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Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Zingiber officinale* ☆

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ABSTRACT

The essential oil and oleoresins (ethanol, methanol, CCl_4 and isooctane) of *Zingiber officinale* were extracted respectively by hydrodistillation and Soxhlet methods and subjected to GC–MS analysis. Geranial (25.9%) was the major component in essential oil; eugenol (49.8%) in ethanol oleoresin, while in the other three oleoresins, zingerone was the major component (33.6%, 33.3% and 30.5% for, methanol, CCl_4 and isooctane oleoresins, respectively). The antioxidant activity of essential oil and oleoresins were evaluated against mustard oil by peroxide, anisidine, thiobarbituric acid (TBA), ferric thiocyanate (FTC) and 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging methods. They were found to be better antioxidants than butylated hydroxyanisole (BHA). The antimicrobial properties were also studied using various food-borne pathogenic fungal and bacterial species. The essential oil and CCl_4 oleoresin showed 100% zone inhibition against *Fusarium moniliforme*. For other tested fungi and bacteriae, the essential oil and all oleoresins showed good to moderate inhibitory effects. Though, both essential oil and oleoresins were found to be effective, essential oil was found to be better than the oleoresins.

1. Introduction

Herbs and spices, which are important part of the human diet, have been used for thousands of years to enhance the flavor, color and aroma of food. In addition to boosting flavor, herbs and spices are also known for their preservative (Neilsen and Rios, 2000), antioxidative (Shobana and Naidu, 2000), antimicrobial (Salie et al., 1996) and various other medicinal values (Wood et al., 2001), which forms one of the oldest sciences. Scientific experiments since the late 19th century have documented the antimicrobial and antioxidative properties of some spices, herbs and their components.

At present, it is estimated that about 80% of the world population relies on botanical preparations as medicines to meet their health needs. Herbs and spices are generally considered safe and proved to be effective against certain ailments. Fortunately, even long-term consumption of these substances is not known to produce any side effects. They have been extensively used in many Asian and African countries since ancient times. However, in recent years, in view of their beneficial effects, use of spices/herbs has been gradually increasing in developed countries also. In traditional Indian medicine or Ayurveda, ginger and many other spices have been used as medicine (Langer, 1998).

Ginger is one of the oldest herbs known by the people and is one of the earliest spices to be known in the east. Ginger of the commerce consists of thick scaly rhizomes of the plant *Zingiber officinale*, belonging to the family Zingiberaceae. The plant is indigenous to warm tropical climates, particularly southeastern Asia. It is now extensively cultivated in India, China, Africa, Jamaica, Mexico and Hawaii (Evans, 1989). Indian ginger plant is an erect perennial, growing from 1–3 ft. in height. Mostly gingers in cultivation are sterile cultivars grown for the edible rhizomes and flowers are rarely seen. The rhizomes (spice of commerce) are aromatic, thick lobed, branched and scaly structures with a spicy lemon-like scent. It is well known that ginger rhizomes contain both aromatic and pungent components. The essential oil and oleoresins extracted from ginger rhizomes are very valuable products responsible for the characteristic ginger flavor and pungency. Both oil and oleoresins are used in many food items, soft drinks, beverages and many types of medicinal substances.

Abbreviations: BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; PG, propyl gallate; TBA, thiobarbituric acid; DPPH, 2,2'-diphenyl-1-picrylhydrazyl; FTC, ferricthiocyanate; GC–MS, gas chromatography–mass spectrometry; PV, peroxide value.

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There are numerous studies on the composition and activities of ginger essential oil; however, ginger oleoresins are not studied so vastly. The present paper deals with the chemistry, antioxidative and antimicrobial behavior of essential oil and oleoresins (extracted in ethanol, methanol, carbon tetrachloride and isooctane) of *Z. officinale*. The objective is the comparative study of antioxidative and antimicrobial properties of ginger essential oil and oleoresins.

2. Experimental

2.1. Chemicals and microbial cultures

All chemicals and solvents used were of analytical grade. Thiobarbituric acid (TBA), diphenylpicrylhydrazyl radical (DPPH) and linoleic acid were purchased from Acros (New Jersey, USA). Butylated hydroxyanisole (BHT), butylated hydroxytoluene (BHA), propyl gallate (PG) and 2,4-dinitrophenylhydrazine were purchased from s. d. fine-chemicals Ltd., Mumbai, India. Streptomycin and chloramphenicol were purchased from Ranbaxy Fine chemicals Ltd., New Delhi, India. Crude mustard oil was purchased from local oil mill, Gorakhpur, India.

In order to determine the antimicrobial efficacy of the volatile oil and oleoresins, various food-borne and pathogenic fungi and bacteriae were used. The fungi, whose sensitivity was checked, are *Aspergillus niger* (AN), *Aspergillus flavus* (AF), *Aspergillus oryzae* (AO), *Aspergillus awamori* (AA) and *Fusarium monoliforme* (FM); and the bacteriae tested are *Escherichia coli*, *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), *Proteus vulgaris* (PV) and *Klebsiella pneumoniae* (KP). The fungi and bacteriae were purchased from Microbial Type Culture Collection MTCC, Chandigarh, India.

2.2. Extraction of essential oil and oleoresins

The mature and healthy rhizomes of ginger were purchased from the local market of Gorakhpur, India. They were properly washed and thinly grated and were used for the extraction of essential oil and oleoresins. Essential oil was extracted by hydrodistillation process using a Clevenger's type apparatus in accordance with the method recommended by European Pharmacopoeia Maisonneuve and Sainte (1983). Light yellow colored oil, with a pleasant odor, was obtained which was separated and dried over the minimum amount of anhydrous sodium sulfate to remove traces of moisture.

The oleoresins were extracted from ginger rhizomes with the help of Soxhlet apparatus using four different solvents viz., ethanol, methanol, CCl_4 and isooctane. The essential oil and oleoresins so obtained were stored at low temperature (4 ± 2 °C) in dark for further use.

