



Indigenous Knowledge Affords Antimicrobial Plants for the Management of Infections

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Authors' contributions

This work was carried out in collaboration among all authors. Authors JA, WA and SAA involved in conception and design of the study, data collection and analysis, and writing of the manuscript. Author FAA involved in conception and design of the study and reviewed the manuscript, corrected and improved the scientific quality of the manuscript. Author IKA contributed to the drafting of the manuscript, reviewed and improved quality the final manuscript. All authors have read and accepted this manuscript.

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ABSTRACT

Aim: The research was carried out to ascertain the antimicrobial effect of the plants *Omphalocarpum ahia*, *Homalium letestui*, and *Coelocaryon oxycarpum*, which are used locally to treat some infectious diseases.

Place and Duration of Study: Department of Biomedical Sciences, School of Allied Health Sciences, University of Cape Coast, Cape Coast, Ghana and Department of Herbal Medicine, KNUST, Ghana from June to August 2014.

Method: The stem barks of the plants were extracted with 70 % methanol and successively

partitioned with petroleum ether, ethyl acetate, methanol to obtain three different fractions. The antimicrobial activities of the extracts and fraction against MRSA, *S. typhi*, *E. coli*, *S. pneumoniae*, *P. aeruginosa*, *E. faecalis*, and *S. aureus* were determined using the disk diffusion method and the minimum inhibition concentration by the serial microplate dilution method with 0.2 mg/ml p-iodonitrotetrazolium as growth indicator whereas gentamycin was used as the positive control. Phytochemical tests on the plant materials were done according to standard methods.

Result: All the fractions of each plant had activity against some of the bacteria. Ethyl acetate (EA) and hydro-methanolic (CE) extracts of *Coelocaryon oxycarpum* exhibited activity against all selected bacteria with MIC ranging from 0.625-5 mg/ml for CE and 0.3125-5 mg/ml for EA. Hydro-methanolic (CE) extracts of *Omphalocarpum ahia* also exhibited antibacterial activity against all the selected bacteria.

Conclusion: The current research showed that *Omphalocarpum ahia* and *Coelocaryon oxycarpum* have considerable antimicrobial activity against all the strains used in the study. Local knowledge may afford lead materials for the development of novel antimicrobial agents.

Keywords: Antimicrobials; *homalium letestui*; *omphalocarpum ahia*; *coelocaryon oxycarpum* phytochemicals; medicinal plants.

1. INTRODUCTION

Previous researches have shown that most human infectious diseases are caused by bacterial pathogens [1]. This does not only highlight the role of infections in the annual records of diseases but also how much of these infections are by bacteria, looking at their prevalence around the world. Four years on, it was documented that 26 per cent of death among children in Africa was due to bacterial disease, exceeding the percentage mortality recorded for malaria [2]. A great number of deaths worldwide can be attributed to microbial infection, according to WHO, nearly 50,000 individuals are dying every day from infectious diseases [3].

Owing to the ubiquitous nature of microorganisms, they are almost impossible to avoid. The arsenal of antimicrobial agents available has kept the fight in our favour. However, there are worrying signs that the efficacies of a number of these agents are on the wane due to microbial resistance [4]. Antimicrobial resistance threatens the effective prevention and treatment of the ever-increasing range of infections caused by microorganisms [5]. So, despite the numerous existing antimicrobial agents, the search continues for new effective antimicrobial agents [6].

During the last several decades, natural products with antimicrobial effects have been investigated to unravel novel agents as replacements for synthetic antibiotics whose efficacies have decreased due to the resistance of microorganisms [5-7]. Several medicinal plants have been shown to possess antimicrobial

activities [7-9]. Thus, medicinal plants offer a pool of resources for novel candidates in the search for safer and more effective antimicrobial agents.

