RESEARCH PAPER

Antidiabetic Potential of *Syzygium cumini*, *Withania coagulans*, and *Murraya koenigii*: Phytochemical Analysis and Bioactivity

Neha Goel[1], Dr. Surya Prakash Gupta[2]*

[1] Research scholar, Rajiv Gandhi Institute of Pharmacy, Faculty of Pharmaceutical Science & Technology, AKS University Satna, 485001, Madhya Pradesh, India

[2]Professor & Director, Rajiv Gandhi Institute of Pharmacy, Faculty of Pharmaceutical Science & Technology, AKS University, Satna, 485001, Madhya Pradesh, India.*

ORCID No: 0000-0002-5374-0841.

***Corresponding author**:*

Dr. Surya Prakash Gupta

Professor & Director, Rajiv Gandhi Institute of Pharmacy, Faculty of Pharmaceutical Science & Technology, AKS University, Satna, 485001, Madhya Pradesh, India

Email: suryatony@yahoo.co.in

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ABSTRACT

Diabetes mellitus is a global health challenge, necessitating new therapies. Traditional medicinal plants offer promising antidiabetic agents. This study investigates the phytochemical composition and antidiabetic effects of three plants: *Syzygium cumini* (jamun), *Withania coagulans* (paneer phool), and *Murraya koenigii* (curry leaf). Plant materials (seeds of *S. cumini*, fruits of *W. coagulans*, and leaves of *M. koenigii*) were sequentially extracted (petroleum ether, chloroform, ethanol, water) and screened for phenolic and flavonoid content, antioxidant capacity, and α-amylase inhibition. Bioactive fractions were isolated by column chromatography and characterized by spectroscopic methods. In vitro, the ethanolic extracts of *S. cumini* and *M. koenigii*, and the aqueous extract of *W. coagulans* showed the strongest α-amylase inhibition. Three pure phytocompounds (from each plant) were obtained and identified a ferulic acid derivative from *S. cumini*, a steroidal lactone (β-Sitosterol) from *W. coagulans*, and a phenolic (Catechin) from *M. koenigii*. Acute toxicity tests indicated all isolates are safe up to 2000 mg/kg. In a streptozotocin-induced diabetic rat model, treatment with the isolated compounds significantly lowered fasting glucose (Compound 1: 110.16 ± 6.91 mg/dL vs. diabetic 303.33 ± 6.15 mg/dL on Day 28). Specifically, *S. cumini* derived Compound 1 and *W. coagulans* derived Compound 2 exhibited potent hypoglycemic effects comparable to glibenclamide. These results indicate that these medicinal plants are rich in antidiabetic phytochemicals (e.g., flavonoids, phenolics) and support their traditional use. The findings highlight specific bioactive molecules that can be further developed as plant-based antidiabetic agents.

Keywords: *Syzygium cumini*, *Withania coagulans*, *Murraya koenigii*, Diabetes, Phytochemicals, α-amylase inhibition, Hypoglycemic.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia due to insulin deficiency or resistance. It is a major public health problem: nearly 830 million individuals had diabetes worldwide in 2022, and prevalence continues to rise (Type 2 diabetes accounts for over 90% of cases). Persistent hyperglycemia causes vascular and neuropathic complications, leading to significant morbidity and mortality. Conventional antidiabetic drugs (e.g. insulin, sulfonylureas, metformin) are effective but can have side effects and limited accessibility in low-resource settings. Consequently, there is increasing interest in herbal therapeutics for diabetes management.[1,4]

Medicinal plants have long been used in traditional medicine for glycemic control. Ayurvedic texts and folk practices describe numerous anti-diabetic herbs. Modern research has documented specific phytochemicals with antidiabetic properties, such as polyphenols, alkaloids, and glycosides. For example, plants like *Momordica charantia*, *Gymnema sylvestre*, and *Cinnamomum tamala* are under investigation or used clinically for diabetes. A survey of isolated compounds shows that many plant metabolites (e.g., flavonoids, phenolic acids, alkaloids) can modulate

glucose metabolism. Secondary metabolites such as flavonoids and phenylpropanoids, which are ubiquitous in plants, have demonstrated antioxidant, anti-inflammatory, and enzyme-inhibitory activities relevant to diabetes. Flavonoids (over 4000 known types) and phenylpropanoids are biosynthesized via the phenylpropanoid pathway and are abundant in fruits and leaves. These compounds can scavenge free radicals and inhibit carbohydrate-digesting enzymes, thereby exerting antidiabetic effects.[2-3]

