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Extraction Phytochemical Screening And Isolation Of Compound From Gardenia Resinifera Hydroalcoholic Extract

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Abstract
The present study was carried out to explore the phytochemical profile and isolate bioactive compounds from the hydroalcoholic extract of Gardenia resinifera Roth. Extraction was performed using a hydroalcoholic solvent system to maximize the yield of both polar and non-polar phytoconstituents. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids, tannins, and phenolic compounds. For isolation, thin layer chromatography (TLC) was initially optimized to identify a suitable solvent system, followed by column chromatography using silica gel as the stationary phase and toluene:ethyl acetate (7.5:2.5) as the mobile phase. Fractions 55–63 exhibited characteristic color reactions in phytochemical tests and distinct UV fluorescence, suggesting the presence of terpenoid-based constituents. Spectroscopic characterization, including UV, FTIR, ^{1}H-NMR, and ESI-MS, confirmed the structure of the isolated compound as a pentacyclic terpenoid derivative with the molecular formula $C_{30}H_{50}O$ and λmax at 535 nm. These findings highlight Gardenia resinifera as a rich source of bioactive terpenoids, supporting its traditional medicinal applications and providing a foundation for further pharmacological investigation.

Keywords: *Gardenia resinifera; Hydroalcoholic extract; Phytochemical screening; TLC; Column chromatography; Terpenoid; UV; FTIR; NMR; Mass spectrometry*

INTRODUCTION

Medicinal plants have long been recognized as valuable sources of bioactive compounds that serve as leads for drug discovery and development (Mustafa et al., 2017). Among them, the genus *Gardenia* (family Rubiaceae) has been widely studied for its diverse phytochemical constituents, including iridoids, terpenoids, flavonoids, and phenolic compounds, which are associated with significant pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, and neuroprotective effects (Zedan et al., 2024).

Gardenia resinifera Roth., commonly known as "Dikamali," is a medicinal shrub native to India and traditionally used in Ayurveda and Siddha systems of medicine. The gum-resin obtained from its bark is reported for its use in the management of respiratory disorders, skin ailments, and inflammatory conditions (Maurya et al., 2017). Phytochemical investigations have revealed the presence of iridoid glycosides, triterpenoids, and sterols that may contribute to its therapeutic potential (Wang et al., 2020).

Extraction of plant materials using hydroalcoholic solvents is considered efficient, as it allows the simultaneous recovery of both polar and moderately non-polar constituents, ensuring a broad spectrum of phytochemicals for further analysis (Darwin et al., 2025). Preliminary phytochemical screening is a crucial step in identifying classes of bioactive compounds, which can then be subjected to chromatographic separation techniques such as thin layer chromatography (TLC) and column chromatography for isolation and purification (Ingle et al., 2017).

In natural product chemistry, isolation and structural characterization of compounds are essential for establishing their role in pharmacological activity. Column chromatography, coupled with spectroscopic techniques including UV, FTIR, NMR, and mass spectrometry, provides a comprehensive approach to identifying the molecular structure of purified compounds (Butler; 2004).

Considering the traditional importance of *Gardenia resinifera* and the lack of extensive phytochemical exploration, this study focuses on the extraction, phytochemical screening, and isolation of bioactive compounds from its hydroalcoholic extract, aiming to provide a scientific basis for its therapeutic applications.

MATERIAL AND METHODS

Material

For the investigation, hydroalcoholic extract of *Gardenia resinifera* was used along with analytical grade solvents such as toluene, ethyl acetate, methanol, ethanol, chloroform, HCl, KH_2PO_4, NaOH, and citric acid, procured from standard suppliers. Silica gel $60F_{254}$ plates (Merck) were used for TLC and silica gel (60–120 mesh) for column chromatography using a glass column (100 × 3 cm) with an isocratic mobile phase of toluene:ethyl acetate (7.5:2.5). Fractions were visualized under UV (254 and 365 nm), normal light, and phytochemical tests. Characterization was carried out by UV, FTIR (Bruker Alpha), ^{1}H-NMR (JEOL 500 MHz, Methanol-D_3, TMS standard), and Mass spectrometry (Waters triple quadrupole system).

Methods

Defatting of plant material

A total of 188 g of shade-dried powdered leaves of *Gardenia resinifera* were subjected to maceration with petroleum ether to remove fatty materials. The process was continued until complete defatting of the plant material was achieved (Devi and Arumughan; 2007).

