



GC-MS and FTIR characterization of 1,3,4-oxadiazole from *Biophytum sensitivum* with potential cardiovascular applications

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ABSTRACT

Cardiovascular diseases (CVDs) remain a leading cause of mortality worldwide, with a rising burden in low- and middle-income countries such as India. As concerns grow over the side effects and long-term safety of synthetic drugs, plant-derived bioactive compounds are gaining attention for their therapeutic potential. In this study, the traditional medicinal plant *Biophytum sensitivum* (L.) DC was investigated for its cardioprotective phytoconstituents. Methanolic (MeOH) extracts of the whole plant were first analyzed using Fourier Transform Infrared Spectroscopy (FTIR), which revealed key functional groups including hydroxyl, ester carbonyl, aromatic, and nitrogenous moieties—features commonly associated with antioxidant and lipid-regulatory activity. Subsequent Gas Chromatography–Mass Spectrometry (GC-MS) profiling identified several secondary metabolites, including two prominent oxadiazole derivatives: 2-p-nitrophenyl-5-isopropoxy-1,3,4-oxadiazole-5-one and 1,2,5-oxadiazole-3-amine-4-(4-methoxyphenoxy). These heterocyclic compounds are associated with lipid-lowering, calcium channel-modulating, and anti-inflammatory properties, supporting the plant's therapeutic potential. The findings validate the ethnomedicinal use of *B. sensitivum* and highlight its promise as a natural source of bioactive molecules for developing safer cardiovascular drugs.

KEYWORDS: *Biophytum sensitivum*, 1,3,4-oxadiazole, GC-MS, FTIR, Cardiovascular therapeutics, Medicinal plant, Heterocyclic compounds

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INTRODUCTION

Cardiovascular diseases (CVDs) remain a primary contributor to global mortality, responsible for nearly 17.9 million deaths each year constituting approximately 32% of total deaths worldwide, as reported by the World Health Organization (Şahin & İlgin, 2022). In India, the burden of non-communicable diseases, particularly CVDs, has significantly increased over the past decade, disproportionately affecting both urban and rural populations. The prevalence of cardiovascular disorders is exacerbated by factors such as sedentary lifestyles, high-fat diets, hypertension, hyperlipidaemia, obesity, and poor mental health. Atherosclerosis, a major pathological condition underpinning CVDs, arises from lipid accumulation in arterial walls, ultimately leading to myocardial infarction, arrhythmias, and cardiac arrest (Turecký *et al.*, 2021; Gao *et al.*, 2022). The strong correlation between hyperlipidaemia and cardiovascular

morbidity has highlighted the urgent need for effective lipid-lowering interventions. Current therapeutic strategies for CVD management often rely on synthetic drugs, which, despite their effectiveness, are associated with various side effects and long-term safety concerns. In response, the exploration of plant-based medicines, especially those derived from traditional folk remedies, has gained momentum. Secondary metabolites, particularly terpenoids, alkaloids, and flavonoids, have shown promising pharmacological potential, including cardioprotective, anti-inflammatory, antioxidant, and antihypertensive properties (Solárová *et al.*, 2020; Eftekhari *et al.*, 2021).

Biophytum sensitivum (L.) DC, a small herbaceous plant traditionally used in Ayurvedic medicine, is known for its therapeutic applications, including treatment for chest complaints. The plant is rich in various secondary metabolites and has shown antioxidant and lipid-lowering properties.

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Recent interest has focused on 1,3,4-oxadiazole, a heterocyclic compound with a five-membered ring structure that includes two nitrogen atoms and one oxygen atom. This compound has been recognized for its roles as an esterase mimic, calcium channel modulator, and lipid scavenger, making it a potential candidate for designing new cardioprotective drugs (Gastaldi *et al.*, 2023; Yang *et al.*, 2023). The present study aims to investigate the presence of 1,3,4-oxadiazole in *B. sensitivum* using advanced spectroscopic techniques, including FTIR and GC-MS. By characterizing the secondary metabolites in this medicinal plant, the study seeks to validate its traditional applications and highlight its potential as a natural source for the development of cardioprotective agents with reduced adverse reactions.