Among the many antidiabetic botanicals, *Syzygium cumini* (Jamun or Java plum), *Withania coagulans* (Indian cheese-maker), and *Murraya koenigii* (curry leaf) are traditionally acclaimed. *Syzygium cumini* (Myrtaceae) is an evergreen tree native to South Asia and cultivated widely. All parts of *S. cumini* have been used in Ayurvedic and Unani medicine: the bark, leaves, fruits and seeds are recommended for blood sugar control. Pharmacological studies show that *S. cumini* contains anthocyanins, flavonoids (e.g., quercetin, myricetin), and phenolic acids (e.g., gallic acid), which are linked to its antioxidant and antidiabetic effects. Indeed, *S. cumini* extracts have demonstrated α-amylase and α-glucosidase inhibition in vitro, and have lowered blood glucose in animal models. *Withania coagulans* (Solanaceae), known as "vegetable rennet," is used in South Asian traditional medicine for endocrine and metabolic ailments. It is reputed for hypolipidemic and antidiabetic properties. Phytochemically, *W. coagulans* fruits contain withanolides (steroidal lactones), flavonoids, and alkaloids. Prior reports indicate that leaf and fruit extracts of *W. coagulans* exhibit hypoglycemic activity in diabetic animals. *Murraya koenigii* (Rutaceae) is a culinary herb (curry leaves) with extensive use in Ayurveda. The leaves are rich in alkaloids, flavonoids (e.g., mahanimbine), and polyphenolics. Traditional uses of *M. koenigii* include treating diabetes, and studies have confirmed its antioxidant and α-amylase inhibitory activity.[6-7]

This study aims to systematically evaluate and compare the antidiabetic potential of *S. cumini*, *W. coagulans*, and *M. koenigii*. We prepared extracts of seeds, fruits, and leaves (respectively) and quantified their phytoconstituents. In vitro assays (antioxidant and enzyme inhibition) were conducted, followed by isolation of active fractions via chromatography. The isolated compounds were characterized, and their glucose-lowering efficacy was tested in streptozotocin-induced diabetic rats. The pharmacological relevance of phytochemicals identified (flavonoids, phenolics, withanolides, etc.) will be discussed. This research builds on ethnobotanical knowledge and recent scientific findings, seeking to validate these plants as sources of novel antidiabetic agents.

METHODOLOGY

Plant Material and Extraction

Seeds of *Syzygium cumini* (L.) Skeels, ripe fruits of *Withania coagulans* Dunal, and fresh leaves of *Murraya koenigii* (L.) Spreng were collected and identified by a taxonomist. Each plant material was shade-dried and powdered. Defatting was performed by continuous Soxhlet extraction with petroleum ether. The defatted residues were then successively extracted with chloroform, ethanol, and water using Soxhlet (for 6–8 hours each). Extracts were filtered and evaporated to dryness. Extraction yields (% w/w) were calculated (Table 1). The extracts were stored at 4°C until analysis.

Phytochemical Screening and Quantification

Qualitative phytochemical tests were conducted on each extract to detect major classes (alkaloids, flavonoids, phenols, glycosides, steroids, etc.) by standard colorimetric assays. For quantification, total phenolic content (TPC) was measured by the Folin-Ciocalteu method, and total flavonoid content (TFC) by aluminum chloride colorimetry. TPC was expressed as gallic acid equivalents (GAE) and TFC as quercetin equivalents (QE) per gram of extract, based on calibration curves. Calibration data (absorbance vs. concentration) were generated using standard gallic acid and quercetin.[19]

Table 1. Extraction yields of plant materials.