Extraction with hydroalcoholic solvent using maceration

The defatted plant powder was extracted using a hydroalcoholic solvent system (ethanol: water, 80:20 v/v) as described by Kokate (1994). The mixture was kept undisturbed for 48 hours under sterile conditions. The resulting extract was filtered through Whatman No. 40 filter paper, and the filtrate was concentrated on a water bath at 80–90 °C until a dry extract was obtained (Sahlabgi et al., .2025)

Thin layer chromatography

Thin Layer Chromatography (TLC) is based on the adsorption principle, where solutes in the mobile phase migrate across the surface of a stationary phase. In this study, silica gel 60F254 plates (7 × 6 cm; Merck) were used with the one-dimensional ascending method. Samples (1 µL) were spotted using glass capillaries at 1 cm distance on five tracks, and plates were developed in a twin trough chamber pre-saturated with the mobile phase for 20 minutes. After development, plates were dried and visualized using freshly prepared iodine reagent. The mobility of active compounds was determined by calculating the retention factor (Rf) values (Cimpoiu; 2006).

Detection and calculation of R_f Value

The R_f Value of the spot was determined using the formula after the chromatogram was formed:

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Isolation of compound by column chromatography

Coloum size: Glass column, 100 x 3 Cms
Stationary Phase: Silica gel (60 to 120 mesh size)
Elution mode: Isocratic elution
Mobile Phase: Toluene: Ethyl acetate (7.5:2.5)
Extract: *Gardenia resinifera* Hydroalcoholic extract
Visualized by: Short UV (254nm), long UV (365nm) and Normal light
Identification of similar fractions: visualized using a phytochemical test, normal light, long UV (365 nm), and short UV (254 nm).

Procedure

Preparation of column

The wet packing method was used to stuff the columns. After being activated at 105°C, silica was taken, suspended in mobile phase, transferred to a column, and given time to settle. To prevent the silica layer from being disturbed during elution, a cotton plug was placed at the top of the silica layer.

Preparation of sample

2.5 grams of extract were placed in a beaker, and enough mobile phase was added, along with silica (60–120 mesh size) for column chromatography. Using a cotton plug, the slurry was prepared and applied on top of the silica gel column.

Isocratic elution technique

The isocratic elution method was employed to fractionate the components. For elution, a toluene: ethyl acetate (7.5:2.5) mobile phase is employed. Ten milliliters of each portion were gathered, and distillation was used to recover the solvent. TLC was applied after the fraction was collected, concentrated, and stored.

Identification of similar fractions

TLC Toluene: Ethyl acetate (7.5:2.5) was used to monitor the various fractions of column chromatographic elution (100); a UV chamber and a phytochemical test with a particular reagent were used to identify a single isolated compound by comparing it to reference compounds. Fractions with comparable TLC fingerprinting profiles were collected and combined. After drying, the fraction revealed a single compound with a comparable R_f value to the reference compound; the product was subsequently purified using the method of recrystallization.

Table 1: Characterization of Isolated compound

S. No	No. of fractions	TLC UV spectra		Salkowski test	Results
		UV-254	UV-366		
1	1-10	No Spot	No Spot	-ve	No colour change
2	11-21	3 Spot	3 Spot	-ve	No colour change
3	22-32	4 Spot	3 Spot	-ve	No colour change
5	33-45	3 Spot	4 Spot	-ve	No colour change
6	46-54	2 Spot	3 Spot	-ve	No colour change
7	55-63	1 Spot	1 Spot	+ve	Reddish brown coloration
8	64-72	2 Spot	3 Spot	-ve	No colour change
9	73-84	3 Spot	2 spot	-ve	No colour change
10	85-96	2 Spot	3 spot	-ve	No colour change
11	97-100	No Spot	No Spot	-ve	No colour change

Characterization of Isolated compound (55-63)

Ten milliliters of each of the 100 fractions were collected, and IR, NMR, and mass spectroscopy analyses were used to characterize the isolated compound.

I.R. Analysis- The IR spectrum of compounds was recorded on (Bruker Alpha) (LNCT College of Pharmacy Bhopal, M.P.) using solid plate technique with KBr.

NMR Analysis- H^1NMR was recorded on JEOL (500MHz FT-NMR) in Methanol-D3 using TMS as internal standard (IISER, Bhopal).