Three of such prospective plants, used locally for the treatment of infections are; *Omphalocarpum ahia*, *Homalium letestui*, and *Coelocaryon oxycarpum*. *Omphalocarpum ahia* is found mostly in West Africa especially in Sierra Leone and Ghana [10]. This plant, as well as other plants from the genus *Omphalocarpum*, have been shown to have various therapeutic uses including the treatment of infections like leishmaniasis and malaria [11]. It is used in traditional medicine for the treatment of pain, inflammation, bacterial, and parasitic diseases [11]. With such great potential, scientific data on this Genus especially *O. ahia* is very scarce hence no sufficient data to back its use in traditional medicine. *Homalium letestui* of the family Flacourtiaceae [12] is a forest tree found in the rainforest of West Africa known to have numerous therapeutic uses traditionally [13]. *Homalium letestui*, especially the stem bark and root, are used traditionally by the Ibibios of the Niger Delta of Nigeria for various treatment including stomach ulcer, malaria and as an aphrodisiac [13] and among the people of Nzema west Municipality in the Western Region of Ghana it is very popular in the treatment of infections. Other members of the genus *Homalium* have been shown to possess antibacterial activities [13]. However, there is no existing published research on the antimicrobial activity of the *Homalium letestui* species even though it is locally used for that purpose just like the other members of the *Homalium* genus. *Coelocaryon oxycarpum* is an aromatic tree that

grows spontaneously to a height of more than 40 m [14]. The genus *Coelocaryon* is used in traditional medicine to treat various diseases. The stem is traditionally taken as purgative [15] and its fruit is used as a spice by the people of the north-east of Côte d'Ivoire [16] and just like the fore mentioned plants it is traditionally used by the Nzema tribe of Ghana for the treatment of infectious diseases.

All the fore mentioned plants have however been shown to be rich in phenols and polyphenols, saponins, and alkaloids. Studies have shown that phenols and polyphenols, saponins, and alkaloids are good antimicrobial compounds [17-20]. The present research was therefore carried out to ascertain the antimicrobial actions of these plants as suggested by folklore.

2. METHODS AND MATERIALS

2.1 Plant Collection and Authentication

The stem bark of *Homalium letestui*, *Coelocaryon oxycarpum*, and *Omphalocarpum ahia* were harvested from Adukrom (4°52'09.4" N 2°12'45.2" W) a village near Axim in the Nzema East Municipality of the Western region of Ghana. The plants were identified and authenticated by Mr Clifford Asare, Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Ghana. The plants with voucher numbers BHM/HOM/0190/2014, BHM/COEL/0190/2014, and BHM/OMPH/018A/2014 were deposited at the herbarium of the Department of Herbal Medicine, KNUST, Ghana.

2.2 Plant Preparation and Extraction

The stem barks of the plants were air-dried for 14 days followed by oven drying at 40°C for 48 hours. The plant materials were pulverised into a fine powder using the hammer mill, packed into brown paper bags and kept until needed for extraction. Powdered air-dried stem barks of *H. letestui* (500 g), *C. oxycarpum* (500 g), and *O. ahia* (500 g) were cold macerated with 70 % methanol for 72 hours. They were then filtered and concentrated under reduced pressure (40 °C) to yield crude extracts with yields of 6.7% ^w/_w, 5.3% ^w/_w, and 6.7% ^w/_w for *H. letestui*, *C. oxycarpum*, and *O. ahia* respectively. A portion of each extract (5 g) was reserved for the crude activity. The remaining portion of each plant extract was successively partitioned with

0.5 L each of petroleum ether, ethyl acetate, and methanol to afford respective fractions of the medicinal plants as summarized in Table 1.

2.3 Phytochemical Screening of the Selected Plants

Tests for secondary metabolites such as saponins, tannins, flavonoids, triterpenes, glycosides, and alkaloids were carried out according to the method described by [21].

2.4 Antimicrobial Assay

2.4.1 Test organisms and media

Bacterial strains such as *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), Methicillin-resistant *Staphylococcus aureus* (MRSA), *Salmonella typhi* clinical isolates, and *Streptococcus pneumoniae* (ATCC 49619) used for the study was obtained from the Department of Biomedical Sciences, University of Cape Coast, Ghana. Mueller-Hinton agar was purchased from Central Drug House (P) Ltd, New Delhi, India.