MATERIALS AND METHODS

Plant Material and Taxonomic Identification

The entire plant (Figure 1) of *Biophytum sensitivum* (L.) DC was harvested from Komaneri village in Thoothukudi District, Tamil Nadu, India. Taxonomic verification was done by S. Mutheeswaran, Scientist, St. Xavier Research Foundation, St. Xavier College, Palayamkottai, Tamil Nadu. A voucher specimen (Ref. No. XCH-40483) has been deposited for future reference.

B. sensitivum belongs to the Oxalidaceae family and is popularly referred to in Tamil as “Theentanaali” or “Little Tree Plant.” *B. sensitivum* is usually found growing in fallow land and features red flowers with a closely compacted growth system of not more than 2-2.5 cm in height. The plant has traditionally been employed in Ayurveda for the management of respiratory diseases and chest disorders (Roopa *et al.*, 2022).

Preparation of Plant Extracts

Following taxonomic verification, the entire plant material of *B. sensitivum* was washed extensively with distilled water to eliminate surface contaminants. The plant material was then shade-dried at room temperature for about 2-3 weeks to



Figure 1: *Biophytum sensitivum* plant displaying the displays the aerial and root parts

maintain thermolabile phytochemicals. The dried plant material was mechanically ground into a coarse powder and then sieved through a 100 mm mesh sieve. The resultant powder was kept at ambient temperature in clean, tight polyethylene bags until additional extraction. Extraction was performed using a modified protocol explained by Pallab *et al.* (2013).

For extraction, 500 g of the powdered plant material was put into a filter paper thimble and loaded into the main chamber of a Soxhlet extractor (10 g sample/200 mL solvent) 70% methanol (analytical grade, 500 mL) was employed as the solvent of extraction and loaded into the distillation flask. The Soxhlet apparatus was set up and run at the boiling point of the solvent (around 65 °C for methanol), with constant extraction performed for 6-8 hrs, until the solvent in the siphon tube turned colorless, showing that the extraction was complete. The MeOH extract was concentrated under reduced pressure with a rotary evaporator at 40 °C. The obtained semi-solid residue (crude extract) was stored, weighed, and kept in a sterile glass vial at 4 °C for further phytochemical and bioactivity analyses.

FTIR Spectroscopy

FTIR spectroscopy was conducted to characterize the functional groups corresponding to phytochemicals in the MeOH extract of *B. sensitivum*. FTIR analysis was done with the Shimadzu IR Affinity-1S FTIR spectrometer (Shimadzu Corporation, Japan). An amount of 2 mg of the lyophilized methanolic extract was homogenized in 200 mg of spectroscopic-grade potassium bromide (KBr) and well mixed using an agate mortar. The mixture was compressed for 2 min, into a transparent pellet under vacuum in a manual hydraulic press at a pressure of 10 tonnes. The prepared KBr pellet was introduced in the sample holder, and spectra were collected at 4000 to 400 cm⁻¹ wavenumber range with 4 cm⁻¹ resolution and 32 scans per sample for the purpose of signal quality. The spectra thus obtained were scanned for typical absorption bands for functional groups. Spectral interpretation was performed through the comparison of observed peaks to reference data from the National Institute of Standards and Technology (NIST) Library (Cárdenas-Escudero *et al.*, 2023). The identification of important functional groups such as aromatic, hydroxyl, carbonyl, and ester linkages, which are characteristic of bioactive secondary metabolites, was made possible by this comparison.

GC-MS Analysis

The MeOH extract of *Biophytum sensitivum* (L.) DC was subjected to GC-MS to scan for secondary metabolites, as per a slightly modified protocol as in Javaid *et al.* (2021). Approximately 1 mL of the concentrated extract was re-dissolved in 10 mL HPLC-grade 70% methanol (final concentration: 100 mg/mL), vortexed for 2 min, and subjected to sonication for 10 min to ensure complete solubilization. The solution was then centrifuged at 10,000 rpm for 5 min at 4 °C using a refrigerated centrifuge. The supernatant obtained was filtered through a 0.22 µm PTFE syringe filter to achieve a

particle-free solution, which was then transferred into GC-MS vials for analysis.

