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Alkaloid, Phenolic Profiling and Biological Activities of *Caralluma russeliana* Methanol Extracts from Al Baha region, Saudi Arabia

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ABSTRACT

The current work highlights the antioxidant, cytotoxicity and antimicrobial activities of the methanol extract of *Caralluma russeliana* and their alkaloids and phenolics composition by HPLC analysis. The antioxidant activity of the methanol extract of *C. russeliana* displayed that it quenched DPPH with IC_{50} 119.17 µg/mL, ABTS with IC_{50} 155.71 µg/mL, NO with IC_{50} 223.40 µg/mL and H_2O_2 with IC_{50} 184.40 µg/mL. Among the tested cell lines, hepatocellular (HepG2) and lung (A549) were the most sensitive cell lines towards the extract which significantly block proliferation with IC_{50} 24.37 µg/mL and 26.84 µg/mL, respectively, and moderately active against HCT-116, skin A-431 and prostate PC-3 cells. Furthermore, the extract was active against the bacterial strains and inactive against the tested fungal strains and showed MIC 3300 µg/mL and 1666.66 µg/mL against *S. aureus* and *P. vulgaris*, respectively in antimicrobial assay. The identified alkaloids and phenolic constituents by HPLC such as berberine, camptothecin, chlorogenic acid, syringic acid, p-coumaric acid, catechol and cycloclavine are known to exert antimicrobial and anticancer effect triggered by oxidative stress through different mechanisms.

Keywords: *Caralluma russeliana*, Cytotoxicity, Antimicrobial, Antioxidant.

INTRODUCTION

Cancer and disease due to microbes are one of the major causes of mortality. The drug candidates currently used for cancer therapy have developed resistance and toxicity despite of the advancement in technology and drug development[1]. Moreover, bacterial infections occur very commonly among cancer patients owing to weak immunity which results from radiotherapy, chemotherapy, prolonged hospitalizations, malnutrition, and invasive procedures[2]. The overuse of antibiotics is the main drivers to drug resistant microbial pathogens, particularly, methicillin resistant *S. aureus* which mostly prevail in hospitals[3]. Natural products based drugs or extracts are safer compared to synthetic drugs and are in used for the cure of different disease since ancient time, due to their lower side effects and better compatibility[4]. Natural products display diverse biological activities such as antioxidant, anti-inflammatory, anticancer, antimicrobial, antitubercular, and antipyretic[5,6] that

are attributed to the plant secondary metabolites such as flavonoids, alkaloids, phenolics, tannins, terpenoids, and glycosides present in plant extract.

Caralluma (fam.Asclepiadaceae), constitute around 200 genera and 2500 species which is widely distributed in Europe, Africa, South Africa, Canary Island and Arabian Peninsula. *Carulluma* species are important part of traditional system of medicine in various countries. *C. fimbriata* is medicinally used in pain, inflammation, diabetes and appetite suppressant, *C. tuberculata* as food and for the treatment of fever, leprosy and diabetic, while *C. attenuata* for migraine and diabetes. *Caralluma* species (*C. fabricata*, *C. edulis, C. tuberculata* and *C. umbellate, C. laciantha, C. stalagmifera, C. arabica*) possess important pharmacological properties like antidiabetic, antiinflammatory, anticancer, antimicrobial, antioxidant and antigastric[7].

Caralluma russeliana, occupy the rocky mountains of Al Baha region, situated on southern part of Saudi Arabia (Fig. 1). This small, erect and fleshy, perennial herbs have been used in folk medicine in the treatment of cancer, diabetes, inflammation, fever, tuberculosis, skin rashes, snake bite and scorpion bite[8-12]. Recent studies have reported that *C. russeliana* stem extracts possess significant antidiabetic potential and caused reduction in complications such as body weight, lipid profile, AST, ALP in STZ induced rats[13]. These medicinal values are attributable to the phytoconstituents particularly pregnane glycosides, russeliosides A–D, flavone glycosides, luteolin, which have been isolated from this plant[14-16]. The above studies encouraged us to explore this plant from AL Baha region, KSA. Therefore, in this work, we investigated the antioxidant, anticancer and antimicrobial studies of *C. russeliana*, which is the first study from this place.