2.3. Phytochemistry

The Ginger oil and each oleoresin was subjected separately to gas chromatography–mass spectrometry (GC–MS) analysis using a Hewlett–Packard gas chromatograph (Model 6890) coupled with a quadruple mass spectrometer (Model HP 5973) and a Perkin Elmer Elite – 5MS capillary column (5% phenylmethylsiloxane; 30 m × 0.25 mm × 0.25 m). The interphase, ion source and selective mass detector temperatures were maintained at 280 °C, 230 °C and 150 °C, respectively. Helium was used as a carrier gas at a flow rate of 1.0 mL/min for essential oil and 1.5 mL/min for oleoresins. For the ginger oil, the oven temperature was programmed linearly as: at 60 °C for one minute; then increased from 60 to 185 °C at the rate of 1.5 °C/min and held at 185 °C for one minute, then again increased from 185 °to 275 °C at the rate of 9 °C/min and held at 275 °C for two minute. The oven temperature for oleoresins was programmed as follows: 70 °C (zero min), increased from 70 °C to 280 °C at the rate of 5 °C /min and held at 280 °C for 20 min.

2.4. Identification of components

The components were identified on the basis of comparison of their retention indices and mass spectra with published data (Gurib-Fakim et al., 2002; Singh et al., 2005a,b,c; Shivanand et al., 2004, 2005) and computer matching was done with the Wiley 275 and National Institute of Standards Technology (NIST 3.0) libraries provided with the computer controlling GC–MS systems. The retention indices were calculated using a homologous series of *n*-alkanes C_6–C_{31}.

2.5. Antioxidant activity

The antioxidant activity of ginger oil and its different oleoresins were compared to BHA, BHT and PG by carrying *in vitro* tests including peroxide, anisidine, thiobarbituric acid value, DPPH radical scavenging and total antioxidant activity by ferric thiocyanate (FTC) methods.

2.6. Sample preparation

The ginger oil and oleoresins were added individually to unrefined crude mustard oil at the concentration of 200 ppm (v/v). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) were also added to mustard oil at the same concentration i.e. 200 ppm (w/v). An equal quantity of mustard oil without any additives was taken as control for peroxide, TBA and anisidine methods.

2.7. Peroxide value

This parameter measures the total peroxide and hydroperoxide oxygen content of the edible oil system. The peroxide values were determined according to the procedure prescribed by IUPAC (Paquot, 1979). Five grams of mustard oil samples were dissolved in 30 mL of gl. acetic acid: chloroform (3:2) solution, 0 5 mL saturated KI solution was added and the mixture was shaken. After one minute 30 mL water was added and the whole solution was titrated with 0.01 N $Na_2S_2O_3$ using 1% starch indicator. Titration was continued, shaking the flask vigorously until the blue color just disappeared. The peroxide value was calculated as milliequivalents of peroxide per kilogram of sample

$$\text{Meq of peroxide/kg of oil} = \frac{S \times M \times 1000}{\text{Wt of sample (gm)}}$$

where S = mL of $Na_2S_2O_3$ consumed, and M = concentration of $Na_2S_2O_3$ (0.01)The reactions for this method are:

$$\mathrm{ROOH} + 2\mathrm{H}^+ + 2\mathrm{I}^- \rightarrow \mathrm{I}_2 + \mathrm{ROH} + \mathrm{H}_2\mathrm{O}$$
$$\mathrm{ROOR} + 2\mathrm{H}^+ + 2\mathrm{I}^- \rightarrow \mathrm{I}_2 + 2\mathrm{ROH}$$
$$\mathrm{I}_2 + 2\mathrm{S}_2\mathrm{O}_3^{2-} \rightarrow \mathrm{S}_4\mathrm{O}_6^{2-} + 2\mathrm{I}^-$$

where ROOH is a lipid hydroperoxide, and ROOR is lipid peroxide.

2.8. TBA value

The test was performed according to the methods previously reported by Kikuzaki and Nakatani (1993) with minor modifications. To 10 g of mustard oil sample, 0.67% thiobarbituric acid (20 mL) and benzene (25 mL) were added. This mixture was shaken continuously for 2 h using mechanical shaker. After 2 h, supernatant was taken and placed in boiling water-bath for 1 h. After cooling, absorbance of supernatant was measured at 540 nm with Hitachi-U-2000 spectrophotometer. The thiobarbituric acid value (meq. of malondialdehyde/g) was calculated as

$$\text{TBA value} = \frac{3.2 \times O.D.}{0.15 \times W}$$

where *O.D.* is absorbance at 540 nm, and W is the weight of oil samples in grams.

In this reaction, malondialdehyde reacted with TBA to form a pink pigment that was measured spectrophotometrically at 540 nm.

2.9. Anisidine value

The anisidine value measures 2-alkenals and was determined according to the method described earlier (Singh et al., 2005a,b,c). Sample (0.5 ± 0.1) g was dissolved in isooctane and volume was made up to 50 mL with isooctane. Five millilitres of this solution was mixed with 1 mL of 0.25% of *p*-anisidine reagent and kept in dark for 10 min. Its absorbance (A_2) was measured at 350 nm using the same spectrophotometer. A blank test (without the addition of anisidine reagent) was also done (A_1). The anisidine value was calculated as:

$$\text{Anisidine value} = \frac{(A_2 - A_1) \times 1.2 \times 50}{\text{Wt of oil sample (g)}}$$

2.10. DPPH free radical scavenging activity

The DPPH radical absorbs at 517 nm and the antioxidant activity can be determined by monitoring the decrease in this absorbance. The capacity of ginger oil, its oleoresins/synthetic antioxidants to scavenge the lipid-soluble DPPH radical was monitored at 517 nm by the method reported earlier (Cuendet et al., 1997). For this, 1 mL methanolic solution of ginger oil and oleoresins at different concentrations (5–20 μL/mL) were mixed with 4 mL of 0.004% methanolic solution of DPPH. The absorbance was measured at 517 nm after 30 min. Control (without any additive) and standards (containing synthetic antioxidants viz. BHA, BHT and PG; in place of oil and oleoresins) were also subjected to the same procedure for comparison. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{A_c - A_t}{A_c} \times 100$$

where A_c is the absorbance of control sample, and A_t is the absorbance of test sample.