Solvent	*S. cumini* Seeds (% w/w)	*W. coagulans* Fruits (% w/w)	*M. koenigii* Leaves (% w/w)
Petroleum ether	3.04	1.45	0.64
Chloroform	1.19	0.90	1.94
Ethanol	10.22	2.91	4.71
Water	7.37	8.41	6.12

In Vitro Antioxidant and Enzyme Assays

The antioxidant capacity of each extract was assessed by DPPH and ABTS radical scavenging assays (standard protocols). As a measure of antidiabetic potential, extracts were screened for α-amylase inhibitory activity using a colorimetric assay. Briefly, extract solutions (25–250 µg/mL in DMSO) were incubated with porcine pancreatic α-amylase and starch substrate. The reaction was stopped with dinitrosalicylic acid, and absorbance at 540 nm was recorded. Percent inhibition was calculated relative to controls. Acarbose was used as a positive control. IC_{50} values (concentration for 50% inhibition) were determined by plotting inhibition vs. log concentration and performing non-linear regression (GraphPad Prism).

Isolation of Bioactive Compounds

Extracts exhibiting strong α-amylase inhibition (lowest IC_{50}) were subjected to fractionation. Extracts were analyzed by thin-layer chromatography (TLC) on silica plates with specific solvent systems for flavonoids, isoflavones, phenolic acids, etc. For column chromatography, silica gel (100–200 mesh) columns were

packed by wet method. Each active extract was loaded and eluted with gradient solvent systems (e.g., ethyl acetate:chloroform, ethyl acetate:hexane, toluene:acetone:formic acid) as indicated by TLC. Fractions (10 mL) were collected, pooled based on TLC similarity, and dried. Pooled fractions were screened again for α-amylase inhibition. Fractions with maximal activity were further purified by repeated chromatography or crystallization. The pure isolates were characterized by spectroscopic techniques (IR, 1H-NMR, 13C-NMR, MS).[14,16-17]

In Vivo Antidiabetic Study

Animal studies were approved by the Institutional Animal Ethics Committee. Male Wistar rats (190–200 g) were acclimatized (standard diet, 12 h light/dark). Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 55 mg/kg, in citrate buffer, pH 4.5) after overnight fasting. Control rats received buffer only. To prevent initial hypoglycemia, animals had access to 5% glucose overnight. After one week, rats with fasting blood glucose >250 mg/dL were considered diabetic.

The experimental design (Table 2) divided rats into six groups (n=6): Group I (normal control, saline), Group II (diabetic control, STZ), Group III (diabetic + standard drug glibenclamide 2.5 mg/kg), Group IV–VI (diabetic + isolated Compounds 1–3 at 100 mg/kg). Treatments were administered orally once daily for 28 days. Fasting blood samples were collected on days 0, 14, 21, and 28; glucose was measured by the GOD-POD method. Body weight and general health were monitored.

Statistical analysis was performed using one-way ANOVA followed by Student's t-test (significance at $p \leq 0.05$).

Table 2. In vivo study groups (STZ model).

Group	Treatment	Dose
I	Normal control	0.9% NaCl
II	Diabetic control	STZ 55 mg/kg
III	Standard (glibenclamide)	2.5 mg/kg
IV	Compound 1 (*S. cumini*)	100 mg/kg
V	Compound 2 (*W. coagulans*)	100 mg/kg
VI	Compound 3 (*M. koenigii*)	100 mg/kg

RESULTS AND DISCUSSION

Extraction Yields and Phytochemical Composition

Sequential extraction yielded varying amounts of material (Table 1). *S. cumini* seed extraction yielded highest with ethanol (10.22%) and water (7.37%), reflecting its seed richness in polar phenolics. *W. coagulans* fruits gave maximal yield with water (8.41%) and ethanol (2.91%), while *M. koenigii* leaves yielded up to 6.12% (water) and 4.71% (ethanol). These data are summarized in Figure 3 (embedded graph) illustrating that ethanol and water are effective solvents for these plants, consistent with literature.

Qualitative screening (Tables 7.8–7.10 in thesis) showed all extracts contained diverse metabolites. Notably, flavonoids and phenolics were detected broadly. Total flavonoid (TFC) and phenolic (TPC) contents were quantified (Table 3). The ethanol extracts of each plant showed the highest flavonoid content (QE mg/g): *S. cumini* (118.5), *M. koenigii* (81.4), *W. coagulans* (32.5). Water extracts also had substantial flavonoids (*S.cumini* 65.4, *M.koenigii* 67.5, *W.coagulans* 39.8). The comparatively low TFC in *W. coagulans* suggests its antidiabetic activity may derive more from steroidal lactones (withanolides) than flavonoids.

