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**PHYTOCHEMICAL PROFILING OF *GYNOCHTHODES RIDSDALEI*  
LEAVES- AN ENDEMIC SPECIES OF SOUTHERN WESTERN GHATS,  
INDIA**

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**ABSTRACT**

*Gynochthodes ridsdalei*, a member of the Rubiaceae family, is an endemic species found in the southern-western Ghats of Kerala and Tamil Nadu, India. This plant is used in traditional medicines. This study aimed to determine the chemical composition of *G. ridsdalei* leaves using preliminary phytochemical screening and gas chromatography-mass spectrometry (GC-MS) analysis. Preliminary phytochemical screening involved the use of different solvents such as petroleum ether, chloroform, ethyl acetate, and methanol. Screening revealed the presence of various compounds, including carbohydrates, saponins, tannins, phenols, flavonoids, proteins, amino acids, steroids, terpenoids, and cardiac glycosides, in the leaf extract. GC-MS analysis of the methanolic leaf extract was performed to obtain a more detailed profile of its constituents. A total of 23 compounds were identified in the extract. The major constituents detected were n-hexadecanoic acid (51%), 2,4-di-tert-butylphenol (3.63%), tetradecanoic acid (2.89%), 2-butenedioic acid (Z)- dibutyl ester (2.60%), methyl glycocholate, 3TMS derivative (2.59%), and

octadecanoic acid (2.21%). Mass spectra were interpreted using the National Institute of Standards and Technology (NIST) and Wiley Library databases. The analysis revealed that the leaves of *G. ridsdalei* contain a significant amount of organic compounds. The findings highlight the potential pharmacological and therapeutic relevance of this species.

**Keywords:** *Gynochthodes ridsdalei*, GC-MS, Phytochemicals, Therapeutic potential

## INTRODUCTION

The family Rubiaceae produces various bioactive compounds with significant pharmacological potential. *Gynochthodes ridsdalei* Razafim. & Bremer, a woody liana, is an endangered species endemic to the southwestern Ghats [1]. The therapeutic importance of this plant has been substantiated by the presence of several bioactive compounds. Previous studies have shown that the methanolic extract of *G. ridsdalei* stem exhibits various pharmacological and therapeutic effects, including antimicrobial, antioxidant, antitumor, muscle relaxant, and anti-inflammatory properties [2]. Phytochemical analysis of the methanolic extract of root revealed the presence of sterols, phenols, terpenes, coumarins, fatty acids, esters, anthraquinones, vitamins, and additional terpenes in both stems and roots [3]. Given that this plant is both endemic and endangered in the southwestern Ghats, it is crucial to further explore and investigate its chemical composition for future research. This study aimed to contribute to the current knowledge

in this area by reporting the qualitative phytochemistry and gas chromatography-mass spectrometry (GC-MS) analysis of the methanolic extract of *G. ridsdalei* leaves.

## MATERIALS AND METHODS

### Collection of Materials

Fresh *G. ridsdalei* leaves were collected from Ponmudi hills, Trivandrum, Kerala. The plant was identified and voucher specimens were deposited in CATH herbarium with voucher number CATH 21027.

### Preparation of Extract

Leaves were chopped and subjected to shade drying at ambient temperature. Dried leaves were ground into a fine powder and stored in a container. A total of 20 g of the leaf powder was used for further extraction. Serial Soxhlet extraction of the leaf powder was performed using different solvents with varying polarities: petroleum ether (40-60°C), chloroform (50-60°C), ethyl acetate (60-77°C), and methanol (60°C). Each extraction process lasted for 4 h.

### Qualitative phytochemical analysis

Phytochemical examinations were carried out for the leaves as per standard methods such as carbohydrates- Molisch's test and Benedict's test [4], phenol- Lead acetate test and Ferric chloride test [4], tannin- Ferric chloride test [5], saponin- Frothing test [6], alkaloid- Dragendroff's test and Mayers test [5], flavonoid- Alkaline reagent test and Ferric chloride test [5], protein and amino acid- Millons test and Biurette test [7] and, sterol and terpenoids- Salkowski's test [5], glycosides- Liebermann's test and Salkowski's test [8].