2.4.2 Antimicrobial susceptibility test of plant extracts

The antimicrobial activities of the plant extracts and fractions were determined using the disk diffusion method described by [22] with some modifications. About 20 mg of each plant extract was dissolved in Tween 80 (2 ml) and sterilized with 0.22µm of Millipore filter. It was then loaded on 8mm diameter sterile filter paper discs to achieve a 20mg/disc final concentration. Bacterial suspensions were prepared by mixing pure colonies in physiological saline and adjusted 0.5M McFarland solution (1.05×10^8 CFU/ml). Sterile swabs were then immersed in the bacterial suspensions and pressed against the edge of the test tube to remove excess moisture. The swabs from each bacterial suspension were then streaked entirely on Mueller Hinton media with exception of that of *Streptococcus pneumoniae* which is supplemented with 7% sterile sheep blood. The discs were then placed over the Mueller-Hinton agar plates. Gentamicin disc (10µg) and Tween 80 served as positive and solvent controls respectively for the study. The plates were stored in the fridge for 2 hours at 5°C to allow the plant

extract to diffuse followed by incubation at 37°C for 24 hours. The antimicrobial activity of the extracts was determined by measuring the zone of inhibition present with a Vernier calliper. Inhibition zones were regarded as antimicrobial activity.

2.5 Determination of Minimum Inhibitory Concentration (MIC) of Plant Extracts

The minimum concentration of a substance that inhibits the growth of a microbe is the MIC [22]. Tests were carried out using Mueller Hinton Broth suspension with Tween 80 to obtain concentration (5% v/v) [7]. The minimum inhibition concentration of the extracts was determined using the serial microplate dilution method with modifications [7]. A twofold serial dilution of the extracts and gentamicin (control) were prepared in a 96-well microplate. Each well of the plate contained varying concentrations of the standard drug (gentamicin) and test drugs (20mg/ml-0.31mg/ml). 10 µl bacterial suspension (10^6 CFU/ml) was added to each well to obtain a concentration of 10^4 CFU/ml. The plates were covered with sterile cling film loosely to prevent bacteria dehydration. The microplates were incubated at the temperature of 37°C for 24 hours. A 0.2mg/ml p-iodonitrotetrazolium was added to each well of the microplate as a growth indicator and incubated at 37°C for 2 hours. The lowest concentration of the extract at which the colour remains violet is considered as the MIC of the extract.

3. RESULTS AND DISCUSSION

3.1 Antimicrobial Activity of Extracts and their Fractions

Determination of the antimicrobial activities of the 70% hydro-methanolic v/v extract (CE), as well as the ethyl acetate (EA), petroleum ether (PE), and methanol (ME) extracts were initially carried out to confirm activities of each plant against the various organisms using the diffusion method.

All the crude extracts (CE) of the plants had activity against all of the test microbes except for *H. letestui* which had no activity against MRSA, *S. aureus*, and *E. faecalis* Table 2. The crude extracts (CE) of *C. oxycarpum* and *O. ahia* showed better activity against MRSA than gentamicin and the crude extracts of *C. oxycarpum* against *S. pneumoniae* had better activity than gentamicin and the activity of the crude extract of *H. letestui* against *P. aeruginosa* was higher than gentamicin Table 2.

Only the ethyl-acetate extract of *Coelocaryon oxycarpum* was effective against all the test microbes, with the extract from *H. letestui* being effective against only *P. aeruginosa* whereas the ethyl-acetate (EA) of *O. ahia* inhibited the activity of *E. coli*, MRSA, and *E. faecalis* Table 4. The ethyl acetate extract of *C. oxycarpum* had better activity against MRSA and *E. coli* as compared to gentamicin.

The methanolic extract (ME) of the plants had some activity against most of the microbes especially for *O. ahia* which was effective against all the microbes except *E. faecalis*. The antimicrobial activity of *H. letestui* and *O. ahia* resided in the methanolic extract Table 5.

The petroleum ether extract from the three plants had the least antimicrobial activity with *C. oxycarpum* inhibiting only MRSA whereas *H. letestui* inhibited only *S. typhi*. *O. ahia* had activity against *S. typhi*, *S. pneumoniae*, and *E. faecalis* Table 3.