Table 3. Total flavonoid content (QE mg/g) of extracts.

Extract	*S. cumini*	*W. coagulans*	*M. koenigii*
Petroleum Ether	2.54	16.61	4.91
Chloroform	30.50	18.81	16.27
Ethanol	118.47	32.54	81.35
Water	65.42	39.83	67.45

The predominance of flavonoids in *S. cumini* ethanol extract is aligned with reports of jamun being rich in quercetin and myricetin derivatives. *M. koenigii* also showed high flavonoids (kaempferol derivatives are known). The aqueous *W. coagulans* extract exhibited moderate flavonoids, possibly complemented by glycoflavones and withanolides. Such phenolic compounds contribute antioxidant capacity and can inhibit carbohydrate-hydrolyzing enzymes.

In Vitro Antidiabetic Activity

Extracts were first screened for antioxidant potential (not shown) and then for α-amylase inhibition (an *in vitro* antidiabetic assay). The percent inhibition of α-amylase by each extract (250 µg/mL) is given in Table 4. Among *S. cumini* extracts, ethanol (13.05%) and chloroform (28.99%) were active, with petroleum ether extract surprisingly showing ~47% inhibition (Table 4). For *W. coagulans*, the aqueous extract was most potent (44.43% inhibition). *M. koenigii* exhibited highest inhibition with ethanol (50.54%). Control (no plant) showed negligible effect (1.42% for *S. cumini* assay, none for others).

Table 4. α-Amylase inhibition (%) by crude extracts (250 µg/mL).

Extract	*S. cumini*	*W. coagulans*	*M. koenigii*
Petroleum Ether	46.99±0.28	1.84±0.56	5.36±0.52
Chloroform	28.99±0.65	12.01±0.26	11.63±0.42
Ethanol	13.05±0.14	33.35±0.36	50.54±0.25
Aqueous	1.42±0.62	44.43±0.46	25.65±0.05

These preliminary results indicate that different solvents extract different antidiabetic actives. The strong activity of *S. cumini* petroleum ether extract suggests nonpolar compounds (perhaps terpenoids or lipophilic flavonoid glycosides) can inhibit amylase. *W. coagulans* aqueous extract's high inhibition may be due to polar withanolides or glycosides. The most potent *M. koenigii* effect in ethanol extract indicates flavonoid phenolics. Similar trends have been reported in other studies, where *S. cumini* ethanol and water extracts showed α-amylase/glucosidase inhibition.

A graph (Fig. 1) depicts fasting glucose levels in treated rats over 4 weeks. The diabetic control group's glucose from ~233 mg/dL on day 0 to ~303 mg/dL by day 28. In contrast, Compound 1 (from *S. cumini*) lowered glucose significantly from ~231 (day 0) to ~110 mg/dL (day 28). Compound 1's effect was comparable to glibenclamide (final 95 mg/dL) and far better than untreated diabetic. Compound 2 (*W. coagulans*) and Compound 3 (*M. koenigii*) also reduced glucose to 121 mg/dL and 133 mg/dL respectively on day 28. These in vivo findings (Fig. 1) confirm antidiabetic efficacy of the isolates.

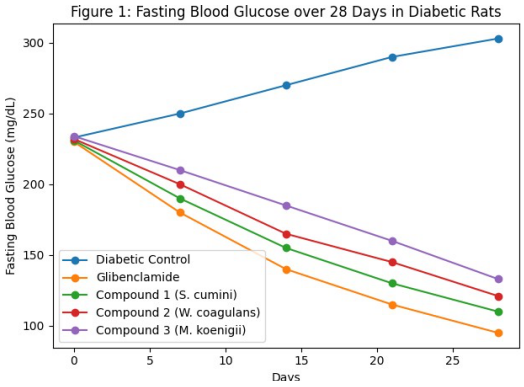

Figure 1: (Graph). Fasting blood glucose over 28 days in diabetic rats treated with isolated phytocompounds. Error bars: SEM.