#### GC-MS analysis and Identification of Components

Methanolic extract (0.5  $\mu$ L) of leaves *G. ridsdalei* was performed using GC-MS model: Shimadzu, QP2020 equipped with an autosampler series AOI 20i. An SH Rxi 5ms fused silica capillary column of 30m length, 0.25mm diameter and 0.25 $\mu$ m film thickness was used for the analysis. For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. The carrier gas was Helium (99.99%) used at a constant flow rate of 1.2 ml/min. The extract was injected into the instrument and oven temperature was programmed from 70  $^{\circ}$ C (1 min); followed by 180  $^{\circ}$ C at the rate of 10  $^{\circ}$ C min<sup>-1</sup>; and 280  $^{\circ}$ C, where it was held for 10

minutes. Injector and ion source temperature were set at 300 $^{\circ}$ C and 220 $^{\circ}$ C respectively. The total running time of GC-MS was 37 min. The identification of compounds was based on comparison of their mass spectra with those of NIST Libraries.

#### RESULTS & DISCUSSION

Initial screening of *G. ridsdalei* leaves was conducted using various solvents, including petroleum ether, chloroform, ethyl acetate, and methanol. Preliminary phytochemical analysis revealed the presence of fatty acids, phenols, terpenoids, esters, and carbohydrates. These compounds have been reported to exhibit a range of biological activities, including anti-inflammatory, antimicrobial, antioxidant, anticancer, diuretic, and anti-apoptotic properties.

**Table 1** presents the results of the Soxhlet extraction of leaves in four solvents, showing that the percentage yield of the extract ranged from 0.45% to 3.85%. **Table 2** provides an overview of the phytochemical compounds present in the crude extract as well as the individual solvents used (petroleum ether, chloroform, ethyl acetate, and methanol). The chromatogram of the compounds is shown in **Figure 1**. **Table 3** showed the constituents in the methanol extract.

Table 1: Yield from leaves of *G. ridsdalei* after Soxhlet extraction in 4 solvents

| Extraction solvents | % of yield (W/W) |
|---------------------|------------------|
| Petroleum ether     | 0.8              |
| Chloroform          | 3.57             |
| Ethyl acetate       | 0.45             |
| Methanol            | 3.85             |

Table 2: Phytochemical analysis of *G. ridsdalei* leaves in four solvents

| Tests                | Petroleum ether | Chloroform | Ethyl acetate | Methanol |
|----------------------|-----------------|------------|---------------|----------|
| Carbohydrate         | +               | +          | +             | +        |
| Tannin               | -               | -          | -             | +        |
| Saponin              | -               | -          | -             | +        |
| Phenol               | +               | -          | +             | +        |
| Alkaloid             | -               | -          | -             | -        |
| Flavonoid            | -               | +          | -             | +        |
| Protein              | +               | -          | +             | -        |
| Sterol and Terpenoid | +               | +          | +             | +        |
| Glycosides           | +               | +          | +             | +        |

