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Original Article

# GC-MS Analysis of Bioactive Compounds Present in the Different Extracts of the Whole Plant *Impatiens minor* (DC.) Bennet

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## ABSTRACT

**Background and Objectives:** The systematic assessment of herbs utilized in the traditional medicine could provide more promising data regarding their therapeutic properties helping to address the increasing need for innovative treatments for infections and illnesses. Considering this perspective, *Impatiens minor*, an important medicinal plant distributed in the wet wastelands throughout India and also in the wet forests of Western Ghats was selected for our research. **Materials and Methods:** In this study, the plant *I. minor* in whole was collected, authenticated and subjected to shade drying for powdering which was done by mechanical grinder. Soxhlation technique was employed for the extraction of powdered plant material using different solvents to prepare the dried extract which was subjected to preliminary phytochemical evaluation and GC-MS analysis. **Results:** Presence of glycosides, phenolic compounds, flavonoids, terpenoids, sterols and carbohydrates was found in the preliminary phytochemical evaluation of different extracts. In the GC-MS analysis, presence of eight major compounds were found in the petroleum ether extract, while the chloroform extract showed the presence of six major compounds and twelve major compounds were found in the ethanol extract. **Conclusion:** It is worth noting that certain compounds identified in the GC-MS analysis have been documented to possess anti-oxidant, anti-inflammatory, and anticancer properties in previous studies. This study has the potential to contribute valuable insights for future in-depth investigations.

**Keywords:** *Impatiens minor* (DC.) Bennet , Extraction, Preliminary phytochemical evaluation, GC-MS analysis.

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## INTRODUCTION

For centuries, nature has served as a rich reservoir of medicinal compounds, providing humanity with a vast array of healing agents. Throughout history, numerous modern pharmaceuticals have been developed by harnessing the power of natural sources. In fact, a significant proportion of these breakthroughs can be traced back to the traditional uses of these agents in ancient medicine.[1] Nowadays, there is a resurgence in the fascination with conventional medicine and a growing need for additional medications derived from plants. The resurgence in the popularity of plant-based medications primarily stems from the prevailing notion that "green medicine" is secure and more reliable compared to expensive synthetic drugs, a significant number of which have undesirable side effects.[2] Phytochemicals play a crucial role in the management of several diseases including asthma, arthritis, cancer, etc. Numerous potent phytocompounds have been extracted and characterized from these green factories, leading to the development of several drugs with exceptional efficacy. [3] In the recent decades, gas chromatography-mass spectrometry (GC-MS) has established firmly as a key technological tool for the characterization of phytocompounds.[4] With this view, the plant *Impatiens minor* (DC.) Bennet (Family of *Balsaminaceae*) (Figure 1) was selected for the present study.

The genus *Impatiens* has around 1000 species of plants and new species are constantly discovered. Most of *Impatiens* species have a palaeotropical origin, however, there are few species endemic from Eurasia, North America, and Central America. Several plants of *Impatiens* genus have relevance for agriculture, ethno medicine and pharmacology.[5-9] The *I. minor* is one among them commonly known in the name of lesser balsam or wild balsam. It is also known in the synonym of *Balsamina minor* DC and *I.*

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*kleinii* Wight and Arn. It is a small succulent annual herb endemic to peninsular India and distributed widely in Western Ghats, usually growing in the rainy season. Leaves are simple, opposite, ovate-lanceolate, serrate, and pubescent. Flowers are rose coloured, axillary, solitary, or in pairs. Fruits, pale green dehiscent capsules, contain small globose black seeds. It well grows in moist places, rock crevices, on roofs or walls, in shade, and also on tree trunks.[10,11] The primary object of this study was to conduct an preliminary phytochemical screening of the different extracts of the whole plant *I. minor* and the characterization of active constituents by GC-MS analysis. The findings of this study would serve as a solid foundation for future investigations.