GC-MS analysis was carried out in an Agilent 7890A gas chromatograph connected to a 5975C mass selective detector (MSD) with a DB-5MS capillary column (30 m x 0.25 mm internal diameter, and film thickness of 0.25 μm). The port for injection was kept at 250 $^{\circ}\text{C}$, and the oven temperature was set to begin at 60 $^{\circ}\text{C}$ (for 2 min), followed by a rise at a rate of 10 $^{\circ}\text{C}/\text{min}$ to a final temperature of 280 $^{\circ}\text{C}$, for 10 min. Helium, the carrier gas was used at a flow rate of 1.0 mL/min. The mass spectrometer was run in electron ionization (EI) mode at 70 eV, scanning 40–600 m/z. Compound identification was done by matching retention times, molecular ion peaks, and fragmentation patterns with entries in the NIST Mass Spectral Library, taking only those with a match quality of more than 85% for further analysis.

RESULT AND DISCUSSION

Plant Authentication and Ethnopharmacological Background

B. sensitivum, which is a diminutive herbaceous plant of the Oxalidaceae family, was taxonomically confirmed and certified. It has been used conventionally in Siddha and Ayurvedic treatment for respiratory ailments, inflammation, and congestive chest diseases (Sivan *et al.*, 2022). Its documented cardioprotective effects, comprising anti-inflammatory as well as lipid-lowering action, have been further validated by previous research (Suja *et al.*, 2025), positioning it as a logical candidate in cardiovascular drug research.

FTIR Analysis and Functional Group Confirmation

FTIR spectroscopy was utilized to determine the functional groups in the MeOH extract of *B. sensitivum*. Spectral interpretation based on conventional frequency assignments and cross-checked with the NIST Library indicated the presence of a wide range of bioactive chemical classes. Figure 2 depicts the FTIR spectrum of the MeOH extract of *B. sensitivum*, with major absorption peaks. Table 1 presents the corresponding functional group assignments, which show the presence of hydroxyl, carbonyl, aromatic, and ether functionalities.

A major absorption band at 3444.63 cm^{-1} is due to the stretching frequencies of O-H and N-H groups, indicating the presence of hydroxyl and amine groups that are often present in phenolic compounds, flavonoids, and heterocycles with amino groups. These groups possess radical-scavenging and anti-inflammatory activity, and their presence indicates antioxidant activity of the extract (Eftekhari *et al.*, 2021). The strong peak at 1749.32 cm^{-1} is due to C=O stretching of ester and lactone functional moieties, common in terpenoid and oxadiazole backbones. The occurrence of such carbonyl functionalities is indicative of lipid-modulating and vasorelaxant activity, which is consistent with the GC-MS-detected oxadiazole derivatives. A spike band at 1625.88 cm^{-1} is due to C=C or C=N stretching, indicative of

