

Gas Chromatography Mass Spectrometry Profiling in Methanolic and Ethyl-acetate Root and Stem Extract of *Corbichonia decumbens* (Forssk.) Exell from Thar Desert of Rajasthan, India

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ABSTRACT

Background: *Corbichonia decumbens* (Forssk.) Exell (*Molluginaceae*), recently has moved to Lophiocarpaceae as per angiospermic plant group (APG) III system, is an annual or short-lived, dwarf, glabrous subshrub, prefers to grow on rocky places and on sand-stones in dry, hot areas of Rajasthan. This is the potential plant with medicinal properties. Vegetative organs under study show antioxidant, anti-inflammatory, antiulcer, antimicrobial, and antinociception activity. **Objective:** This study was carried out to identify the phytoconstituents present in the methanolic and ethyl-acetate extract of root and stem of *C. decumbens* by GC-MS analysis. **Materials and Methods:** Powdered test samples were sequentially extracted with methanol and ethyl-acetate. The compounds obtained as a result of GC-MS screening were identified on the basis of their retention time, peak area and compared with that of literature available and by interpretation of mass spectra. **Results:** GC-MS analysis of a methanolic extract of root detected mome-inositol (49.53%), guanosine (20.91%), and cis-vaccenic acid (9.25%). While ethyl-acetate extract of root analyzed pentadecanoic acid (17.91%), octadecanoic acid (15.01%) and cis-vaccenic acid (12.04%). Methanolic extract of stem detected mome-inositol (75.47%), pentadecanoic acid (6.04%), and 7-tetradecenal, (Z) (4.54%) while ethyl-acetate extract of stem revealed the presence of 1-heptacosanol (17.35%), hexadecanoic acid (17.17%), and octadecanal (12.64%). **Conclusion:** The results suggest that *C. decumbens* (Forssk.) Exell is a plant of potential medicinal value, yielding various bioactive compounds that confirm the application of this plant as a plant-based drug in pharmacy-industry.

Key words: Bioactive compounds, *Corbichonia decumbens*, gas chromatography mass spectrometry screening, lophiocarpaceae, phytochemical screening, retention time

SUMMARY

- Extraction is the most important step in the analysis of bioactive compounds present in botanical preparations. The strength of solvent plays a key role in this process, methanol as well as ethyl-acetate showed better response as far as extraction potency is concerned. Gas chromatography mass spectrometry analysis is highly reliable, and the interpretations of the

results are of high-quality. This tool is in particular useful for confirming of the presence of bioactive-substances. The results suggest that *Corbichonia decumbens* (Forssk.) Exell can be used for drug formulations against some major disorders, i.e., cancer, ulcer, tuberculosis, arthritis, etc.



Abbreviations Used: GC-MS: Gas Chromatography-Mass Spectrometry, kPa: Kilopascal, RT: Retention time, MF: Molecular formula, MW: Molecular weight

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INTRODUCTION

Plants have contributed a lot of medicinal compounds being used today to treat diseases such as cancer, hormonal imbalances, jaundice, diabetes, inflammation, etc. Plants are commonly available in abundance, especially in the tropics.^[1] Most of the world's population relies upon traditional medicine, particularly plant-based drug for the primary health care.^[2] In developing countries, low-income people such as farmers, people of small isolated villages, and native communities use folk medicine for the treatment of common infections.^[3] Researches into medicinal plants have shown that they contain secondary metabolites which possess a variety of structural arrangement and properties.^[4]

The plant family, lophiocarpaceae is comprised of two genera with approximately 25 species.^[5] These plants are commonly known as stone plants or carpet weeds. *Corbichonia decumbens* is a creeping,

well-branched, diffuse-ascending, and prostrate to procumbent, semi-succulent, and annual herb of Rajasthan.^[6]

The methanol extract of leaves of this plant has shown significant anti-inflammatory effects in various animal models and is used as an antiulcer.^[7,8] Leaves of this plant are used as a herbal alternative for heal

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of various diseases.^[9] The present investigation was carried out to detect phytochemicals from root and stem using gas chromatography coupled with mass spectrometry. The molecular weight and structure of the compounds of test materials were ascertained by interpretation on mass spectrum of gas chromatography-mass spectrometry (GC-MS) using the database of National Institute of Standard and Technology (NIST). The GC-MS analysis will provide a representative spectral output of all the compounds that get separated from the sample.^[10] This tool facilitates the relationship between the compounds extracted from plants and their pharmacological efficacies.