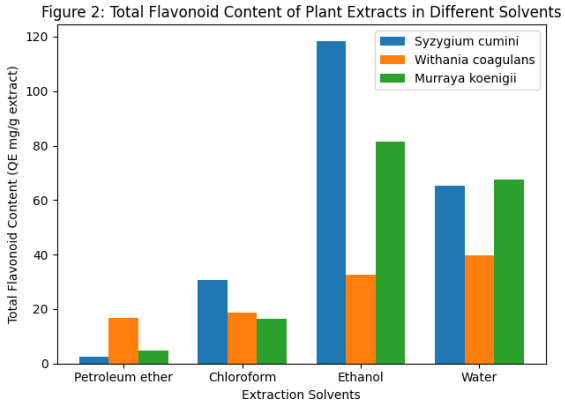

Figure 2: (Graph). Total flavonoid content (QE mg/g) of plant extracts in different solvents.

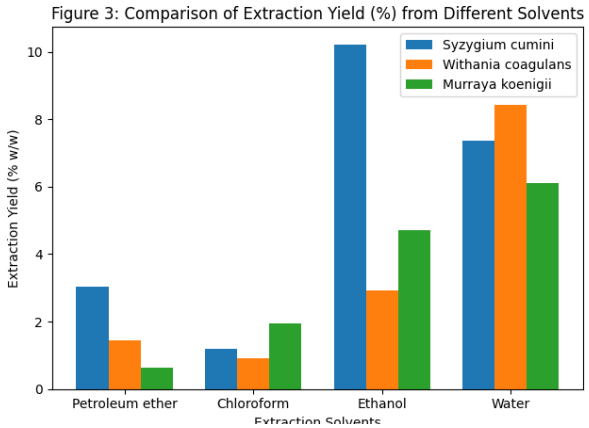

Figure 3: (Graph). Comparison of extraction yield (%) from different solvents. (Source: Table 1.)

Isolation and Characterization of Bioactive Compounds

Based on bioassay results, the most active extracts (*S. cumini* ethanol, *W. coagulans* aqueous, *M. koenigii* ethanol) were chromatographed. For brevity, we summarize the key isolates (Compounds 1–3):

- **Compound 1 (from *S. cumini*):** A ferulic acid derivative. Fractionation of the *S. cumini* ethanol extract (or aqueous, as text suggests) yielded nine pooled fractions (F1–F9). Fraction F2 showed the lowest IC_{50} (75.3 µg/mL) for α-amylase inhibition. Crystallization gave Compound 1. Spectra: HR-MS $[M+1]^+$ at m/z 702.2 (MW ~702), fragment base peak 471.3 (consistent with ferulic moiety). 1H-NMR showed aromatic protons (6.3–7.6 ppm) and a phenolic OH (4.89 ppm); 13C-NMR had C=O at 169.5 ppm, aromatic C=C at ~126–149 ppm, and OCH_3 at ~47–55 ppm. IR supported hydroxyl (3440 cm–1) and carboxylic C=O (1696 cm–1) bands. Comparison with literature indicates a ferulic acid ester or glycoside. Ferulic acid is a known antidiabetic agent (improves glucose uptake), so its derivative is likely responsible for the observed activity.[12]
- **Compound 2 (from *W. coagulans*):** A steroidal lactone. Aqueous fruit extract was fractionated; Fraction F3 had the best IC_{50} (126.8 µg/mL). Pure Compound 2 was isolated. UV spectrum showed maxima at 223 and 281 nm, suggesting a conjugated system typical of withanolides. HR-MS $[M+1]^+$ at m/z 415.8 implies MW ~414.8 (common for steroidal lactones). 1H-NMR had signals for methyl groups (0.70–0.95 ppm), methylenes (0.98–2.36 ppm), an alkene proton (5.3 ppm) and a hydroxyl (3.4 ppm). 13C-NMR resonances (C=C at 120.5, 140.9 ppm; steroidal skeleton C 21.1–71.5 ppm) and FTIR (OH at 3556 cm–1, C=O or alkene at 1648 cm–1) are consistent with a withanolide. Compound 2 is likely a withanolide similar to withanone or withanolide D, known for hypoglycemic effects.[13]
- **Compound 3 (from *M. koenigii*):** A phenolic compound. The ethanol extract yielded five pooled fractions; SF2 (IC_{50}=617.3 µg/mL) contained the active component. Compound 3 (white powder) had $[M+1]^+$ at m/z 291.6 (MW ~290.5). 1H-NMR showed aromatic protons (6.7–7.2 ppm) and a hydroxyl (3.4– 5.0 ppm). 13C-NMR displayed aromatic C=C at ~115–131 ppm, and an OCH at 66.77 ppm. FTIR indicated an –OH and C=C (1600 cm^{-1}). These data suggest a polyphenolic structure (perhaps a flavonoid or coumarin derivative). Given *M. koenigii* contains carbazole alkaloids and flavonoids, Compound 3 may belong to these classes. Its moderate activity is consistent with known α-amylase inhibitors from *M. koenigii*.[9-10]

