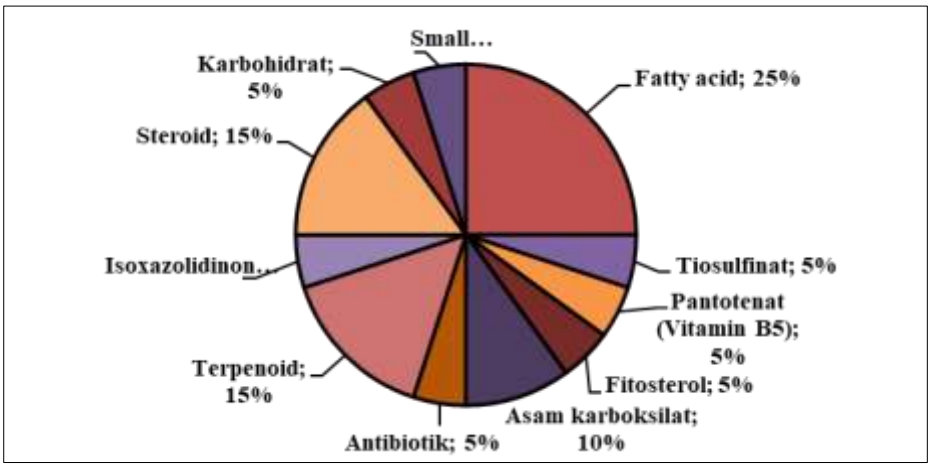

Figure 2 Abundance of metabolite compound classes in the leaves of belulang grass

Based on (Figure 2), it can be observed that the number of different types of sensitisants in each class that can be identified from the leaf grasses of locusts is different. These types of sensuality include components from the classes of fatty acids, thiosulfinic, pantothenic, phytosterols, carboxylic acids, antibiotics, terpenoids, isoxazolidinone, steroids, carbohydrates, and small molecules, all of which have different distribution ranges compared to the percentage of sensuality of these classes, which is about 88.86 per cent (Table 1). The dominant compound components in the methanolic extract of grapevine leaves analysed by GC-MS were 25% of the fatty acid class. Furthermore, they are involved with other groups, which provide abundant pharmaceutical potential for the use of compounds obtained from the leaves of locust grass.

Based on the results of metabolite profile analysis on the leaves of belulang grass, fatty acids were identified as the most common due to their volatile nature, while terpenoids were identified as the most common of the other classes due to their aromatised nature. According to (Isa, 2011) [24], methyl esters have volatile properties (easily soluble) similar to 7,10-octadecadienoic acid. This compound is also present in the linoleic acid class, which is used to make fats. Linoleic and linolematic are examples of non-essential fats that have long chains. According to (Zheng, C. J., Yoo, J. S., Lee, T. G., Cho, H. Y., Kim, Y. H., & Kim, 2005) [25], the mechanism of action of unsaturated fatty acids as antibacterial agents is related to their ability to interfere with fatty acid synthesis. Bacterial synthesis in foods is very important because it produces a large number of lipid-containing components, such as membranes. On the other hand, according to (Nur, R., Mu'nisa, A., & Hala, 2019) [26], terpenoids are a type of chemical compound that has a fragrant aroma. These bioactive substances can be used in pharmaceuticals as antimicrobial and anticancer agents. (Heliawati, 2018) [27] states that terpenoids function as antiseptics, anaesthetics, antispasmodics, sedatives, components of food aromas and perfumes (monoterpenoids), and as growth regulators.

4. Conclusion

Based on the results of the study, it was concluded that the metabolite profile of belulang grass (*Eleusine indica* (L.) Gaertn) leaves using GC-MS obtained 69 peaks of compounds that were successfully identified with 20 compounds with the highest peak and percent area. The highest peak has a percent area of 31.25% which consists of n-hexadecanoic acid, I-(+)-ascorbic acid 2,6-dihydroxy-decanoic acid and pentadecanoic acid. While the results of the metabolite biosensory pathway search obtained 11 classes of metabolite classes with the highest class being fatty acids by 25%, terpenoids and steroids 15% and carboxylic acids 10%. The compounds contained in belulang grass leaves all have different roles of activity and can have potential as medicinal materials for the future. Further research needs to be carried out related to activity tests and IC50 on the leaves of belulang grass (*Eleusine indica* (L.) Gaertn).

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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