Fig. 1. *Caralluma russeliana* plant[17]

EXPERIMENTAL

Extraction

The aerial parts of *Caralluma russeliana* was collected from Albaha region, KSA and authenticated by taxonomist. The plant was Soxhlet extracted using methanol as a solvent to afford the methanol extract, dried completely under vacuum and kept in refrigerator. The extract was then screened for its phytochemical analysis, antioxidant, antimicrobial, anticancer and HPLC analysis.

Phytochemical analysis

The amount of total phenolic (TPC), total flavonoid (TFC), total tannins and total alkaloids was quantified using method of Folin–Ciocalteu colorimetric[18], Chang *et al.,*[19], Broadhurst and Jones[20] and Shamsa *et al.,*21.

Antioxidant activity

The extract was screened for antioxidant potential at Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University by DPPH, ABTS, NO, H_2O_2 and ferric reducing power method as previously reported[22].

Anticancer activity

The extract was screened for cytotoxicity by MTT as previously reported[23] against skin (A-431), lung (A549), colorectal (HCT-116), hepatocellular (HepG2), prostate (PC-3) and breast (MCF-7) and a normal healthy lung fibroblast (MRC-5), which were procured from American Type Culture Collection. Doxorubicin was used for comparison.

Antimicrobial activity

The plant methanolic extract was evaluated against different bacterial and fungal strains at Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. Two *Gram-positive* strains (*Staphylococcus aureus* and *Bacillus subtilis*) and two *Gram-negative* bacterial strains (*Escherichia coli*, *Proteus vulgaris*) were taken and the extract was tested against them. The plant extract was also tested against two fungal strains (*Aspergillus fumigatus*, *Candida albicans*) using a modified well diffusion method. The

extract was dissolved in DMSO to prepare solution of 10 mg/mL as stock solution. The agar plates were spread with bacterial and fungal strains and 100 µL of extract solution was added in well of each plate. The plates were kept at 37^{0}C for 24-48 hours. After incubation microorganism were grown on to the plates and zone of inhibition was measured. For this experiment, DMSO was taken as negative control, Gentamycin was taken as positive control for bacterial strains and Ketoconazole was taken as positive control for fungal strains at a dose of 1 mg/mL, 100 µL of each control was added in the well. The microbial strains sensitive towards the extract was subjected to determine MIC by using broth dilution method.

HPLC analysis

The phenolics and alkaloids in the extract was analyzed by HPLC using Agilent 1100 instrument according to method of Lin *et al.,*[24]

RESULTS AND DISCUSSION

Phytochemical analysis

C. russeliana contains important classes of compounds like phenols, flavonoids, tannins and alkaloids as exhibited by the phytochemical analysis of its methanol extract. As illustrated in Fig. 2, the extract contains 16.75 mg/g total phenolic content, 7.41 mg/g total flavonoid, 1.29 mg/g total tannin and 2.96 mg/g total alkaloid content (TAC). These different classes of compounds in the extract were further assayed by HPLC for determination of alkaloid and phenolics.

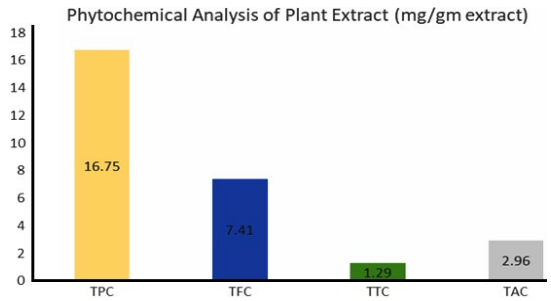


Fig. 2. Phytochemical analysis of methanol extract of *Caralluma russeliana*. TPC: total phenolic content, TFC: total flavonoid cotent, TTC: total tannin content, TAC: total alkaloid content, data are shown in triplicate