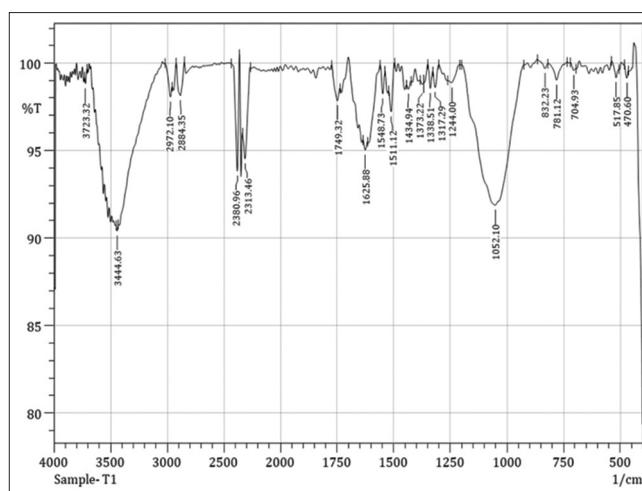


Figure 2: FTIR spectrum of the MeOH extract of *Biophytum sensitivum*

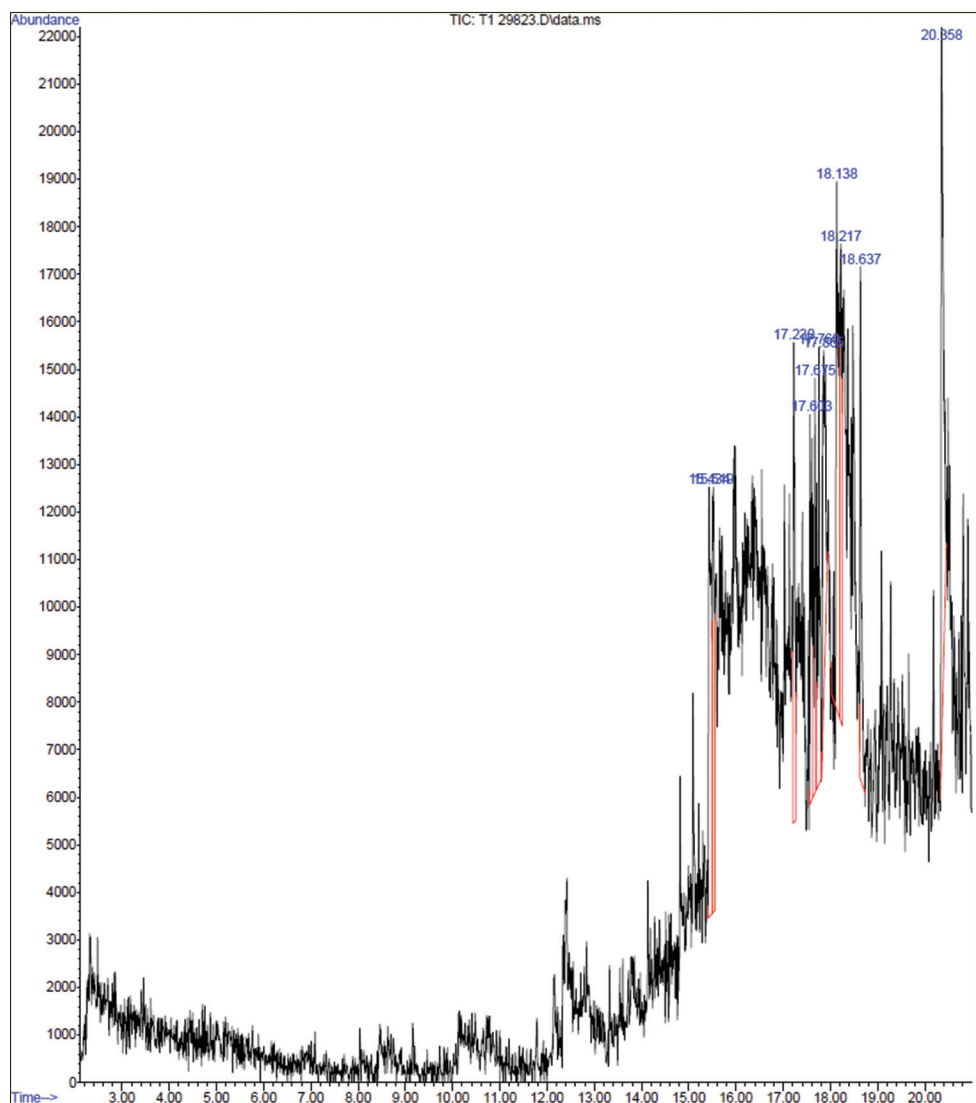
aromatic systems and nitrogenous heterocycles like oxadiazoles. This also indicates the presence of the identified compound 2-p-nitrophenyl-5-isopropoxy-1,3,4-oxadiazole-5-one, which is known for cardiovascular use (Luczynski & Kudelko, 2022). The band at 1434.94 cm^{-1} is due to N=N stretching vibrations, further indicating the presence of azo or azole moieties, a characteristic of bioactive heterocyclic compounds. Likewise, the aromatic substitution patterns at 781.12 cm^{-1} and 704.93 cm^{-1} are due to mono- and di-substituted phenyl rings, supporting the aromatic nature of the phytochemicals. Lastly, the 2380.96 cm^{-1} absorption could be attributed to C-O stretching or ester bond deformation, consistent once more with oxadiazole esters. These features in combination are typical of complicated phytochemicals with multiple biological activities.