2.11. FTC method

The antioxidant activities of ginger oil, oleoresins/synthetic compounds were determined according to FTC method in linoleic acid emulsion (Mitsuda et al., 1996). The reaction medium contained ginger oil and oleoresins at the concentration of 1 mg/100 mL of absolute ethanol (2 mL), an emulsion of 2.51% linoleic acid in ethanol (2 mL), 4 mL of 0.05 M-phosphate buffer (pH 7.0) and 2 mL of distilled water. The solution (10 mL) was mixed and incubated at 40 °C in dark. The same reaction medium, without any additive was taken as control sample. Synthetic antioxidants (BHA, BHT and PG) were used for comparison, in the same concentration. At regular intervals during incubation, a 0.1 mL aliquot of the mixture was diluted with 9.7 mL of 75% ethanol, followed by the addition of 0.2 mL of 30% ammonium thiocyanate and 0.1 mL of 20 mM of $FeCl_2$ in 3.5% HCl. The absorbance was measured at 500 nm. These steps were repeated every 24 h until the control reached its maximum absorbance value. The low absorbance value indicates the efficiency of the test samples to inhibit lipid oxidation.

3. Antimicrobial investigations

3.1. Antifungal activity

The selected fungi were grown on Czapeck dox agar (CDA) medium and plates were incubated at 37 °C. The mycelial discs of 5 mm diameter were cut along with adhering agar from the seven days old cultures and were used as inoculums throughout the present study. The antifungal activity of the volatile oils and oleoresins against fungi were undertaken using inverted petri plate and poison food techniques (Ramdas et al., 1998).

3.2. Poisoned food technique

The calculated amount (2, 4 and 6 μL) of each of the crude ginger oil and oleoresins were mixed with the 25 mL of the sterilized culture medium (≅45 °C) and it was poured in previously sterilized petri plates. The mycelial discs (as made above) of the test fungi were inoculated in these plates under aseptic conditions and the plates were incubated at 37 °C. In the control set, the essential oil/oleoresins were replaced by an equal amount of water. Radial growths of fungi in terms of average diameter (mm) were recorded on the 5th day. The data were used for calculating percent inhibition of mycelial growth

$$\% \text{ Mycelial zone inhibition} = \frac{d_c - d_t}{d_c} \times 100$$

where d_c and d_t are average diameters of mycelia colony of control and treated sets, respectively.

3.3. Inverted petri plate technique

The petri plates seeded with respective test fungi were inverted upside down and pre-sterilized filter paper disc (Whatman no. 1, 10 mm, d) were placed on the center of its inverted lid. The required doses (2, 4 and 6 μL) of undiluted sample (ginger oil and oleoresins) were aseptically soaked on filter paper discs. During the entire experiment, complete aseptic conditions were maintained. Petri plates were then incubated at 37 °C for five days in inverted position. Each test was replicated for three times and fungal sensitivity was calculated in terms of percent mycelial zone inhibition.

3.4. Antibacterial activity

The antibacterial properties of ginger essential oil and oleoresins were studied by agar well and disc diffusion methods (Bauer et al., 1996).

3.5. Agar well method

The selected bacterial strains were inoculated into 10 mL of sterile nutrient broth, and incubated at 37 °C for 16–18 h. Using a sterile cotton swab, the nutrient broth cultures were swabbed on the surface of sterile nutrient agar plates. Agar wells were prepared with the help of sterilized cork borer with 8 mm diameter. Using a micropipette, 100 μL solution of ginger oil and oleoresins (obtained by dissolving 10 μL of each in 1 mL DMSO), were added to different wells in the plates. The plates were incubated in an upright position at 37 °C for 24 h. The diameters of inhibition zones (in mm) were measured.

3.6. Disc diffusion method

Filter paper discs (Whatman no. 1) of 8 mm diameter were prepared and sterilized. Using an ethanol dipped and flamed forceps, these discs were aseptically placed over nutrient agar plates seeded with the respective test microorganisms. Crude ginger oil and oleoresins (10 μL) were aseptically transferred to these discs. The plates were incubated in an upright position at 37 °C for 24 h. The diameters of inhibition zones (in mm) were measured.

3.7. Antibiotic sensitivity testing

The cultures were enriched in sterile nutrient broth for 16–18 h at 37 °C. Using sterile cotton swabs; the cultures were aseptically swabbed on the surface of sterile Nutrient Agar plates. Using an ethanol dipped and flamed forceps, the antibiotics (streptomycin and chloramphenicol at the concentration 10 mg/Ml in DMSO) discs were aseptically placed over the seeded agar plates. The plates were incubated at 37 °C for 24 h and the diameter of the inhibition zones (in mm) were measured.

4. Results and discussion

4.1. Phytochemistry

Careful and detailed interpretations of the experimental data (EM fragmentation, retention indices) were carried out which permitted identification of a large number of components (Tables 1 and 2). Table 1 shows identification of 57 components in ginger oil, representing about 92.7% of the total amount. From Table 2, it is evident that in ethanol oleoresin, 55 components constituting 92.9% of the total weight; in the methanol oleoresin, a total of 72 components making 89.7% of the whole mass; in case of carbon tetrachloride oleoresin, 69 compounds constituting about 95% of the total weight and in isooctane oleoresin 51 compounds representing 87.2% of the total amount were identified. Five major components of essential oil and each of the oleoresins are as given under:

Ginger oil: Geranial (25.9%), α-zingiberene (9.5%), (*E*,*E*)-α-farnesene (7.6%), neral (7.6%), ar-curcumene (6.6%); EtOH oleoresin: eugenol (49.8%), zingerone (14.5%), *trans*-6-shogaol (5.9%), geraniol (3.7%), borneol (1.9%); MeOH oleoresin: zingerone (33.6%), *trans*-6-shogaol (14.9%), diacetoxy-[6]-gingerdiol (4.9%), decanal (3.8%), α-zingiberene (2.7%); CCl_4 oleoresin: zingerone (33.3%), *trans*-6-shogaol (10.4%), geranial (7.5%), neral (4.9%), methyldiacetoxy-[6]-gingerdione (3.5%) and isooctane oleoresin: zingerone (30.5%), palmitoleic acid (10.9%), *trans*-6-shogaol (9.3%), palmitic acid (8.9%), diacetoxy-[6]-gingerdiol (3.3%).