HPLC Analysis of the methanol extract

The HPLC analysis of methanol extract of *Caralluma russeliana* was performed to detect different alkaloids and phenolics (Fig. 3, 4). The different alkaloids namely Evodiamine, berberine, protopine, camptothecin and cycloclavin whereas different phenolics namely chlorogenic, catechol, syringic acid, *p*-coumaric, pyrogallol, gallic acid and salicyclic acid were observed in different concentration at different retention time (RT). Among the identified alkaloids, cycloclavine was the major components (9.16 µg/mg) followed by camptothecin (6.33 µg/mg) and berberine (4.13µg/mg). Evodiamine was present in lower concentration (3.22 µg/mg) while protopine in a fairly low concentration (0.79 µg/mg). In case of identified phenolics, *p*-coumaric acid, gallic acid and syringic acid was the major with concentration of 11.69 µg/mg, 6.14 µg/mg and 4.0 µg/mg, respectively. Chlorogenic, syringic, pyragallol and salicyclic acid were present in lower concentration of 3.02, 4.0, 3.88, and 2.10 µg/mg, respectively whereas catechol was less than 1% (Table 1, 2). This analysis indicates that the extract contains biological active alkaloids and phenolics.

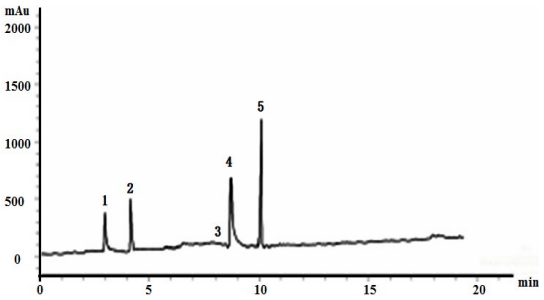


Fig. 3. Alkaloids identified in the methanol extract by HPLC analysis

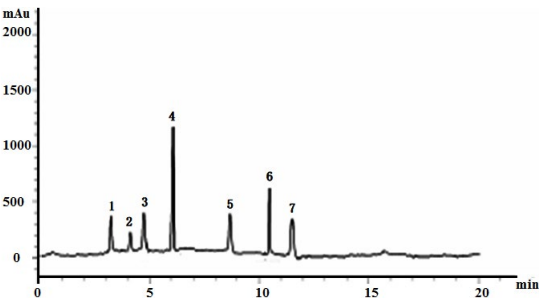


Fig. 4. Phenolics identified in the methanol extract by HPLC analysis

Table 1: Alkaloids identified in methanol extract of *C. russeliana*

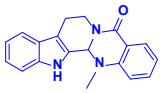
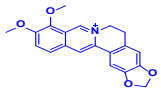
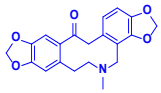
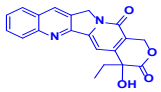
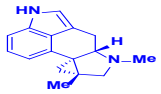
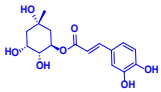
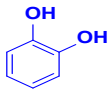
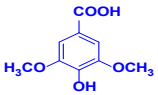
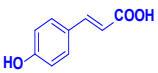
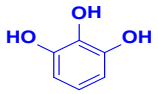
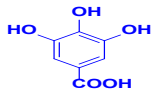
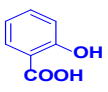
S. No	Compound name	Structure	RT	Conc. (µg/mg)
1	Evodiamine		3.1	3.22
2	Berberine		4.0	4.13
3	Protopine		8.1	0.79
4	Camptothecin		8.95	6.33
5	Cycloclavine		10	9.16

Table 2: Phenolics identified in methanol extract of *C. russeliana*

S. No	Compound name	Structure	RT	Conc. (µg/mg)
1	Chlorogenic acid		3.0	3.02
2	Catechol		4.0	0.87
3	Syringic acid		5.0	4.0
4	*p*-coumaric acid		6.2	11.69
5	Pyragallol		8.8	3.88
6	Gallic acid		10.2	6.14
7	Salicylic acid		11.8	2.10

Antioxidant activity

The antioxidant activity of the methanol extract of *C. russeliana* as measured by DPPH, ABTS, NO, H_2O_2 and FRAP are depicted in Table 3 as IC_{50} in µg/mL. The extract exhibited concentration dependent scavenging activity. The extract strongly quenched DPPH with IC_{50} of 119.17 µg/mL, compared to BHT (22.14 µg/mL) and Ascorbic acid (10.21 µg/mL). The ABTS, NO and H_2O_2 scavenging by the extract was found to be 155.71 µg/mL, 223.40 µg/mL and 184.40 µg/mL, respectively. The FRAP of the extract (203.48 µg/mL) was significantly lower than BHT (50.35 µg/mL) and ascorbic acid (20.89 µg/mL).