MATERIALS AND METHODS

Fresh and healthy plant material was collected from rocky places of Jodhpur (Beri-Ganga, Machia-Safari, Bheem-Bhadak and Ossian) District of Rajasthan in the month of July–October 2016. The specimen authentication and taxonomic identification were done by Botanical Survey of India Jodhpur, Rajasthan.

The plant material was washed 2–3 times with running fresh water, shade-dried, and grinded to powder. The powdered plant material was kept in small and labeled plastic bags. Two gram powder was transferred to round bottom flask each containing 100 ml of solvent, i.e., methanol and ethyl-acetate, boiled at 65°–75°C for 6 h using Soxhlet extractor. Extract was filtered using Whatman filter no. 1 and evaporated to dryness. A volume of 2 µl of this solution was employed for GC-MS analysis.^[11] The GC-MS analysis was performed at Advanced Instrumentation Research Facility (AIRF) JNU, Delhi. Syringe insertion and injection speed were high, and it was pumped for five times. Temperature of injection port was maintained at 260.0°C, column oven temperature was set at 80°C. For GC pressure was

maintained at 81.9 kPa. Split ratio was 50.0, and ion source temperature was maintained at 230°C.

Standard analytical procedures were used for screening of preliminary phytochemicals, i.e., Wagner's Test (for alkaloids), Braymer's Test (for tannins), Salkowski's Test (for steroids), Sodium Hydroxide Test (for flavonoids), Frothing Test (for saponins), and Molisch's Test (for carbohydrates). The extract contained polar as well as nonpolar phytoconstituents. The spectrum of unknown components was compared with spectrum stored in the NIST library version. The eluted compounds were characterized on the basis of their molecular formula, structure, retention time, and peak % area.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of root and stem extract in methanol and ethyl-acetate as solvent showed the presence of various metabolic compounds such as alkaloids, tannins, steroids, flavonoids, and carbohydrates. The compounds have shown an affirmative and strong response in both the solvents under the study of the stem as compared to root. Methanolic stem extract and ethyl-acetate root extract showed better response as compared to methanolic root and ethyl-acetate stem extract.

The more precise information in qualitative analysis can be obtained by GC-MS.^[12] Root extract in methanol revealed 44 peaks [Figure 1] indicating the presence of 39 compounds and stem revealed 38 peaks [Figure 2] indicating 34 compound. 14 compounds showed biological activity in methanolic root and stem extract [Table 1]. Ethyl-acetate extract of root show the presence of 60 peaks [Figure 3] indicating 46 compounds and stem shows 56 peaks [Figure 4] indicating 44 compounds. Twenty compounds showed biological activity in ethyl-acetate root and stem extract [Table 2].

The first compound identified in methanolic root extract of *C. decumbens* with less retention time (RT = 6.365) was 1,3,5-triazine-2,4,6-triamine (0.88%), whereas 1-H-cyclopenta (A) pentalene, 2,3B,6,6A,7,7A-hexahydro-2, 2, 3B-trimethyl, (3B. Alpha., 6A. Alpha. 7A. Beta-(0.44%) was the last compound which take longest retention time (RT = 39.241). Mome-inositol [Figure 5] showed highest % area (49.53%) in methanolic root extract. In ethyl-acetate root extract, decane, 3-methyl showed less retention time (RT = 8.068) with 0.32% area whereas tetrakis (2,3-ditert-butylphenyl)-4,4'-biphenylene diphosphonate was the last compound which was retained for longest