[2]-Paradol (0.1%) has been found in MeOH oleoresin (Table 2), which was not reported by earlier workers (Gurib-Fakim et al., 2002; Singh et al., 2005a,b,c; Shivanand et al., 2004, 2005). In literature, many variations have been found in the chemical composition of ginger oil. Agrawal et al. (2001) reported curcumene as the major constituent in the fresh ginger rhizomes, while Menut et al. (1994) identified citral as the main constituent of ginger oil. In our previous report on ginger oil (Singh et al., 2005a,b,c), α-zingiberene was found to be the major constituent. These differences in

Table 1
Chemical composition of *Z. officinale* essential oil analyzed by GC–MS

Compounds	%	RI[a]	Identification[b]
2-Heptanol	0.1	899	MS, RI
Tricyclene	Trace	919	MS, RI, co-GC
Alpha-pinene	0.8	928	MS, RI, co-GC
Camphene	3.0	945	MS, RI, co-GC
Sabinene	Trace	968	MS, RI, co-GC
Beta-pinene	0.1	973	MS, RI, co-GC
6-Methyl-5-hepten-2-one	0.1	980	MS, RI, co-GC
Myrcene	0.5	985	MS, RI, co-GC
Octanal	Trace	1002	MS, RI, co-GC
Alpha-phellandrene	0.1	1004	MS, RI, co-GC
3-Carene	Trace	1006	MS, RI, co-GC
p-Cymene	0.1	1020	MS, RI, co-GC
Limonene	0.5	1025	MS, RI, co-GC
Beta-phellandrene	1.4	1026	MS, RI, co-GC
1,8-Cineole	1.9	1027	MS, RI, co-GC
Terpinolene	0.1	1081	MS, RI, co-GC
2-Nonanone	Trace	1088	MS, RI
Linalool	0.9	1097	MS, RI, co-GC
Camphor	Trace	1139	MS, RI, co-GC
Citronellal	0.3	1147	MS, RI, co-GC
Borneol	2.1	1165	MS, RI, co-GC
Terpinen-4-ol	0.1	1174	MS, RI, co-GC
Criptone	0.1	1180	MS, RI
Alpha-terpineol	0.9	1190	MS, RI, co-GC
Citronellol	0.7	1228	MS, RI, co-GC
Neral	7.4	1238	MS, RI, co-GC
Geraniol	3.4	1254	MS, RI, co-GC
Geranial	*25.9*	*1274*	*MS, RI, co-GC*
Endo-bornylacetate	0.3	1282	MS, RI
2-Undecanone	0.2	1294	MS, RI
Alpha-copaene	0.2	1366	MS, RI
Geranyl acetate	0.6	1376	MS, RI
Beta-elemene	0.2	1381	MS, RI
cis -Alpha -bergamotene	0.1	1396	MS, RI
cis-Beta-farnesene	0.4	1445	MS, RI
trans-Beta-farnesene	0.2	1448	MS, RI
trans-Cadina-1(6), 4-diene	0.1	1463	MS, RI
Gamma-muurolene	0.5	1467	MS, RI
ar-Curcumene	6.6	1474	MS, RI
delta-selinene	0.6	1482	MS, RI
trans-Muurola-4(14), 5-diene	0.9	1485	MS, RI
Alpha-zingiberene	9.5	1489	MS, RI
(*E,E*)-alpha-farnesene	7.6	1501	MS, RI
7-Epi-alpha-selinene	0.3	1505	MS, RI
Delta-cadinene	0.1	1507	MS, RI
Beta-sesquiphellandrene	5.1	1516	MS, RI
trans-Gamma-bisabolene	0.2	1518	MS, RI
Elemol	0.5	1538	MS, RI
Germacrene-B	0.3	1543	MS, RI
trans-Nerolidol	1.5	1556	MS, RI
trans-Sesquisabinene hydrate	0.7	1582	MS, RI
Zingiberenol	1.7	1606	MS
Guaiol	0.6	1614	MS, RI
Beta-eudesmol	1.0	1640	MS, RI
Alpha-eudesmol	0.7	1642	MS, RI
Acorenone B	0.3	1654	MS, RI
(*E,E*) Farnesal	0.2	1730	MS
Total	92.7%		

Percentages are the mean of three runs and were obtained from electronic integration measurements using selective mass detector.
Trace: <0.05.
˚Co-elution with plasticizer.
[a] The retention index was calculated using a homologous series of *n*-alkanes c6-c31.
[b] co-gc: Co-injection with an authentic sample.

the chemical composition of the oil and oleoresins from the same plant/plant part could be due to the environmental, developmental, genetic or some other factors. Yield and composition of oil and oleoresins differ widely with the production conditions (Blair et al., 2001), variety, cultivars or population (Galambosi and Peura, 1996) and on climatic and soil factors. Moreover, chemical constitution of oleoresins also depends on the nature of solvent used for extraction.

4.2. Antioxidant activity

Antioxidant activities of volatile oil and oleoresins have been carried out on mustard oil. Fig 1 shows the changes in peroxide values of different mustard oil samples. The parameter peroxide value measures the total peroxide and hydro peroxide oxygen content of the edible oil system. During the storage period of 28 days, PV of control sample increased from 44.2 meq/kg to 129.5 meq/kg, which is significantly higher than the other samples containing ginger oil, oleoresins and/or synthetic antioxidants. The ginger oil and oleoresins are found to be better antioxidants than BHA, however, their activity is lesser than BHT and PG. Peroxides are the primary products of lipid oxidation and play a central role in auto oxidation of lipids and decomposed into carbonyls and other compounds. The peroxide decomposition products, now present in the oil, may catalyze further oxidation. To measure such secondary oxidation products, anisidine and TBA values of mustard oil samples were also recorded during the storage period.

TBA value measures the malondialdehyde formed from unsaturated fatty acids resulting from oxidation of a lipid system. Fig. 2 shows changes in the TBA values of different samples. Anisidine value measures the level of aldehydes, principally 2-alkenals, present in the oil samples. The results obtained from this method are presented in Fig. 3, which showed that the samples containing essential oil and/or oleoresins had significantly, lowers anisidine value than the control set. It is also evident that the antioxidant potential of essential oil and oleoresins is comparable to BHA and BHT; however, they are less effective than PG. From Figs. 1–3; it can be interpreted that the essential oil and oleoresins can control both primary as well as secondary oxidation processes and their antioxidative potentials are comparable to BHA and BHT. In fact, they are found to be better than BHA in a few experiments.