Based on these observations, the constituents identified in extract have the ability to scavenge DPPH$^{\bullet}$ and quench ABTS$^{+\bullet}$ and reduce Fe^{3+} through various mechanisms.

Table 3: Antioxidant activity (IC_{50}) of methanol extract of *Caralluma russeliana*

Extract	DPPH (µg/mL)	ABTS (µg/mL)	NO (µg/mL)	H_2O_2 (µg/mL)	FRAP (µg/mL)
Methanol	119.17±5.97	155.71±9.59	223.40±5.77	184.40±22.39	203.48±5.41
BHT	22.14±1.82	50.69±1.25	19.92±2.21	116.18±4.58	50.35±4.99
Ascorbic acid	10.21±0.77	10.66±0.89	17.95±2.24	14.77±0.69	20.89±1.25

Results are mean of three experiments ±SD.

Oxidative stress is the imbalance between reactive oxygen species (ROS) and antioxidative defence system. ROS hampers the antioxidant system in the cells that damage biomolecules like DNA, RNA, etc. Constituents derived from plants play a crucial part in the treatment of many diseases by targeting different mechanisms. These natural product protect the cells from oxidative stress causing amelioration of many diseases. *C. russeliana* have been traditionally used for various ailments which may attributable to important phytoconstituents such as alkaloids and phenolics identified in HPLC. Evodiamine, chemically (+)-(S)-8, 13, 13b, 14-tetrahydro-14-methylindolo [2,3:3,4] pyrido[2,1-b]quinazolin-5(7H)-one), is a quinozole alkaloid that has protective role in acute kidney injury and decreases the blood urea and creatinine levele in LPS induced rats[25]. Berberine, an isoquinoline alkaloid has been used in Ayurvedic, Persian and Chinese traditional medicine since time immemorial. This alkaloid can strongly scavenge free radicals and lessen oxidative stress throughPI3K/Akt/Bcl2/Nrf2/SIRT1 and AMPK pathways[26]. Chlorogenic acid, an ester of caffiec acid and quinic acid is a well known antioxidant which scavenge oxygen and nitrogen species. Due to its polyhydroxy structure, it can activate Nrf-2/HO-1 antioxidant signalling pathways and enhances antioxidant ability by regulating expression of various genes[27]. Syringic acid is chemically known as 4-hydroxy-3,5-dimethoxy benzoic acid that hamprs different oxidative stress markers, and inhibit the oxidation of LDL and NADPH oxidase[28]. Gallic acid bearing three hydroxy can scavenge free radical strongly which bestow protection from oxidative stress[29]. *p*-coumaric acid is a phenolic compound with capability to scavenge free radicals. It can inhibit 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH) induced ROS generation and can increase the expression of antioxidant genes[30]. Salicylic acid generates ROS and acts as radical scavenger therefore is involved in cell oxidative stress[31]. The different mechanisms of these alkaloids and phenolics in the methanol extract of *C. russeliana* might be imparting the antioxidant protection against oxidative stress.

Anticancer activity

The anticancer activity of the methanol extract of *C. russeliana* as screened by MTT protocol against a panel of six human adenocarcinomas is illustrated in Table 4. The six adenocarcinomas, namely skin (A-431), lung (A549), colorectal (HCT-116), hepatocellular (HepG2), prostate (PC-3) and breast (MCF-7) while a normal healthy lung fibroblast were procured from American Type Culture Collection. For comparison, doxorubicin was used as a positive control. The methanol extract demonstrated moderate to promising antiproliferative activity towards most of the tested cancer cell lines. Among the tested cell lines, hepatocellular (HepG2) and lung (A549) were the most sensitive cell lines towards the extract. The extract significantly block proliferation with IC_{50} 24.37 µg/mL in HepG2 and 26.84 µg/mL in A549 cells, compared to Doxorubicin which inhibited proliferation with IC_{50} 0.37 µg/mL and 1.01 µg/mL, respectively. The extract was also cytotoxic towards colorectal HCT-116 and skin A-431 with IC_{50} 49.07 µg/mL and 40.78µg/mL, respectively whereas moderately inhibited prostate PC-3 cells with IC_{50} 57.11 µg/mL. The extract displayed mild cytotoxicity on breast MCF-7 cells. However, the anticancer activity of the extract was lower than the positive control, Doxorubicin. From safety point of view, the extract was also tested on normal lung cells (MRC-5) and found to be non-toxic on normal cells. Cancer, the second global cause of mortality is increasing at an alarming rate, therefore prevention and treatment of cancer requires urgent startegies[32]. Bioactive molecules present in different plant