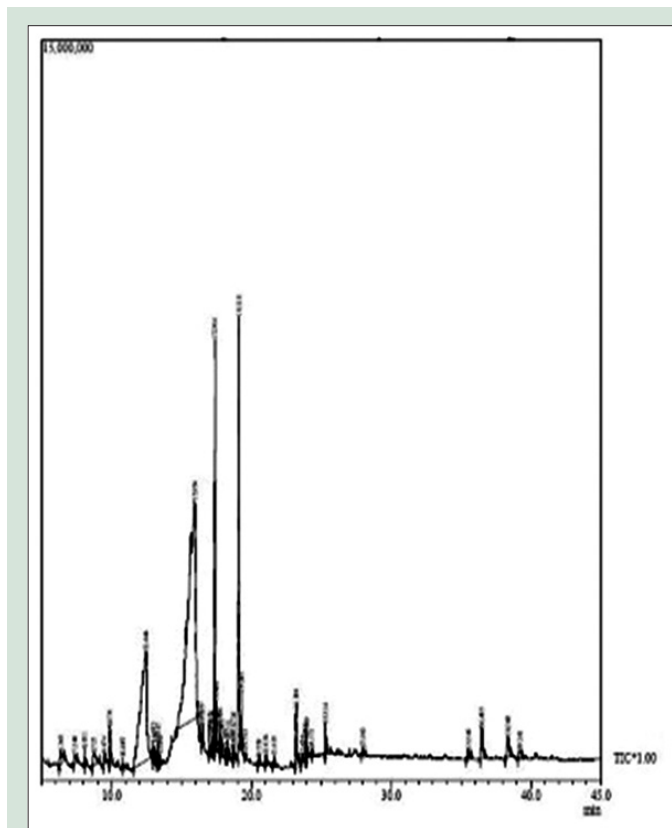


Figure 1: Gas chromatography-mass spectrometry chromatogram of methanolic root extract

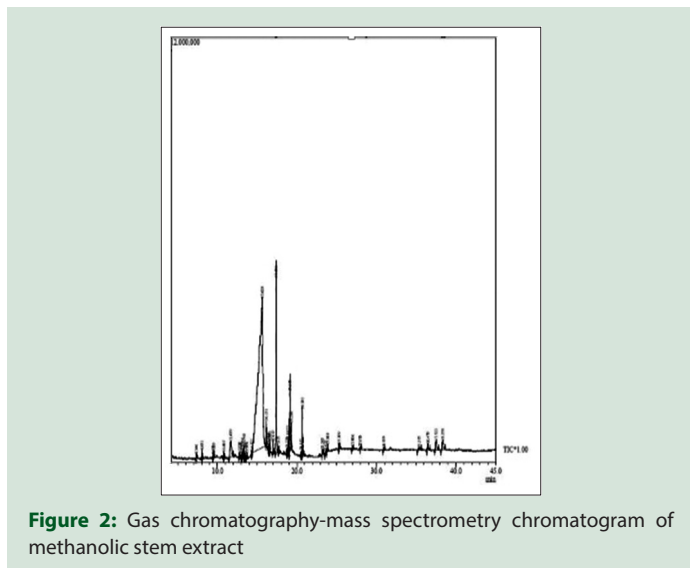
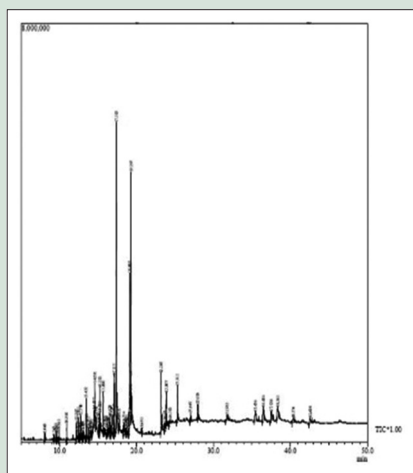


Figure 2: Gas chromatography-mass spectrometry chromatogram of methanolic stem extract

Table 1: Bioactive compounds from vegetative parts (in methanol extract)