Antioxidants react with DPPH (a stable free radical) to convert it into 1,1-diphenyl-2-picrylhydrazine. The degree of discoloration indicates the radical scavenging potential of the antioxidants. The results of this experiment are shown in Fig 4. Ginger oil and oleoresins exhibited marked DPPH free radical scavenging activity in a concentration dependent manner. Fig. 4 illustrates a significant decrease in the concentration of DPPH radical due to the scavenging ability of ginger oil, oleoresins and synthetic antioxidants.

The thiocyanate method measures the amount of peroxide formed during the initial stages of oxidation (Glucin et al., 2007). Total antioxidant activity of ginger oil and oleoresins was determined by ferric thiocyanate method in linoleic acid system. Fig. 5 clearly shows the antioxidative effects of various additives. The essential oil and ethanol oleoresin exhibited better activity as compared to other oleoresins and synthetic compounds.

As different testing methods provide only limited and particular information about antioxidant activity of any compound, we used different methods to evaluate antioxidant potential of ginger oil and its oleoresins. This had helped us to get a clearer picture. In all the experiments, essential oil and oleoresins were found to possess better antioxidative properties than BHA, however, their activities were less than that of PG, Moreover, the activity of all the four oleoresins were almost equivalent and were lesser than that of the ginger essential oil.

The antioxidative potential of ginger essential oil and oleoresins may be due to the presence of various types of compounds in them. There are many reports that emphasize that the phenolic group plays an important role in antioxidant activity (Huang and Frankel, 1997; Baratta et al., 1998 and Singh et al., 2005a,b,c). Silva et al. (2000) reported the significant scavenging effects of phenolic compounds against the DPPH free radical. Hence, the presence of phe-

Table 2
Chemical composition (%) of oleoresins (in various solvents) of *Z. officinale* analyzed by GC–MS

Compounds	EtOH	MeOH	CCl_4	Isooctane	RI[a]	Identification[b]
Butanediol	–	Trace	–	–	794	MS, RI
Octanal	0.4	1.8	1.5	–	1001	MS, RI, co-GC
Beta-phellandrene + 1,8-cineole	–	–	0.8	–	1035	MS, RI, co-GC
Terpinolene	Trace	Trace	0.9	Trace	1092	MS, RI, co-GC
Borneol	1.9	Trace	1.2	–	1173	MS, RI, co-GC
Alpha-terpineol	0.9	0.2	0.9	Trace	1194	MS, RI, co-GC
Decanal	1.2	3.8	2.1	2.7	1204	MS, RI, co-GC
Nerol	0.8	0.2	Trace	Trace	1224	MS, RI, co-GC
Neral	Trace	Trace	4.9	1.2	1243	MS, RI, co-GC
Geraniol	3.7	0.6	1.3	1.0	1256	MS, RI, co-GC
Geranial	Trace	Trace	7.5	Trace	1274	MS, RI, co-GC
Eugenol	*49.8*	0.2	0.2	1.6	*1351*	*MS, RI, co-GC*
Geranyl acetate	Trace	Trace	0.1	–	1357	MS, RI, co-GC
Methyl eugenol	0.5	Trace	–	–	1401	MS, RI, co-GC
Dodecanal	–	0.2	Trace	Trace	1409	MS, RI, co-GC
Gamma-elemene	–	0.1	Trace	Trace	1428	MS, RI
trans-Isoeugenol + *trans*-Beta-farnesene	0.5	0.2	0.1	Trace	1451	MS, RI, co-GC
ar-Curcumene	0.5	1.0	2.8	Trace	1476	MS, RI
Gamma-curcumene	0.3	0.4	Trace	Trace	1479	MS, RI
Alpha-zingiberene	0.9	2.7	2.9	Trace	1494	MS, RI
Gamma-amorphene	Trace	0.2	0.2	Trace	1496	MS, RI
(*E*,*E*)-Alpha-farnesene	0.2	0.7	0.9	Trace	1502	MS, RI
Beta-bisabolene	Trace	0.5	0.7	Trace	1505	MS, RI
Delta-cadinene	Trace	0.1	0.2	Trace	1517	MS, RI
Beta-sesquiphellandrene	0.7	1.4	1.7	0.4	1523	MS, RI
Alpha-calacorene	0.3	0.1	0.1	Trace	1538	MS, RI
Elemol	0.3	0.2	0.2	Trace	1546	MS, RI
trans-Nerolidol	1.2	0.7	0.6	Trace	1555	MS, RI, co-GC
Lauric acid	0.4	Trace	Trace	Trace	1563	MS
Spathulenol	0.9	0.4	1.9	Trace	1572	MS, RI, co-GC
trans-Sesquisabinene hydrate	Trace	0.5	–	–	1582	MS, RI
Globulol	Trace	–	Trace	Trace	1586	MS, RI
Zingiberenol	Trace	0.3	Trace	Trace	1605	MS
10-Epi-gamma-eudesmol	Trace	Trace	0.1	–	1615	MS, RI
Zingerone	14.5	*33.6*	*33.3*	*30.5*	*1675*	*MS*
(*E*,*E*) Farnesal	Trace	0.1	0.1	Trace	1736	MS
Xanthorrhizol	Trace	Trace	0.1	Trace	1746	MS, RI
Myristic acid	Trace	0.3	0.1	1.0	1761	MS, co-GC
Palmitoleic acid, methyl ester	–	–	–	2.7	1892	MS, co-GC
Palmitic acid, methyl ester	–	0.5	–	3.1	1913	MS, co-GC
Palmitoleic acid	–	–	–	10.9	1938	MS, co-GC
Palmitic acid	0.8	1.0	0.7	8.9	1960	MS, co-GC
Palmitic acid, ethyl ester	–	0.1	–	–	1979	MS, co-GC
[2]-Paradol[c]	–	0.1	–	–	2008	MS
(*E*)-[4]-Shogaol	Trace	0.1	0.3	Trace	2071	MS
linoleic acid, methyl Ester	–	0.3	–	–	2077	MS, co-GC
Oleic acid, methyl ester	–	0.1	0.2	0.6	2085	MS, co-GC
1-(4-Hydroxy-3-methoxyphenyl)-2,4-dehydro-6-decanone	Trace	Trace	Trace	0.7	2091	MS
Stearic acid, methyl ester	–	Trace	–	Trace	2110	MS, co-GC
Linoleic acid	Trace	0.6	0.4	0.6	2124	MS, co-GC
Oleic acid	0.2	0.8	0.4	1.8	2128	MS, co-GC
Linoleic acid, ethyl ester	–	0.3	–	–	2140	MS, co-GC
Oleic acid, ethyl ester	–	0.2	–	–	2146	MS, co-GC
Stearic acid	0.3	0.2	0.2	0.9	2150	MS, co-GC
cis-[6]-Shogaol	1.5	0.6	1.8	2.4	2199	MS
[6]-Paradol	0.3	2.6	0.5	1.0	2210	MS
trans-[6]-Shogaol	5.9	14.9	10.4	9.3	2289	MS
[6]-Gingerdione	0.4	–	–	Trace	2306	MS
Diacetoxy-[4]-gingerdiol	–	1.4	2.3	–	2322	MS
[6]-Gingerol	0.8	1.4	0.5	Trace	2383	MS
cis-[8]-Shogaol	Trace	0.7	0.2	–	2412	MS
[8]-Paradol	–	0.1	Trace	–	2425	MS
Acetoxy-[6]-gingerol	Trace	–	0.2	–	2438	MS
trans-[8]-Shogaol	0.6	2.0	1.5	–	2489	MS
Diacetoxy-[6]-gingerdiol	1.5	4.9	1.0	3.3	2495	MS
Methyl diacetoxy-[6]-gingerdiol	0.3 *	1.0	3.5 *	–	2511	MS
Plasticizer (phtalate)	–	–	–	2.5	2511	MS
[8]-Gingerdione	Trace	–	0.4	–	2528	MS
1-Dehydro-[6]-gingerdione	–	0.4	0.6	–	2586	MS
cis-[10]-Shogaol	Trace	0.8	0.2	Trace	2621	MS
[10]-Paradol	–	Trace	Trace	–	2640	MS
Diacetoxy-[8]-gingerdiol	–	Trace	Trace	–	2683	MS
trans-[10]-Shogaol	0.4	2.2	0.6	Trace	2704	MS
[10]-Gingerdione	Trace	0.9	0.6	–	2751	MS
Octacosane	–	–	Trace	–	2800	MS, RI, co-GC