3.2 Minimum Inhibitory Concentration (MIC)

None of the fractions of all the plants had a better minimum inhibition concentration as compared to the gentamicin as shown in Tables 2,3,4 and 5. The crude extract or 70% hydro-methanolic extract (CE) of *O. ahia* had MIC (1.25mg/ml) against MRSA as compared to the crude extract of all the plants as well as the other fractions

Table 1. Yield of crude and fractions of the various plant materials

Plant material	Mass of stem bark (g)	Crude yield g (%)	Fractions (g)		
			PE	EA	ME
<i>C. oxycarpum</i>	500	26.5(5.3)	5.2	7.9	6.1
<i>H. letestui</i>	500	33.5(6.7)	5.8	9.3	10..2
<i>O. ahia</i>	500	32.5(6.5)	7.6	10.8	8.6

PE: Petroleum ether fraction; EA: Ethyl acetate fraction; ME: methanol fraction

Table 2. Susceptibility of test microbes to 70% v/v hydro-methanolic (CE) plant extracts and reference drug

Bacteria	Gentamycin		<i>Coelocaryon oxycarpum</i>		<i>Homalium letestui</i>		<i>Omphalocarpum ahia</i>	
	ZD (mm)	MIC ($\mu\text{g/ml}$)	ZD (mm)	MIC (mg/ml)	ZD (mm)	MIC (mg/ml)	ZD (mm)	MIC (mg/ml)
MRSA	20	10	28	2.5	-	-	25	1.25
<i>S. typhi</i>	28	1.25	24	5	20	>5	19	2.5
<i>E. coli</i>	27	1.25	30	1.25	23	5	23	0.31
<i>S. pneumoniae</i>	28	1.5	29	5	22	>5	19	5
<i>P. aeruginosa</i>	24	0.625	24	2.4	26	>5	20	2.5
<i>E. faecalis</i>	30	0.31	23	0.62	-	-	21	>5
<i>S. aureus</i>	26	2.5	21	2.5	-	-	22	1.25

This symbol (-) represents no antimicrobial activity; MRSA: Methicillin-resistant *Staphylococcus aureus*; MIC: Minimum Inhibitory Concentration; ZD: Zone Diameter. It should be noted that the concentration for gentamicin is in $\mu\text{g/mL}$ while the extracts were in mg/mL.

Table 3. Susceptibility of test microbes to petroleum ether (PE) plant extracts and reference drug

Bacteria	Gentamycin		<i>Coelocaryon oxycarpum</i>		<i>Homalium letestui</i>		<i>Omphalocarpum ahia</i>	
	ZD (mm)	MIC ($\mu\text{g/ml}$)	ZD (mm)	MIC (mg/ml)	ZD (mm)	MIC (mg/ml)	ZD (mm)	MIC (mg/ml)
MRSA	20	10	14	>5	-	-	-	-
<i>S. typhi</i>	28	1.25	-	-	11	>5	12	>5
<i>E. coli</i>	27	1.25	-	-	-	-	-	-
<i>S. pneumoniae</i>	28	1.5	-	-	-	-	11	>5
<i>P. aeruginosa</i>	24	0.625	-	-	-	-	-	-
<i>E. faecalis</i>	30	0.31	-	-	-	-	18	>5
<i>S. aureus</i>	26	2.5	-	-	-	-	-	-

This symbol (-) represents no antimicrobial activity; MRSA: Methicillin-resistant *Staphylococcus aureus*; MIC: Minimum Inhibitory Concentration; ZD: Zone Diameter. It should be noted that the concentration for gentamicin is in $\mu\text{g/mL}$ while the extracts were in mg/mL.

Table 2. The CE of *O. ahia* effectively inhibited *S. typhi* Table 2 but not as effective as the ethyl acetate (EA) extract of *C. oxycarpum* which in turn had better MIC than the EA of the other plants Table 4. Even though *C. oxycarpum* had the highest zone of inhibition against *E. coli* (30mm), its MIC was high as compared to the CE of *O. ahia* as well as the EA and methanolic extract (ME) of *O. ahia*. EA of *O. ahia* had a lower MIC when compared to the EA of the other plants Table 4. The fractions of *C. oxycarpum* exhibited the best inhibitions (lowest MICs) against *P. aeruginosa*. The EA of *C. oxycarpum* has the best MIC against *E. faecalis* Table 4. The various fractions of *H. letestui* had the least inhibitory activity.