Plant part	RT	Compounds	Percentage area	MF	MW	Biological activity
Root	7.348	2,3-dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one	0.36	C ₆ H ₈ O ₄	144	Antimicrobial, anti-inflammatory
Stem	7.346		0.14			
Root	8.051	Naphthalene	0.19	C ₁₀ H ₈	128	Antiseptic, carcinogenic
Stem	8.051		0.25			
Root	10.807	1-tetradecene	0.09	C ₁₄ H ₂₈	196	Anti- tuberculosis activity
Stem	10.807		0.11			
Root	12.973	Dodecanoic acid	0.17	C ₁₂ H ₂₄ O ₂	200	Pharmaceuticals activity
Stem	12.968		0.10			
Root	13.347	9-eicosene, (E)-	0.18	C ₂₀ H ₄₀	280	Antimicrobial and cytotoxic properties
Stem	13.348		0.20			
Root	15.954	Mome inositol	49.53	C ₇ H ₁₄ O ₆	194	Antialopethic, anticirrhotic, antineuropathic,
Stem	15.628		75.47			cholesterolytic, lipotropic
Root	16.333	Pentadecanoic acid	5.79	C ₁₅ H ₃₀ O ₂	242	Lubricants, additives, adhesive agents
Stem	17.382		6.04			
Root	16.515	8-pentadecanone	0.24	C ₁₅ H ₃₀ O	226	Heptatotoxic, demyelination, conjunctivitis activity
Stem	14.321		0.23			
Root	17.676	n-nonadecanol-1	0.16	C ₁₉ H ₄₀ O	284	Antimicrobial and cytotoxic properties
Stem	17.675		0.05			
Root	19.285	Octadecanoic- acid	0.92	C ₁₈ H ₃₆ O ₂	284	Antibacterial action, cosmetic, flavor,
Stem	19.276		0.53			hypocholesterolemic
Root	20.558	3-cyclopentylpropionic acid, 2-dimethylaminoethyl	0.04	C ₂₃ H ₃₈ O ₂	344	Analgesic, anti-inflammatory
Stem	20.552	ester	0.14			
Root	23.206	n-tetracosanol-1	1.20	C ₂₄ H ₅₀ O	354	Antibacterial activity
Stem	23.205		0.31			
Root	36.495	Stigmasta-5,22-dien-3-ol	1.61	C ₂₉ H ₄₈ O	412	Synthetic progesterone
Stem	36.479		0.73			
Root	38.368	Stigmast-5-en-3-ol, (3.beta.)-	1.34	C ₂₉ H ₅₀ O	414	Anti-inflammatory, anti-pyretic, anti-ulcer,
Stem	38.358		1.27			antiarthritic

MF: Molecular formula; MW: Molecular weight; RT: Retention time

**Figure 3:** Gas chromatography-mass spectrometry chromatogram of ethyl-acetate root extract

time (RT = 42.600) with 1.27% area. Pentadecanoic acid [Figure 6] showed highest % area (17.91%).

The first compound identified in methanolic stem extract of *C. decumbens* with less retention time (RT = 7.346) was 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one with 0.14% area whereas stigmast-5-en-3-ol, (3 beta)-take longest retention time (RT = 38.358) with 1.27% area. Highest % area in methanolic stem extract is taken by mome-inositol (75.47%). In ethyl-acetate stem extract, dodecane showed less retention time (RT = 8.067) with 0.26% area, whereas

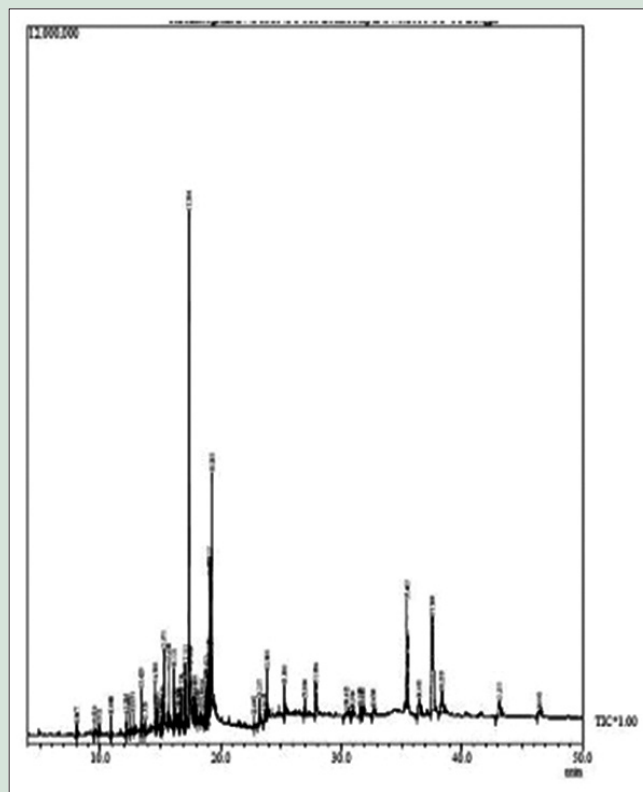
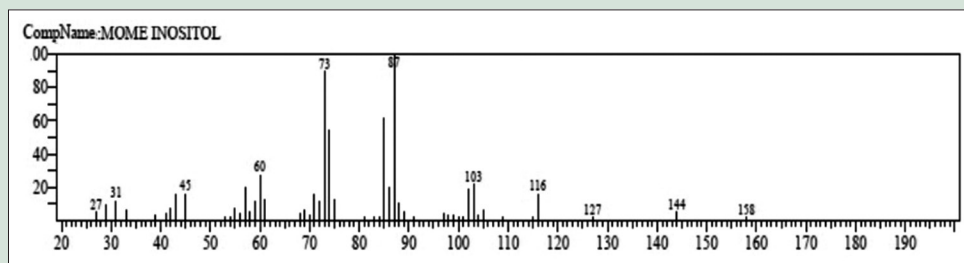
**Figure 4:** Gas chromatography-mass spectrometry chromatogram of ethyl-acetate stem extract