(*continued on next page*)

Table 2 (*continued*)

Compounds	EtOH	MeOH	CCl_4	Isooctane	RI[a]	Identification[b]
(2*E*)-Geranial acetal of [4]-gingerdiol	–	Trace	Trace	–	2887	MS
Nonacosane	–	–	Trace	–	2900	MS, RI, co-GC
trans-[12]-Shogaol	–	Trace	Trace	–	2927	MS
[12]-Gingerdione	–	Trace	Trace	–	2967	MS
Triacontane	–	–	Trace	–	3000	MS, RI, co-GC
(2*Z*)-Neral acetal of [6]-gingerdiol	–	0.1	0.3	–	3010	MS
(2*E*)-Geranial acetal of [6]-gingerdiol	–	0.6	0.5	–	3073	MS
Total (%)	92.9	89.7	95.0	87.1		

Percentages are the mean of three runs and were obtained from electronic integration measurements using selective mass detector.
Trace: <0.05.
[a] The retention index was calculated using a homologous series of *n*-alkanes C_6–C_{31}.
[b] co-gc: Co-injection with an authentic sample.
[c] Not reported in references 17 and 18.
* Co-elution with plasticizer.

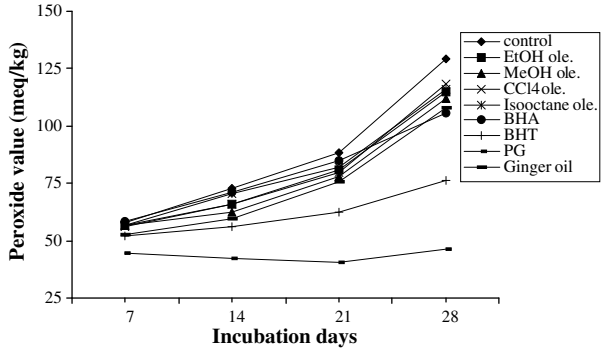

Fig. 1. Antioxidant activity of ginger oil and oleoresins in terms of peroxide values.

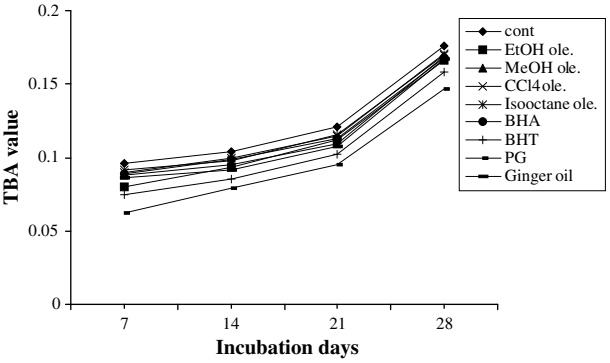

Fig. 2. Antioxidative effect of ginger essential oil and oleoresins in terms of thiobarbituric acid values.

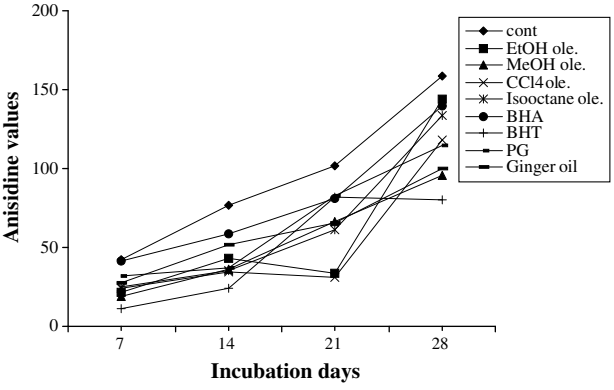

Fig. 3. Antioxidative effect of ginger oil and oleoresins in terms of anisidine values.

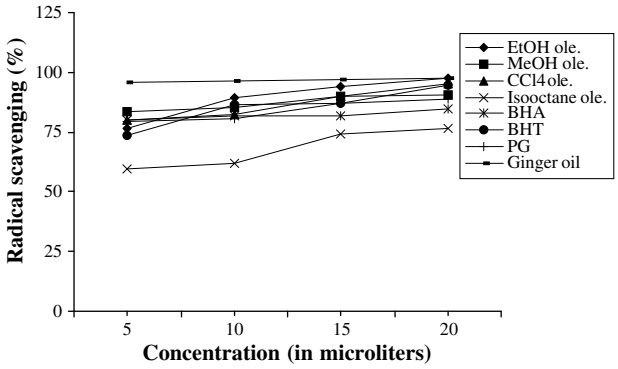

Fig. 4. Radical scavenging effect of ginger essential oil and oleoresins on 2,2′-diphenyl-1-picrylhydazyl radical.