GC-MS Analysis and Identification of Bioactives

The GC-MS profiling of *B. sensitivum* MeOH extract (Figure 3) displayed multiple compounds, with the major fragmentation patterns of every compound illustrated in the spectrum. The mass spectrum of the two major compounds, i.e., 2-p-nitrophenyl-5-isopropoxy-1,3,4-oxadiazole-5-one and 1,2,5-oxadiazole-3-amine-4-(4-methoxy phenoxy) (Figure 4), emphasized the structural details that were essential for their identification. A comprehensive list of the bioactive compounds isolated from the MeOH extract is given in Table 2. They are 2-p-nitrophenyl-5-isopropoxy-1,3,4-oxadiazole-5-one (RT: 17.679 min) and 1,2,5-oxadiazole-3-amine, 4-(4-methoxyphenoxy)- (RT: 18.218 min), both of which had dominant mass fragments at m/z 207 and structurally significant secondary peaks. The typical retention times and spectral profiles established the occurrence of oxadiazole rings bearing electron-withdrawing and electron-donating groups, known to affect biological activity. The 1,3,4-oxadiazole ring is a privileged scaffold in the field of drug discovery, specifically for cardiovascular drug development. These derivatives have previously been shown to have antihypertensive, vasodilatory, antithrombotic, and cardioprotective activity, often through nitric oxide signaling, inhibition of calcium channels, and antioxidant effects (Rubina *et al.*, 2019). As an example, Rani

Table 1: Functional group characterization of MeOH extract of *Biophytum sensitivum* using FTIR spectroscopy

Peak No.	Wavenumber (cm ⁻¹)	Functional Group Identified	Bond Type/Assignment	Interpretation
1	704.93	Mono-substituted phenyl ring	Aromatic C–H out-of-plane bending	Confirms mono-substitution on aromatic ring
2	781.12	Di-substituted phenyl ring	Aromatic C–H out-of-plane bending	Indicates presence of di-substituted benzene rings
3	1434.94	Azo or azole group	N=N stretching	Suggests presence of nitrogen-containing heterocycles (oxadiazoles)
4	1625.88	Aromatic ring/conjugated C=O	C=C/C=O stretching	Indicates aromatic rings or conjugated carbonyl groups
5	1749.32	Ester carbonyl group	C=O stretching	Presence of ester or lactone functional groups
6	2380.96	Alkynes or nitriles (tentative)	C≡C/C≡N stretching	Possibly conjugated alkynes or nitriles
7	3444.63	Hydroxyl/amine group	O–H/N–H stretching (broad)	Suggests phenols, alcohols, or hydrogen-bonded amines
8	1043.32	C–O–C (ether linkage)	C–O stretching	Indicates ether functionality (e.g., methoxy group)
9	1240.52	Aromatic C–O stretch	C–O stretching in aryl ethers	Supports presence of methoxy-substituted aromatics
10	2920.28	Aliphatic C–H	C–H asymmetric and symmetric stretching	Characteristic of –CH ₂ – and –CH ₃ – groups in alkyl chains


Figure 3: GC-MS spectra of bioactive compounds MeOH extract of *Biophytum sensitivum* and its principle fragmentations

et al. (2016) prepared and characterized 1,3,4-oxadiazoles bearing nitrophenyl substitutions, with substantial antihypertensive activity through ACE inhibition. Likewise, oxadiazoles

substituted with methoxy have been associated with increased vasorelaxant and lipid-lowering activities because of increased lipophilicity and target binding affinity (Gandhi *et al.*, 2023).