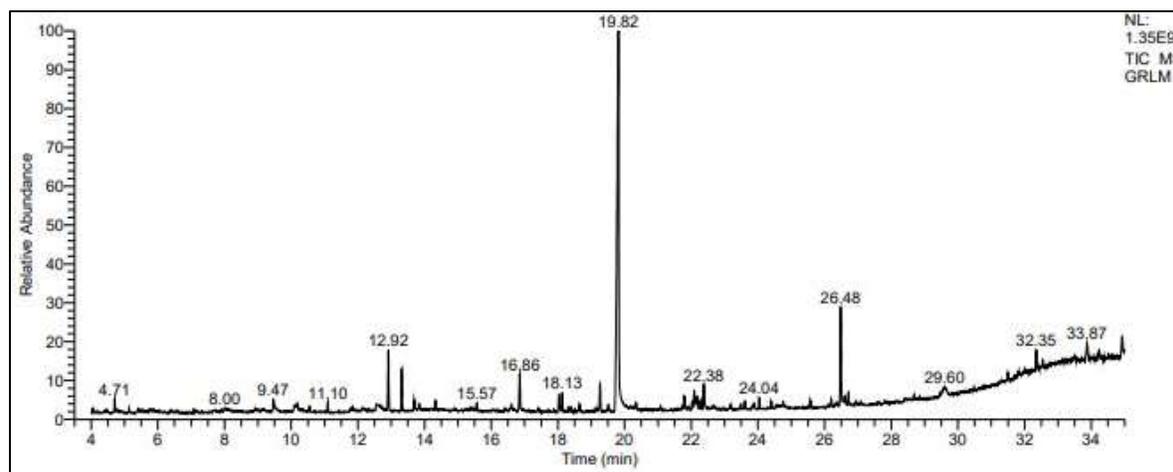
Figure 1: Chromatogram obtained from the GC-MS methanolic leaves extract of *G. ridsdalei*

Table 3: Predominant components detected in methanol extract of *G. ridsdalei* leaves

| S. No. | RT    | Name of the compound  | Area % | Pharmacological action   |
|--------|-------|---|--------|--|
| 1      | 4.71  | Z-3-methyl-2-hexenoic acid  | 0.98   | Not reported   |
| 2      | 9.47  | 2-methoxy-4-vinylphenol   | 1.67   | Antimicrobial and anti-oxidant [9]   |
| 3      | 10.19 | d-mannose   | 0.91   | Antimicrobial activity [10]  |
| 4      | 12.92 | 2,4-di-tert-butylphenol   | 3.63   | Antioxidant activity [11]  |
| 5      | 13.32 | 2-butenedioic acid (Z)-, dibutyl ester  | 2.60   | Anti-bacterial [12]  |
| 6      | 13.69 | 2-bromotetradecanoic acid   | 0.93   | Antioxidant [12]   |
| 7      | 15.57 | Hexadecane, 1,1-bis (dodecyloxy)-   | 0.78   | Antioxidant [13]   |
| 8      | 16.60 | (E)-4-(3-hydroxyprop-1-en-1-yl)-2-methoxyphenol   | 0.87   | Antimicrobial, antioxidant, anti-inflammatory and analgesic [14]                   |
| 9      | 16.86 | Tetradecanoic acid  | 2.89   | Anti-oxidant, anti-microbial, anti-cancerous, cosmetics [15]                       |
| 10     | 18.03 | Ethanol, 2-(9-octadecenyloxy)-, (Z)-  | 1.18   | Anticancer, anti-gonorrhoeal [10]  |
| 11     | 18.13 | 2-Pentadecanone, 6,10,14-trimethyl-   | 1.44   | Hypocholesterolemic, antioxidant and lubrication [10]                              |
| 12     | 19.26 | Cyclopropanebutanoic acid, 2-[[2-[[2-(2-pentylcyclopropyl) methyl] cyclopropyl] methyl] cyclopropyl] methyl]-, methyl ester | 2.15   | Not reported   |
| 13     | 19.51 | Benzothiazole, 2-(2-hydroxyethylthio)-  | 0.79   | Anthelmintic activity[16]  |
| 14     | 19.82 | n-hexadecanoic acid   | 51.07  | Anti-inflammatory, antioxidant, hemolytic, nematicide, anti-androgenic flavor [15] |
| 15     | 21.79 | Phytol  | 1.37   | Antimicrobial, anti-cancerous, anti-inflammatory and diuretic properties [15]      |
| 16     | 22.18 | Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate   | 1.06   | Anti-tumour [17]   |
| 17     | 22.38 | Octadecanoic acid   | 2.21   | Antimicrobial activity [15]  |
| 18     | 22.60 | Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester   | 1.04   | Anti-microbial activity[18]  |
| 19     | 26.71 | Octadecanal, 2-bromo-   | 0.99   | Anti-inflammatory and anti-apoptotic [10]  |
| 20     | 29.60 | (+)-Prostaglandin F <sub>2</sub> α, 4TMS derivative   | 1.84   | Not reported   |
| 21     | 32.35 | Androsta-1,4-dien-3-one, 6,17-bis[(trimethylsilyloxy)-, (6α,17α)-   | 1.61   | Not reported   |
| 22     | 33.87 | Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-   | 1.92   | Antimicrobial[19]  |
| 23     | 34.92 | Methyl glycocholate, 3TMS derivative  | 2.59   | Anti-oxidant [20]  |