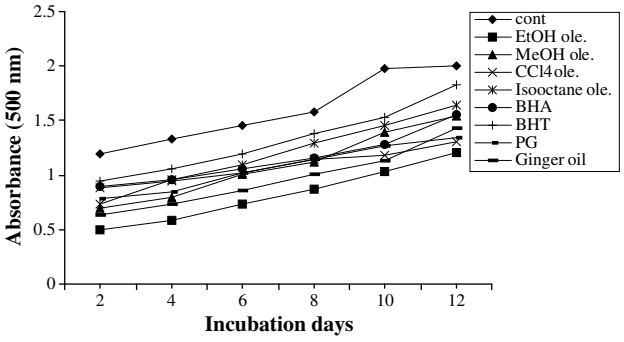

Fig. 5. Antioxidative effect of ginger essential oil and oleoresins in linoleic acid system.

nolic compounds such as eugenol, shogaols, zingerone, gingerdiols, gingerols, diacetoxy-[6]-gingerdiol, etc., in ginger oil and oleoresins (Tables 1 and 2) may be responsible for their antioxidant properties. Moreover, the antioxidant activities observed in ginger oil and oleoresins could be due to the synergistic effects of two or more compounds present in them. Lu and Foo (1995, 2001) reported that most natural antioxidative compounds often work synergistically with each other to produce a broad spectrum of antioxidative properties that create an effective defense system against free radicals. Ginger oil and oleoresins consist of a very complex mixture of various classes of organic compounds (Tables 1 and 2), which may produce either synergistic or antagonistic effects on the process of lipid oxidation.

4.3. Antimicrobial investigations

The results of antifungal investigations for ginger oil and oleoresins as obtained by food poison and inverted petri plate methods are given in Tables 3 and 4. Ginger oil and oleoresin have shown moderate to good inhibitory effects against all the tested fungal isolates. Ginger oil was found to be100% effective against *F. moniliforme* at 6 μL dose in food poison method. Its CCl_4 oleoresin has also shown complete inhibition of FM at 6 μL dose. This observation is in accordance with that of Mishra (1990) that reported the complete control of FM on mung seeds by ginger oil. The various *Aspergillus* species have also shown sensitivity towards the ginger oil and oleoresins. Nanir and Kadu (1987) and Kapoor (1997) have also reported the inhibition of *Aspergillus* species by ginger. Crude ginger extracts have also been reported earlier to exhibit activity against AF and AN in orange and pineapple juices (Akpomedaye and Ejechi, 1998). The ginger oleoresins were, however, less active than ginger oil. There are several reports on the inhibitory effect of ginger on the growth of *E. coli, Proteus species, Staphylococci, Streptococci and Salmonella* (Janes et al., 1999). However, in the present study, *E. coli* was found to be completely resistant towards all the tested samples i.e. essential oil, oleoresins and synthetic antibiotics. *S. aureus* showed sensitivity only towards essential oil and streptomycin.

The results of antibacterial investigations are given in Tables 5 and 6, which indicate that different bacterial species exhibited different levels of sensitivities towards the tested ginger oil and oleoresins. *E. coli* was found to be completely resistant towards all the tested samples i.e. essential oil, oleoresins and synthetic antibiotics. *S. aureus* showed sensitivity only towards essential oil and streptomycin. In agar well method, all the oleoresins and the essential oil showed strong inhibitory effects on *P. vulgaris* and *P. aeruginosa* while for *K. pneumoniae*, only essential oil and CCl_4 oleoresin were effective. On the other hand, in disc diffusion method, only essential oil showed activity against all the tested bacteriae except *E. coli*. From the data it is clear that the antibacterial effects of ginger essential oil is comparable to streptomycin and is better than chloramphenicol for the tested bacterial strains. The oleoresins, however, are less effective than essential oil.

The marked antimicrobial activity of essential oils and oleoresins from spices and herbs is believed to be due to phenolic compounds. From GC–MS studies (Tables 1 and 2), it is clear that the ginger essential oil and oleoresins contain considerable amounts of phenolic compounds (eugenol, shogaols, zingerone, gingerdiols, gingerols, etc.), which might be responsible for the observed antimicrobial potency. However, it is likely that the overall efficiency

Table 3
Antifungal activity of essential oil and oleoresins of *Z. officinale* using food poison method

Samples tested	Dose (μL)	% Mycelial zone inhibition (mm)[a]				
		AF	AS	AO	AN	FM
Ginger oil	2	7.0 ± 0.4	44.4 ± 1.0	25.2 ± 1.6	27.9 ± 1.8	49.4 ± 0.9
	4	12.2 ± 2.6	55.8 ± 3.0	40.2 ± 1.7	38.9 ± 1.4	67.2 ± 0.9
	6	20.6 ± 3.2	66.3 ± 5.0	51.3 ± 0.4	66.7 ± 3.1	100 ± 0
EtOH ole.	2	9.2 ± 1.2	35.6 ± 1.1	29.2 ± 1.0	25.3 ± 0.4	20.6 ± 1.1
	4	15.8 ± 2.5	42.3 ± 1.8	37.5 ± 1.0	28.4 ± 1.2	35.2 ± 0.6
	6	27.8 ± 2.2	50.0 ± 2.8	53.1 ± 1.6	36.2 ± 1.7	45.9 ± 0.8
MeOH ole.	2	6.2 ± 1.7	2.2 ± 1.9	20.9 ± 2.2	8.1 ± 3.2	8.0 ± 1.8
	4	10.3 ± 3.9	15.5 ± 1.4	26.4 ± 0.5	16.5 ± 1.1	10.6 ± 0.7
	6	16.3 ± 3.0	39.9 ± 2.3	27.9 ± 1.8	21.8 ± 0.9	16.1 ± 0.6
CCl_4 ole.	2	5.4 ± 1.7	33.3 ± 2.0	11.5 ± 4.4	2.2 ± 2.0	32.2 ± 0.5
	4	15.6 ± 3.5	46.2 ± 2.9	15.2 ± 0.9	3.2 ± 1.6	54.8 ± 1.8
	6	29.2 ± 3.4	57.8 ± 2.4	24.6 ± 1.2	8.4 ± 1.0	100 ± 0
Isooctane ole.	2	2.4 ± 1.1	22.2 ± 2.2	17.7 ± 0.9	7.8 ± 1.2	18.4 ± 1.4
	4	2.9 ± 1.0	40.6 ± 1.6	36.7 ± 1.7	21.4 ± 1.0	20.6 ± 0.8
	6	4.3 ± 2.3	56.3 ± 2.5	45.6 ± 1.1	47.8 ± 1.8	28.8 ± 1.2