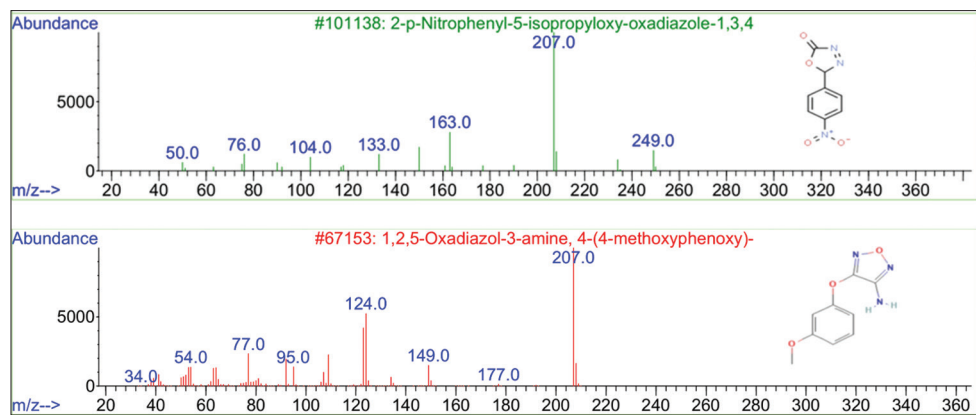


Figure 4: Mass spectrum of a) 2-p-nitrophenyl-5-isopropoxy-1,3,4-oxadiazole-5-one and b) 1,2,5-oxadiazole-3-amine-4-(4-methoxy phenoxy)

Table 2: Bioactive compounds identified in the of MeOH extract of *Biophytum sensitivum* – GCMS analysis

S. No.	Retention Time (min)	Compound	Base Peak (s) (m/z)	Molecular Formula	Molecular Weight (g/mol)	Peak Area	% Area	Probable Pharmacological Relevance
1	17.679	2-p-Nitrophenyl-5-isopropoxy-1,3,4-oxadiazole-5-one	207, 163, 249	$C_8H_5N_3O_4$	207.14	317044	7.65	Antihypertensive, ACE inhibitory, vasodilatory
2	18.218	1,2,5-Oxadiazole-3-amine, 4-(4-methoxyphenoxy)-	207, 124, 149	$C_9H_9N_3O_3$	207.19	263441	6.35	Cardioprotective, antioxidant
3	18.634	2-Hydroxychalcone	105, 147, 178	$C_{15}H_{12}O_2$	224.25	284150	6.85	Antioxidant, anti-inflammatory, vasoprotective
4	18.142	1-Monolinoleoylglycerol trimethylsilyl ether	207, 281, 429	$C_{27}H_{56}O_4Si_2$	500.9	419855	10.14	Anti-inflammatory, endothelial-protective
5	17.764	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine	207, 105, 135	$C_{16}H_{14}N_2O_4$	298.29	281045	6.79	Antioxidant, potential neuroprotective
6	17.679	Dodecahydropyrido[1,2-b] isoquinolin-6-one	207, 178, 125	$C_{13}H_{21}NO$	207.31	298342	7.20	Smooth muscle relaxant, cardiotoxic
7	18.634	2-Myristinoyl-glycinamide	207, 236, 262	$C_{16}H_{28}N_2O_2$	280.41	275143	6.65	Lipid-lowering, potential cardiovascular modulator
8	17.603	Benzoic acid, 2,4-bis [(trimethylsilyl) oxy]-, trimethylsilyl ester	207, 281, 355	$C_{13}H_{22}O_3Si_2$	282.48	245121	5.92	Antioxidant, antimicrobial
9	18.142	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-, isopropyl ester	165, 238, 266	$C_{21}H_{25}NO_4$	355.53	332940	8.05	Anti-inflammatory, neuroprotective
10	17.868	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	207, 281, 429	$C_{14}H_{44}O_6Si_7$	505.1	261193	6.30	Possible phytosiloxane residue or derivatization product

Besides the oxadiazoles, some co-occurring phytoconstituents were detected: 2-Hydroxychalcone, a flavonoid precursor with high antioxidant and vasoprotective activity, already described in cardiovascular active plants (Zhuang *et al.*, 2017). 1-Monolinoleoylglycerol trimethylsilyl ether, lipid derivative with anti-bacterial, anti-inflammatory and endothelial-protective activities, commonly associated with enhanced vascular homeostasis (Tyagi & Agarwal, 2017). 4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine, a phenylalkylamine compound with possible free radical scavenging activity, is structurally similar to known bioactive alkaloids (Nasrin *et al.*, 2022). Dodecahydropyrido[1,2-b] isoquinolin-6-one, a nitrogen heterocycle linked with smooth muscle relaxant and permeates blood brain barrier (Faleye *et al.*, 2023). Although some siloxane derivatives (e.g., octasiloxane, heptasiloxane) were also found present, these are typically seen with GC-MS due to interactions with the column or silylation

artifacts; however, they are present abundantly enough as to suggest a possible endogenous phytosiloxane or plant origin.