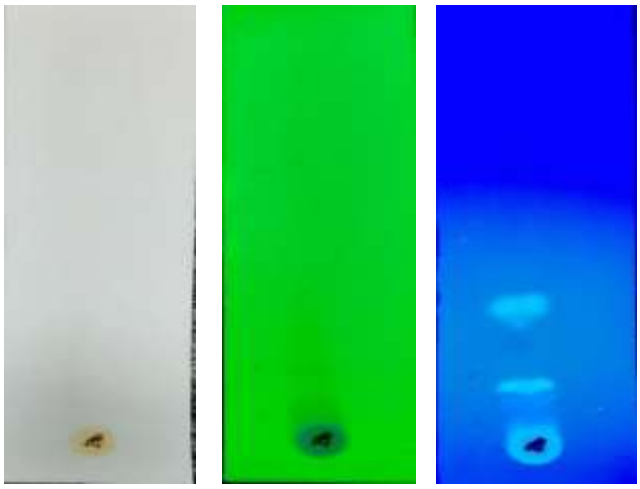
Mass Analysis- MASS spectrum of compounds was recorded Waters Equity *triple* quadrupole MS system.

RESULTS AND DISCUSSION

Table 2: Optimization of thin layer chromatography for column chromatography

S. No.	Mobile Phase	Results
1	Toluene: Ethyl acetate (7:3)	Not Suitable
2	Toluene: Ethyl acetate (8:2)	Not Suitable
3	Chloroform : Methanol (5:5)	Not Suitable

4	Ethyl acetate : Methanol (5: 5)	Not Suitable
5	Ethyl acetate : Methanol (4: 6)	Not Suitable
6	Toluene : Ethyl acetate (6:4)	Not Suitable
7	Chloroform :Methanol (9: 1)	Not Suitable
8	Toluene: Ethyl acetate (9:1)	Not Suitable
9	**Toluene: Ethyl acetate (7.5:2.5)**	**Most Suitable**



Normal Light Short UV Long UV

Figure 1: Optimized mobile phase Toluene: Ethyl acetate (7.5:2.5)

Results of Characterization of Isolated compound

UV spectra of isolated compound (55-63)

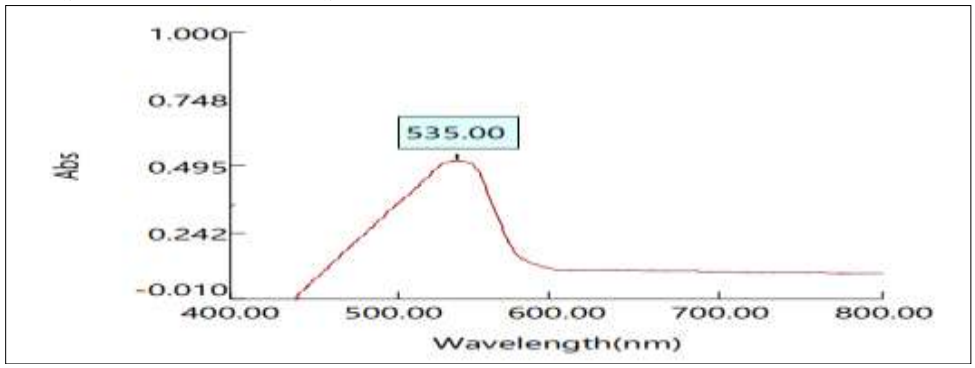


Figure 2: Graph of UV spectra of isolated compound (55-63)

IR Spectra of isolated compound (55-63)

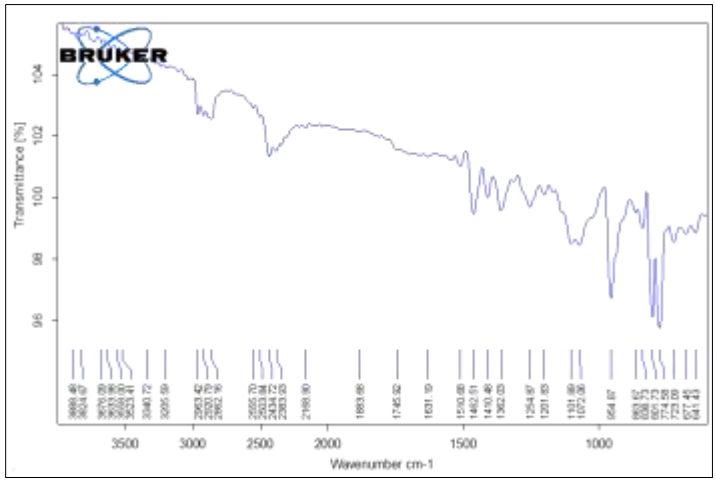


Figure 3: Graph of IR spectra of isolated compound (55-63)

NMR Spectra of isolated compound (55-63)