extracts have emerged as potential anticancer agents. In plants, phenolics and alkaloids have gain considerable interests in drug discovery as they have provided many drugs for various treatment[33]. These bioactive agents regulates apoptotic proteins, oxidative stree, or various transcription signalling pathways[34]. Berberine are known to block proliferation by cell cycle regulation, cell autophagy, inhibits cell invasion, metastasis, downregulates/upregulates vaious metastasis and apoptosis proteins by suppressing signalling pathways like C-myc, cyclin-D1, and MMP-3 expressions and inhibition of PI3K/AKT/mTOR signaling[35,36]. Camptothecin is a quinoline based pentacyclic monoterpenoid indole alkaloid which is a potent topoisomerase II inhibitor and NRF2 inhibitor. This natural product by semi synthesis has afforded many more potent anticancer drugs like Irinotecan, Topotecan or Camtosar which have been granted by WHO for use in sevaral cancers like ovarian, brain, uterine, lung, and colon[37,38]. Evodiamine, acts as modulator of topoisomerase I inhibitor, NF-B, Bcl2, MAPK, suppress H60 expression in KATO III stomach cancer and HeLa S3 uterine cancer and induces apoptosis in many cancer cells like breast, prostate, cervical, melanoma, leukemia, colon and lung[39-41]. Previous study reports that gallic acid induce BIK-BAK/BAK, caspase-3 and 9 and Bcl-2 pathways involved in apoptosis, and regulate oxidative stress[42]. Catechol has been known to have suppressing action on ERK2/c-Myc signals in lung cancer, EMT-related proteins via AMPK/Hippo signals in pancreas cancer[43]. Syringic acid acts as anticancer agent by regulating various expression like Cdk4 and Cdk6 of cell cycle and pre and proapoptotic genes like Bax, Bak, Bcl2, and FLIP[28]. *p*-coumaric acid is capable of inhibiting cell proliferation and migration and triggers apoptosis in lung and colon cancer[44]. Chlorogenic acid is an effective natural anticancer drug approved by the China Food and Drug Administration. It is a safe differentiation inducer for solid tumors, inhibits HIF-1α/AKT pathway, inhibits MAPK/ERK activation via ROS overproduction and induces DNA damage and formed topoisomerase-DNA complexes involved in apoptosis and a chemosensitizing chemotherapy agent[45-48]. Pyrogallol, is known to have antitumor effects in breast and colon cancers *via* miR-134 activation-mediated S-phase arrest and inhibition of PI3K/AKT/Skp2/cMyc signaling[49,50]. Salicylic acid is known to trigger ER stress-induced apoptosis *via* Akt/mTOR/AMPK pathways and upregulation of nitric oxide production[51]. These biactive may be attributable to the anticancer activity by the methanol extract of *C. russeliana*.

Table 4: Anticancer activity (IC_{50}, μg/mL) of the methanol extract of *Caralluma russeliana*

Cell lines	Origin	IC_{50} (µg/mL)	
Cancerous cells		Extract	Doxorubicina
breast	MCF7	111.21±3.38	0.5 ± 0.03
Hepatocellular	HepG2	24.37±1.01	0.37±0.02
Colorectal	HCT-116	49.07±2.40	0.49±0.04
Lung	A549	26.84±2.14	1.01±0.20
Prostate	PC3	57.11±0.86	3.34±0.31
Skin	A-431	40.78±2.59	11.26±0.54
Normal cells			
Lung	MRC-5	521.65±25.32	3.13±0.29

Antimicrobial activity

The methanol plant extract was tested against different bacterial and fungal strains using disc diffusion method. The results of antimicrobial activity are shown in Table 5. The plant extract was active against the bacterial strains and inactive against the tested fungal strains. The extract was active against one *Gram-positive* bacterial strain *S. aureus* and one *Gram-negative* bacterial strain *P. vulgaris* and showed zone of inhibition 9.86mm and 10.90mm respectively. When results were compared with the standard compound Gentamycin, the extract displayed mild antimicrobial activity. The plant extract was also evaluated for its MIC, against these two bacterial strains and showed MIC 3300 µg/mL and 1666.66 µg/mL against *S. aureus* and *P. vulgaris*, respectively.