AF = *Aspergillus flavus*, AS = *Aspergillus solani*, AO = *Aspergillus oryzae*, AN = *Aspergillus niger* and FM = *Fusarium moniliforme*.
[a] Data are the mean of three replicates.

Table 4
Antifungal activity of essential oil and oleoresins of *Z. officinale* using Inverted petri plate methods

Samples tested	Dose (μL)	% Mycelial zone inhibition (mm)[a]				
		AF	AS	AO	AN	FM
(Ginger oil	2	44.4 ± 1.1	35.6 ± 2.3	44.6 ± 1.8	20.1 ± 1.4	44.6 ± 1.2
	4	50.2 ± 1.8	48.6 ± 1.6	50.5 ± 1.5	26.2 ± 2.4	46.0 ± 2.6
	6	59.1 ± 0.9	55.0 ± 2.8	58.0 ± 1.9	35.1 ± 2.5	57.9 ± 0.9
EtOH ole.	2	27.8 ± 0.7	26.4 ± 2.8	10.1 ± 0.3	19.9 ± 1.0	10.1 ± 0.6
	4	36.8 ± 1.0	39.5 ± 0.9	32.6 ± 2.3	20.1 ± 1.1	32.7 ± 1.8
	6	52.9 ± 0.9	58.0 ± 1.8	53.1 ± 2.3	21.9 ± 1.4	53.1 ± 1.5
MeOH ole.	2	18.0 ± 1.7	11.2 ± 0.8	23.3 ± 1.6	–	3.3 ± 2.9
	4	25.0 ± 0.4	27.6 ± 0.5	42.2 ± 2.3	2.1 ± 1.9	28.5 ± 2.1
	6	43.6 ± 0.9	48.8 ± 2.5	56.0 ± 0.9	6.9 ± 1.5	56.0 ± 1.8
CCl_4 ole.	2	38.7 ± 1.1	20.2 ± 0.3	45.9 ± 2.6	–	45.7 ± 0.5
	4	42.5 ± 1.9	35.0 ± 1.3	47.1 ± 2.1	–	45.5 ± 1.0
	6	50.5 ± 1.6	46.9 ± 1.4	50.2 ± 0.6	2.2 ± 2.0	50.2 ± 2.4
Isooctane ole.	2	10.7 ± 2.0	21.1 ± 1.0	35.8 ± 0.8	2.5 ± 1.2	35.8 ± 2.2
	4	15.2 ± 0.8	29.6 ± 1.8	37.6 ± 2.0	8.6 ± 1.0	38.8 ± 1.4
	6	18.0 ± 0.5	45.8 ± 1.8	41.7 ± 1.9	14.7 ± 1.8	41.7 ± 1.4

– No inhibition was observed.
AF = *Aspergillus flavus*, AS = *Aspergillus solani*, AO = *Aspergillus oryzae*, AN = *Aspergillus niger* and FM = *Fusarium moniliforme*.
[a] Data are the mean of three replicates.

Table 5
Antibacterial activity of ginger oil and oleoresins against a few bacterial species using agar well diffusion method

Samples tested	Diameter of inhibition zone (mm)[a]				
	SA	EC	PV	PA	KP
Ginger oil	–	–	18.4 ± 2.5	18.8 ± 1.8	20.5 ± 1.0
EtOH ole.	–	–	13.6 ± 1.4	15.4 ± 0.8	–
MeOH ole.	–	–	–	15.7 ± 1.7	–
CCl_4 ole.	–	–	15.4 ± 1.0	16.8 ± 3.0	16.4 ± 1.6
Isooctane ole.	–	–	14.2 ± 2.2	19.1 ± 0.2	–
Streptomycin	11 ± 0.6	–	16.2 ± 0.7	19.2 ± 1.8	15.5 ± 2.6
Chloramphenicol	–	–	–	–	–
DMSO	–	–	–	–	–

– No inhibition was observed.
SA = *Staphylococcus aureus*, EC = *Escherichia coli*, PV = *Proteus vulgaris*, PA = *Pseudomonas aeruginosa* and KP = *Klebsiella pneumoniae*.
[a] Data are the means of three replicates.

Table 6
Antibacterial activity of ginger oil and oleoresins against a few bacterial species using disc diffusion method

Samples tested	Diameter of inhibition zone (mm)[a]				
	SA	EC	PV	PA	KP
Ginger oil	61.4 ± 3.2	10.4 ± 1.8	–	57.8 ± 1.4	16.2 ± 1.2
EtOH ole.	–	–	–	–	–
MeOH ole.	–	–	–	–	–
CCl_4 ole.	–	–	–	–	–
Isooctane ole.	–	–	–	–	–
Streptomycin	16.3 ± 0.4	–	18.0 ± 0.8	14.5 ± 2.4	16.0 ± 1.5
Chloramphenicol	–	–	–	–	–

– No inhibition was observed.
SA = *Staphylococcus aureus*, EC = *Escherichia coli*, PV = *Proteus vulgaris*, PA = *Pseudomonas aeruginosa* and KP = *Klebsiella pneumoniae*.
[a] Data are the means of three replicates.

of essential oils and oleoresins result from the synergistic action of all constituents.

The essential oil and oleoresins of *Z. officinale* exhibited significant antioxidative properties. It can be inferred that ginger essential oil and oleoresins could be better natural antioxidants having equivalent activity to that of synthetic ones. Since, they have exhibited moderate to good antimicrobial properties, hence, they can be used for preserving edible oils and various other foodstuffs against autoxidation and microbial spoilage.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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