Together, the detection of 1,3,4-oxadiazole derivatives and supportive phytochemicals of *B. sensitivum* highlights the cardiovascular therapeutic potential of the plant. These results confirm its folklore usage and provide new avenues for the lead discovery of oxadiazole-based bioactives for cardiovascular drug development. *In silico* docking, enzyme inhibition assays, and *in vivo* cardiovascular models' validation are suggested to affirm their efficacy and mode of action.

Pharmacological Significance of Oxadiazoles in Cardiovascular Therapy

The oxadiazole nucleus is a well-established pharmacophore in cardiovascular drug design. Compounds like raltegravir

and zibotentan have 1,3,4-oxadiazole units and have been evaluated for endothelial modulation, anti-hypertensive, and atherosclerotic plaque-reducing activity. The oxadiazole molecule can also be a calcium channel modulator in myocardial patients (Gastaldi *et al.*, 2023). In addition, recent studies have demonstrated that oxadiazoles inhibit acid sphingomyelinase, a major enzyme involved in lipid metabolism and inflammation pathways involved in atherosclerosis. Oxadiazole was established in a recent research article as an acid sphingomyelinase inhibitor in Atherosclerosis was involved in sphingomyelin metabolism. It is a novel effective drug target to treat atherosclerosis (Yang *et al.*, 2023). Our results from *B. sensitivum* are consistent with these pharmacological activities, showing the plant as a good natural source of bioactive oxadiazole analogs. This is consistent with the trend of current research towards plant-derived cardiovascular drugs with fewer side effects compared to synthetic statins (Eftekhari *et al.*, 2021).

Therapeutic Implications and Future Directions

This research further establishes the evidence base for *B. sensitivum* as a cardioprotective agent source. The oxadiazole derivatives isolated are most promising because they possess multiple mechanisms, such as lipid-lowering, calcium channel modulation, and antioxidant activities. Such multifunctional activities are crucial in controlling the multifactorial pathophysiology of cardiovascular disease. The significance of this work is based on the first-time reporting of these particular oxadiazole analogs from *B. sensitivum* through GC-MS and FTIR, with direct implications for their inclusion in natural drug formulation pipelines. But to prove pharmacological efficacy and safety, additional *in vitro* and *in vivo* studies, along with molecular docking and pharmacokinetic assessments, are required.

CONCLUSION

This research presents *B. sensitivum*, a medicinal plant with traditional application, as a bioresource with high potential for the discovery of eco-friendly cardiovascular therapeutics. GC-MS and FTIR spectroscopy identified two pharmacologically relevant oxadiazole derivatives: 2-p-nitrophenyl-5-isopropoxy-1,3,4-oxadiazole-5-one and 1,2,5-oxadiazole-3-amine-4-(4-methoxyphenoxy). These heterocyclic molecules have lipid-lowering and anti-inflammatory activities, which are critical in the control of cardiovascular diseases. The presence of functional groups of the identified compounds was verified by FTIR spectroscopy, confirming the structural integrity and bioactivity of the extract. The results are significant in that they validate the use of *B. sensitivum* based on traditional knowledge and offer a sustainable substitute to synthetic medicines, which could minimize environmental pollution and side effects commonly related to traditional pharmaceuticals. Considering the expanding cardiovascular disease burden and the rising necessity for environmentally sound healthcare solutions worldwide, the application of plant bioactive compounds into drug development is a sustainable, socially acceptable, and environmentally sound approach. The pharmacological assay

and environmental life-cycle assessment were suggested to maximally utilize this species' therapeutic and ecological worth.

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