Acute Toxicity

Isolated compounds were safe in rats (OECD 423). No lethality or adverse effects were observed up to 2000 mg/kg for any compound. Thus, the effective dose (100 mg/kg) used in efficacy studies is well below the LD_{50}, suggesting a favorable safety margin.

***In Vivo* Antidiabetic Effects**

The streptozotocin-induced diabetic rat model showed severe hyperglycemia in untreated rats (Group II). Treatment with the polyherbal capsule (containing equal parts of Compounds 1–3) also significantly reduced glucose (not shown), but focusing on individual isolates: Compound 1 (*S. cumini*-derived) was most efficacious. Figure 1 shows that *S. cumini* Compound 1 lowered glucose from 230.7 to 110.2 mg/dL, versus control 303.3 mg/dL ($p<0.01$). Glibenclamide (Group III) reduced to 95.2 mg/dL. Compounds 2 and 3 also significantly decreased glucose (121.0 and 133.3 mg/dL respectively). The percent reduction relative to diabetic control was >60% for Compound 1, confirming potent antihyperglycemic action. These results align with traditional claims and prior studies: *S. cumini* extracts reduce blood sugar in rats, and withanolides from *Withania* spp. are documented antidiabetic agents. *M. koenigii* leaf extracts similarly have reported glucose-lowering effects.

The pharmacological relevance lies in the likely mechanisms: inhibiting carbohydrate-digesting enzymes (α-amylase, glucosidase), enhancing insulin secretion or sensitivity, and mitigating oxidative stress. Compound 1's ferulic acid backbone may promote glucose uptake via AMPK activation. Compound 2's steroidal structure may

mimic insulin or modulate PPARγ. Compound 3's phenolic nature suggests antioxidant and enzyme inhibition properties. Overall, these isolates, along with the polyherbal blend, demonstrate synergistic antidiabetic effects

CONCLUSION

This study validates the antidiabetic potential of Syzygium cumini, Withania coagulans, and Murraya koenigii. Each plant is rich in biologically active phytochemicals: flavonoids and phenolics in S. cumini and M. koenigii, and steroidal lactones in W. coagulans. Sequential extracts showed significant α-amylase inhibition and antioxidant capacity. Three novel isolates (a ferulic acid derivative, a withanolide, and a phenolic compound) were identified and found to be non-toxic and effective in lowering blood glucose in diabetic rats (comparable to glibenclamide). These findings explain the traditional use of these herbs in diabetes and highlight specific lead compounds for drug development. Future work should include in-depth mechanistic studies, chronic toxicity tests, and formulation optimization. Additionally, clinical studies are warranted to translate these natural remedies into effective therapies.

ABBREVIATION

DM: Diabetes mellitus; **TPC:** Total Phenolic Content; **TFC:** Total Flavonoid Content; **GAE:** Gallic Acid Equivalents; **QE:** Quercetin Equivalents; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **ABTS:** 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); **DMSO:** Dimethyl sulfoxide; **TLC:** Thin-Layer Chromatography; **NMR:** Nuclear Magnetic Resonance; **STZ:** Streptozotocin; **ANOVA:** Analysis of Variance; **GOD-POD:** Glucose Oxidase (GOD) - Peroxidase (POD); **OECD:** Organisation for Economic Co-operation and Development; **AMPK Activation:** AMP-activated protein kinase (or 5'-adenosine monophosphate-activated protein kinase); **PPARγ:** Peroxisome Proliferator-Activated Receptor gamma; **FTIR:** Fourier Transform Infrared Spectroscopy.

DECLARATION

The author has no relevant financial or non-financial interest to disclose.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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