Table 5: Antimicrobial activity of the methanol extract showing zone of inhibition (mm) and MIC (µg/mL)

Test samples	Bacterial strains			Fungal strains		
	S.aureus ATCC25923	*B. Subtilis* NRRLB-543	*E. coli* ATCC25922	*P. vulgaris* ATCC13315	*A. fumigatus* ATCC13073	*C. albicans* ATCC10231
Extract	9.86±1.22	NA	NA	10.9±1.12	NA	NA
Positive control	24.4±2.28	26.63±3.12	26.73±3.28	25.46±3.18	17.33±1.45	20.56±2.04
MIC	3300	NT	NT	1666.66	NT	NT

MIC: Minimum Inhibition Concentration (µg/mL); NA: Not Active; NT: Not tested

Antimicrobial resistance is a major burden which occurs when drugs acquire resistance towards many microbes like bacteria and fungi. As drug resistance is the main hurdle in infectious diseases, control of infections has been difficult. Natural products or their plant extracts are good choice for the prevention and treatment of infections[52]. Natural products are reservoir of antimicrobial drugs, for instance, Penicillin a notable antibiotic, Lysergic acid diethylamide, Cabergoline, Streptomycin, Gentamycin, Polymyxcin, Daptomycin, and so on have been isolated from bacteria and fungi[53]. In the present work, identified natural products are endowed with antimicrobial property. Berberine displayed significant antimicrobial effect by inhibtiing antibiotic resistant microbes, damage cell wall and cell membrane of methicillin resistant *S. aureus* (MRSA) and *E. coli*, inhibit biofilm formation, supress protein synthesis and bacterial division[54]. Evodiamine have antibacterial effect by inhibiting the relaxation of plasmid DNA while bactericidal against *K. pneumoniae*[55]. Chlorogenic acid has displayed antifungal activity against fluconazole resistant *Candida* species by mitochondrial depolarization, ROS production and phosphatidylserine externalization[56]. Catechol has been shown to have antifungal effects on Colletotrichum circinans fungus and possess antibacterial effect[57]. Syringic acid possess antimicrobial effect against various *Gram-positive* and *Gram-negative* bacteria. It could inhibit the growth of MRSA, Cronobacter sakazakii, *E. coli*, and influenced cell membrane permeability by proton influx and ion leakage[58]. *Salicyclic* acid could inhibit the growh of many fungal pathogens like *Botrytis cinerea, P. expansum* and *R. stolonifer* via intracellular disorganization, lipid damage and outflow of pathogen's proteins[59]. Also, *Salicyclic* acid microcapsules ruptures cell wall and cell membranes of *E.coli* and *S. aureus* therefore possess significant antibacterial potential[60]. These mechaism of the isolated compounds may be responsible for the antimicrobial activity exerted by the methanol extract of *C. russelliana*.

CONCLUSION

In conclusion, the results of the present study, to some extent are in line with the traditional use of this plant as antioxidant, anticancer and antimicrobial agents. The methanol extract of *Caralluma russeliana* quenched DPPH, ABTS, NO and H_2O_2 radical significantly thus have antioxidant property. The extract was also found to be active against all the tested cancerous cell lines, among which HepG2 and A549 were the most sensitive in exerting toxicity by the extract. Also, the extract was active against *S. aureus* and *P. vulgaris* as observed by the antimicrobial assay. These biological activities might be due to presence of evodiamine, berberine, camptothecin, chlorogenic acid, syringic acid and *p*-coumaric acid which are known for their mode of action as antimicrobial and anticancer agents triggered by oxidative stress. Studies for the isolation and structure elucidation of anticancer, antimicrobial and antioxidant active constituents from the methanol extract of *C. russeliana* are in progress which could provide leads for the treatment of these illnesses in future.

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Conflicts of Interest

No conflict of interest

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