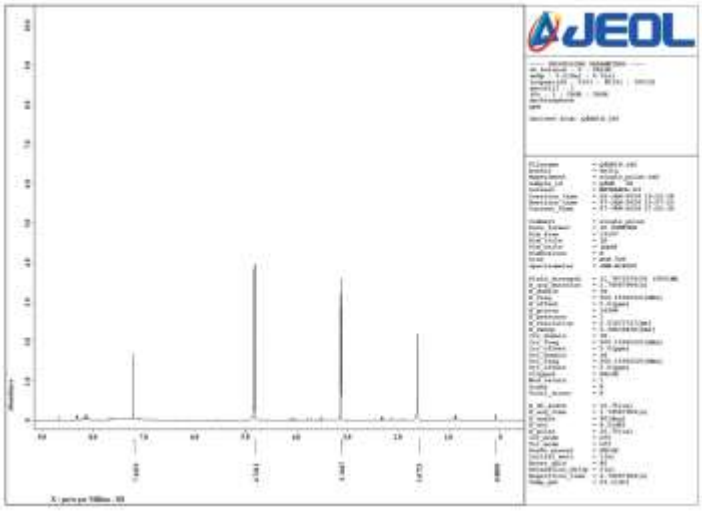


Figure 4: Graph of NMR spectra of isolated compound (55-63)

MASS Spectra of isolated compound (55-63)

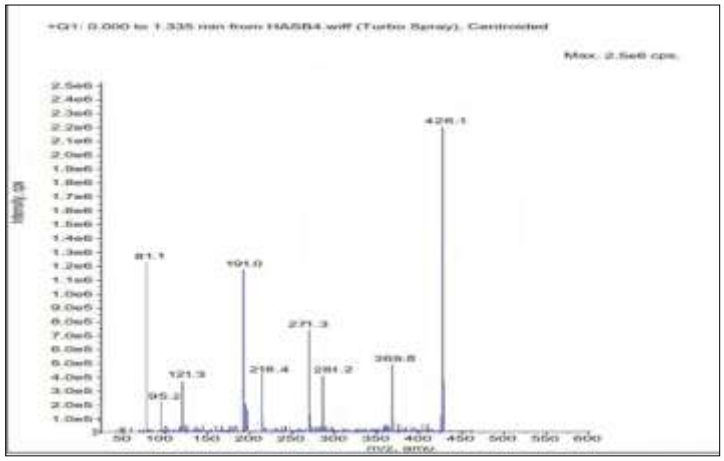
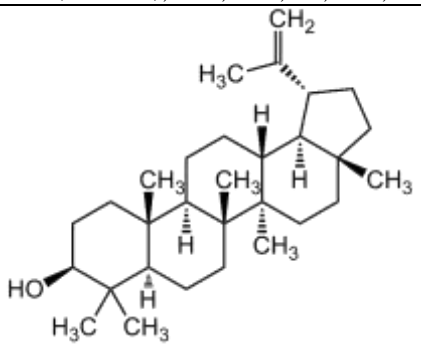


Figure 5: Graph of NMR spectra of isolated compound (55-63)

Table 3: Showing interpreted data of different spectra of isolated compound (55-63)

Method	Spectral interpretation
Lambda Max	535nm
IR	A broad O-H stretching peak around 3200-3600 cm^{-1}. Distinct aliphatic C-H stretching peaks in the 2850-2960 cm^{-1} range. C=C stretching peaks around 1600-1680 cm^{-1}. C-H bending vibrations in the 1350-1470 cm^{-1} range. C-O stretching peaks around 1050-1150 cm^{-1}. A complex fingerprint region between 600-1400 cm^{-1}.
^{1}HNMR (ppm)	δ, 1.6721 (s, H), 3.1367 (d, H-3), 4.7411 (d, H), 7.2423 (s, 1H,-OH) ppm.
ESI-MS (m/z)	426 (100.0%), 191, 218, 81, 271,281, 121, 95,369
Structure	
IUPAC Name	1,2,5,14,18,18-hexamethyl-8-(prop-1-en-2-yl)pentacyclo[11.8.0.0^{2},^{10}.0^{5,9}.0^{14},^{19}]henicosan-17-ol
Molecular Formula	$C_{30}H_{50}O$

CONCLUSION

The present study successfully isolated and characterized a bioactive terpenoid compound from the hydroalcoholic extract of *Gardenia resinifera* using chromatographic and spectroscopic techniques. Thin layer chromatography and column chromatography enabled efficient separation, while UV, FTIR, ^{1}H-NMR, and mass spectrometry provided structural confirmation of the compound as a pentacyclic terpenoid derivative. The findings validate the presence of pharmacologically relevant phytoconstituents in *Gardenia resinifera*, supporting its traditional medicinal use and highlighting its potential as a promising natural source for future drug development.

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