Table 2: Bioactive compounds from vegetative parts (in ethyl-acetate extract)

Plant part	RT	Compound	Percentage area	MF	MW	Biological activity
Root	9.533	Heptadecane	1.36	C ₁₇ H ₃₆	240	Antioxidant
Stem	14.588		0.99			
Root	9.913	Octadecane	0.77	C ₁₈ H ₃₈	254	Lubricants, anticorrosion agents
Stem	9.911		0.22			
Root	10.910	Tetradecane	0.65	C ₁₄ H ₃₀	198	Antifungal, antibacterial, nematocidal
Stem	10.908		0.45			
Root	12.207	Pentadecane	1.20	C ₁₅ H ₃₂	212	Antibacterial
Stem	12.205		0.78			
Root	12.431	Phenol, 2,4-bis (1,1-dimethylethyl)-	0.78	C ₁₄ H ₂₂ O	206	Antibacterial, antioxidant
Stem	12.431		0.44			
Root	12.740	Eicosane	4.28	C ₂₀ H ₄₂	282	Antifungal, antibacterial, antitumor, cytotoxic
Stem	12.737		1.28			
Root	13.432	Hexadecane	1.65	C ₁₆ H ₃₄	226	Antifungal, antibacterial, antioxidant
Stem	13.429		1.05			
Root	13.752	Dodecanoic acid, 1 methylethyl ester	0.45	C ₁₅ H ₃₀ O ₂	242	Cosmetics, lubricants
Stem	13.750		0.29			
Root	15.272	Tetradecanoic acid	2.29	C ₁₄ H ₂₈ O ₂	228	Antifungal, antibacterial, antioxidant, cancer preventive, hypercholesterolemic
Stem	15.273		2.10			
Root	16.135	2-hexadecen-1-ol,	0.30	C ₂₀ H ₄₀ O	296	Antimicrobial, sedatives and anesthetics
Stem	16.582	3,7,11,15-tetramethyl-, [R-[R*, R*-(E)]]	1.88			
Root	16.203	2-pentadecanone, 6,10,14-trimethyl-	0.26	C ₁₈ H ₃₆ O	268	Antibacterial
Stem	16.201		0.42			
Root	16.528	1,2-benzenedicarboxylic acid,	0.76	C ₁₆ H ₂₂ O ₄	278	Antimicrobial, antifouling
Stem	16.526	bis (2-methylpropyl) ester	0.43			
Root	17.015	Octadecanoic acid, methyl ester	0.94	C ₁₉ H ₃₈ O ₂	298	Antimicrobial, antifungal, antibacterial
Stem	17.012		1.17			
Root	17.115	7,9-di-tert-butyl-1-oxaspiro (4,5)	2.63	C ₁₇ H ₂₄ O ₃	276	Antimicrobial
Stem	17.111	deca-6,9-diene-2,8-dione	1.52			
Root	19.297	Octadecanoic acid	15.01	C ₁₈ H ₃₆ O ₂	284	Antifungal, antitumor, antibacterial
Stem	19.293		7.75			
Root	20.693	2-methyloctacosane	0.15	C ₂₉ H ₆₀	409	Antifungal
Stem	22.807		0.34			
Root	23.628	Octadecanal	0.35	C ₁₈ H ₃₆ O	268	Alkane-lyase activity
Stem	23.624		12.64			
Root	25.312	1-heptacosanol	6.39	C ₂₇ H ₅₆ O	396	Nematicidal, anticancer, antioxidant and antimicrobial
Stem	31.801		17.35			
Root	27.007	Squalene	0.65	C ₃₀ H ₅₀	410	Antibacterial, antioxidant, antitumor, immunostimulant, pesticide, chemo preventive, lipoxigenase-inhibitor
Stem	27.004		0.61			
Root	36.484	Stigmasta-5,22-dien-3-ol	3.08	C ₂₉ H ₄₈ O	412	Synthetic progesterone
Stem	36.481		2.05			