## DISCUSSION

Notably, the analysis identified n-hexadecanoic acid as a saturated fatty acid with the highest peak area (51.07%), which has been reported to possess various beneficial properties, including anti-inflammatory, antioxidant, hemolytic, nematicidal, and anti-androgenic activities [15]. Another compound, phytol, which is

categorized as a diterpene, has antimicrobial, anticancer, anti-inflammatory, and diuretic properties [15], while also serving as a precursor to vitamins E and K1. Fatty acids such as octadecanoic acid exhibit antimicrobial activity, and tetradecanoic acid possesses antimicrobial, antioxidant, anticancer, and cosmetic attributes [15]. Among the identified phytochemicals,

phenolic compounds such as 2,4-di-tert-butylphenol exhibit antioxidant activity, and 2-methoxy-4-vinylphenol has reported antimicrobial and antioxidant properties [9, 11]. In addition, d-mannose, octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadeca methyl- displayed antimicrobial activity [19]. Other notable compounds, such as octadecanal, 2-bromo-, possess anti-inflammatory and anti-apoptotic properties [10]. Sesquiterpene 2-pentadecanone, 6,10,14-trimethyl-, exhibits hypocholesterolemic and antioxidant activities [10]. Benzothiazole, 2-(2-hydroxyethylthio), reportedly exhibits anthelmintic activity [16]. Fatty acid esters, namely 2-butenedioic acid (Z)- and dibutyl ester, display antibacterial properties [12], whereas hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, is associated with antimicrobial activity [18]. In addition, hexadecane, 1,1-bis(dodecyloxy)-, methyl glycocholate, 3TMS derivative, 2-bromotetradecanoic acid, and (E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol exhibited antioxidant activity [12, 13, 14, 20]. Furthermore, octadecanal, 2-bromo- demonstrate anti-inflammatory and anti-apoptotic properties, whereas ethanol, 2-(9-octadecenyloxy)-, (Z)- possess anti-cancer and anti-gonorrhoeal properties [10]. Finally,

Z-(13,14-epoxy) tetradec-11-en-1-ol acetate, a cyclic ester, is associated with antitumor activity [17]. Overall, this study highlights the prominent bioactive compounds in the methanol extract.

## CONCLUSION

An initial investigation of the chemical composition of the methanolic extract obtained from the leaves of *G. ridsdalei* was conducted through preliminary phytochemical screening and gas chromatography-mass spectrometry (GC-MS) analysis. The results revealed the presence of various organic compounds, including carbohydrates, saponins, tannins, phenols, flavonoids, proteins, amino acids, steroids, terpenoids, and cardiac glycosides. Subsequent GC-MS analysis identified 23 compounds within the leaf extract of *G. ridsdalei*, all of which exhibited significant biological activity. Particularly noteworthy is the fact that this study represents the first report of GC-MS analysis conducted on the methanol leaf extract of *G. ridsdalei*, thereby reinforcing the significance of this plant species due to its abundant content of various bioactive compounds.

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