3.3 Phytochemical Screening of Plant Extract

The preliminary phytochemical tests on the 70 % methanol extracts and the fractions of *C. oxycarpum*, *O. ahia*, and *H. letestui* revealed the presence of the major secondary metabolites shown in Table 6.

Plants' bioactive constituents have been reported to exhibit resistance against bacteria, fungi, and pests and this explains the antimicrobial activities demonstrated by some plants [23]. In this study, the antimicrobial potential of three plants; *Coelocaryon oxycarpum*, *Omphalocarpum ahia*, and *Homalium letestui* were evaluated whereas gentamycin was used as the positive control as it is known to be effective against both gram-positive and gram-negative bacteria including methicillin-resistant *Staphylococcus aureus* [24-25]. The antibacterial properties of the plant extracts were determined by testing them against *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *MRSA*, *Salmonella typhi* and *Streptococcus pneumoniae*. The current study found the presence of other phytochemicals including tannins, flavonoids, and terpenoids in the crude extracts of all three plants (Table 6) and these phytochemicals have been previously reported to have an antimicrobial effect. [11,18,20,26].

The CE and ME of *Coelocaryon oxycarpum* exhibited antibacterial properties against all the bacterial strains used in the study as shown in Table 2 and Table 5. This indicates that these fractions have some activity against gram-negative and gram-positive bacteria just as gentamycin. Moreover, these fractions showed

higher activity (zone diameter breakpoints) to methicillin-resistant *Staphylococcus aureus* (MRSA), *E. coli*, *S. pneumoniae* as compared to gentamycin even though the minimum inhibitory concentrations were poor. Comparing the result of the gentamycin to the extracts it was realized that smaller quantities (μg) of gentamycin were required to produce a similar effect as larger quantities (mg) of the plant extracts. This may be due to the pure nature of gentamycin compared to the 70% hydro-methanolic v/v extract which has several constituents that may be causing a dilutional effect on the overall activities of the extracts. The PE of *Coelocaryon oxycarpum* showed activity against only methicillin-resistant *Staphylococcus aureus*. ME of *Coelocaryon oxycarpum* was also active against *E. coli*, *P. aeruginosa*, and *S. pneumoniae* but showed less activity as compared to EA and CE of the plant.

For *Omphalocarpum ahia*, it was realized that CE exhibited inhibition zone diameters between (19-25mm) and was quite similar to CE of *Coelocaryon oxycarpum* (21-30mm). The CE of *Omphalocarpum ahia* showed activity against all bacterial strains and best minimum inhibitory concentrations even though all plants showed similar results in the preliminary phytochemical screening. This difference in the activity could be associated with the relative quantities and the specific type of phytochemicals present. As noticed in table 3, the PE of *Omphalocarpum ahia* and the other plants showed the lowest activity or no activity against most bacterial species compared to the other fractions. As demonstrated in table 5 a few of the phytochemicals are found in the PE fraction. This indicates that most of the bioactive agents are in the polar fraction. These fractions have to be further targeted in the future to explore the possible specific phytochemicals responsible for the activities.

Previous reports, as well as our preliminary phytochemical screening of *Homalium letestui*, have revealed similar result [27] of containing phytochemicals which are effective against microbial organisms [18-19] However, in the present study, CE, PE, ME, and EA of *Homalium letestui* was not as effective as compared to the other plants. It showed a considerable level of antimicrobial activity against only a few of the organisms with all the minimum inhibition concentrations being 5mg/ml and/or above. This may also be due to the specific types or quantities of tested phytochemicals present in the fractions.