MF: Molecular formula; MW: Molecular weight; RT: Retention time

**Figure 5:** Mass spectrum of mome inositol

1-heptacosanol [Figure 7] was the last compound which take longest retention time (RT = 46.441) and highest % area (17.35%).

These observations support stronger extraction capacity of methanol and ethyl-acetate extract that could have been produced a number of active constituents responsible for many biological activities. The compounds identified by preliminary qualitative analysis and GC-MS

analysis are medicinally important as they possess unique structure with specific biological activities. Many plant parts are used in Ayurvedic, traditional, folk, and homoeopathic medicines to treat several ailments, including liver and spleen enlargement, hepatitis, nervous disorders, renal disorders, bronchitis and asthma, and whooping cough.^[13,14] For extraction of these bioactive compounds effects on extraction rate of

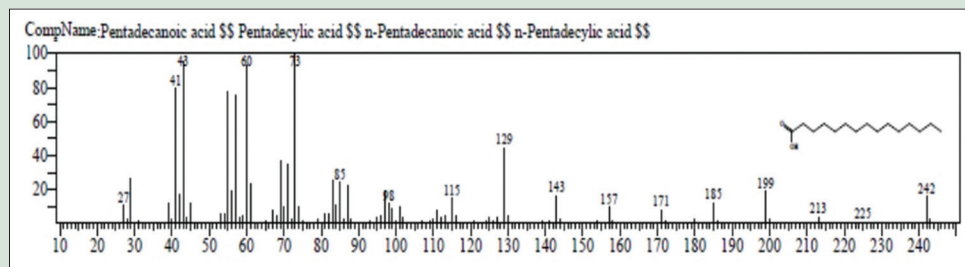


Figure 6: Mass spectrum of pentadecanoic acid

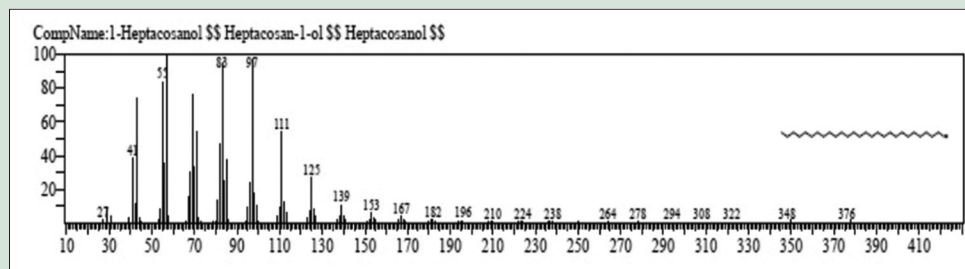


Figure 7: Mass spectrum of 1-heptacosanol

solvents should be investigated.^[15] The accuracy of a method can be measured through comparing with the external standard method.^[16] Before using herbal or plant-based drugs further toxicity studies using human cell lines are needed to determine the suitability of these preparations.^[17]

CONCLUSION

These results may help in standardization, characterization and identification of bioactive compounds to carry out further research. The presence of various bioactive compounds justifies the use of root and stem to cure various ailments by conventional practitioners. Hence, it is recommended as a plant of potential pharmaceutical importance. Thus, this plant can be used as a potential source in the field of drug formulations. Further research is desired to resolve ethical and legal challenges.

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Conflicts of interest

There are no conflicts of interest.

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