Table 4. Susceptibility of test microbes to ethyl acetate (EA) plant extracts and reference drug

Bacteria	Gentamicin		<i>Coelocaryon oxycarpum</i>		<i>Homalium letestui</i>		<i>Omphalocarpum ahia</i>	
	ZD (mm)	MIC ($\mu\text{g/ml}$)	ZD (mm)	MIC (mg/ml)	ZD(mm)	MIC (mg/ml)	ZD (mm)	MIC (mg/ml)
MRSA	20	10	23	5	-	-	11	>5
<i>S. typhi</i>	28	1.25	25	1.25	-	-	-	-
<i>E. coli</i>	27	1.25	29	1.25	-	-	11	0.625
<i>S. pneumoniae</i>	28	1.5	23	5	-	-	-	-
<i>P. aeruginosa</i>	24	0.625	19	1.25	19	>5	-	-
<i>E. faecalis</i>	30	0.31	19	0.3125	-	-	9	5
<i>S. aureus</i>	26	2.5	22	1.25	-	-	-	-

This symbol (-) represents no antimicrobial activity; MRSA: Methicillin-resistant *Staphylococcus aureus*; MIC: Minimum Inhibitory Concentration; ZD: Zone Diameter. It should be noted that the concentration for gentamicin is in $\mu\text{g/mL}$ while the extracts were in mg/mL

Table 5. Susceptibility of test microbes to methanol (ME) plant extracts and reference drug

Bacteria	Gentamicin		<i>Coelocaryon oxycarpum</i>		<i>Homalium letestui</i>		<i>Omphalocarpum ahia</i>	
	ZD (mm)	MIC ($\mu\text{g/ml}$)	ZD (mm)	MIC (mg/ml)	ZD (mm)	MIC (mg/ml)	ZD (mm)	MIC (mg/ml)
MRSA	20	10	17	5	-	-	19	>5
<i>S. typhi</i>	28	1.25	-	-	14	>5	14	>5
<i>E. coli</i>	27	1.25	17	>5	16	5	18	0.31
<i>S. pneumoniae</i>	28	1.5	18	5	18	>5	15	5
<i>P. aeruginosa</i>	24	0.625	20	1.25	-	-	21	5
<i>E. faecalis</i>	30	0.31	-	-	-	-	-	-
<i>S. aureus</i>	26	2.5	-	-	-	-	20	1.25

This symbol (-) represents no antimicrobial activity; MRSA: Methicillin-resistant *Staphylococcus aureus*; MIC: Minimum Inhibitory Concentration; ZD: Zone Diameter. It should be noted that the concentration for gentamicin is in $\mu\text{g/mL}$ while the extracts were in mg/mL

Table 6. Secondary metabolites of selected plants

Plant constituents	<i>Coelocaryon oxycarpum</i>				<i>Homalium letestui</i>				<i>Omphalocarpum ahia</i>			
	CE	PE	EA	ME	CE	PE	EA	ME	CE	PE	EA	ME
Tannins	+	-	-	+	+	-	-	+	+	-	-	+
Flavonoid	+	-	+	+	+	-	+	+	+	-	-	+
Terpenoids	+	+	+	-	+	+	-	-	+	+	+	-
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-
Glycosides	+	-	+	+	+	-	+	+	+	-	-	+
Saponins	+	-	-	+	+	+	+	+	+	-	+	-
Phytosterols	+	+	+	-	+	+	-	-	+	+	+	-

The symbol (+) indicates positive and (-) indicates negative. CE: Crude extract, PE: Pet. ether Extract, EA: Ethyl acetate extract, ME: Methanol extract

As already established, the antibacterial activity of the plants observed could be attributed to the presence of phytochemicals [17]. Also worth noting that the activities of the fractions and extract observed could be affected by the dilutional power (within the test medium) of the component compound which may be present in the plants [28]. In all instances, the crude extract of each plant gave a better activity. This could be a result of an additive effect of all phytochemicals present. More work must be done to characterise the specific compounds in these plants.

4. CONCLUSION

Plant extracts, especially *Omphalocarpum ahia* and *Coelocaryon oxycarpum* may possess compounds with antimicrobial properties that can be harnessed in the development of new drugs. Further investigations can be done to ascertain and isolate the compounds effective against the strains of resistant bacteria. The activity against *MRSA* especially needs to be further explored for a possible bioactive agent for the methicillin resistance.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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