## MATERIALS AND METHODS

### Plant material-Collection and Identification

The whole plant of *Impatiens minor* (DC.) *Bennet* was collected from Seethangoli, a small village located nearer to Kasaragod town of Kerala, India. Mr. Biju P., Assistant Professor, Department of Botany, Government College, Vidyanagar, Kasaragod, Kerala, India identified and authenticated the collected material.

### Plant material-Powdering and Extraction

The collected whole plant material was powdered and extracted in reference with previous literature.[12-16] After 30 days of shade drying, the plant material was powdered with mechanical grinder and stored in airtight container for further study. The successive solvent extraction of the coarse powdered material (35 g) was done with the solvents (500 mL) of ascending order of polarity viz., petroleum ether, chloroform, and ethanol (70%) by soxhlet extraction procedure. Each extract was filtered, distilled off the solvent to obtain pure dried extract which were preserved for further studies.

### Preliminary phytochemical screening

Preliminary phytochemical screening of the prepared extracts was carried out in accordance with the procedure in the published literatures.[12-17]

### Analysis of extracts by GC-MS

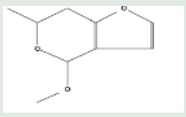
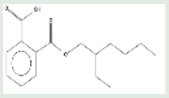
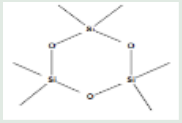
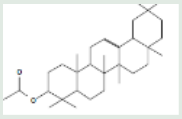
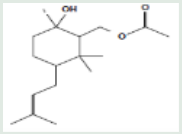
GC-MS analysis of petroleum ether, chloroform, and ethanol extract was carried out using a Perkin Elmer GC/MS Claurus 680/600 system provided with an Elite-5MS column (30.0 m, 0.25 mm ID, 250 µm $d_f$). For detection in GC/MS, an electron ionization system with ionization energy of 70eV was used. Helium gas (99.999%) was employed as the carrier gas at a constant flow rate of 1 mL/min and an injection volume of 1 µL was used (split ratio of 10:1). Injection port temperature was ensured as 250°C and ion-source temperature 230°C. The instrument was set at an initial temperature of 60°C and maintained this temperature for 2 min. At the end of this period, the oven temperature reached 300°C, at the rate of rise of 10°C/min, and maintained for 6 min. Mass spectra were taken at 70 eV; a scan interval of 0.2 sec and fragments from 50 to 600Da. Total GC running time was 32 min. The relative percentage amount of each component was calculated by comparing its average peak

**Figure 1:** *Impatiens minor* (DC.) Bennet.

**Table 1: Preliminary qualitative phytochemical screening of the *Impatiens minor* extracts.**

| Sl. No. | Phytochemical | Petroleum ether extract | Chloroform extract | Ethanol extract |
|---|---|---|---|---|
| 1. | Alkaloids | - | - | - |
| 2. | Glycosides | - | - | + |
| 3. | Phenolic compounds and Tannins | - | - | + |
| 4. | Flavones and Flavonoids | - | - | + |
| 5. | Carbohydrates | - | - | + |
| 6. | Proteins and Amino acids | - | - | - |
| 7. | Terpenoids | + | + | + |
| 8. | Sterols | + | + | - |
| 9. | Saponins | - | - | - |
| 10. | Gum and mucilage | - | - | - |

**Table 2:** **Compound detected in the GC-MS scanning of petroleum ether extract of *I. minor*.**

| Sl. No. | Retention time | Name of the compound | Mol. Formula | Structure | Mol. Wt. | Peak Area % |
|---|---|---|---|---|---|---|
| 1. | 16.90 | Z,Z-6,2 8-Heptatriactontadien-2-one | $C_{37}H_{70}O$ |  | 530 | 0.705 |
| 2 | 16.98 | 4-methoxy-6-methyl-6,7-dihydro-4H-Furo(3,2-C) pyran | $C_9H_{12}O_3$ |  | 168 | 0.567 |
| 3. | 19.60 | Phytol | $C_{20}H_{40}O$ |  | 296 | 2.58 |
| 4. | 23.17 | 1,2-Benzenedicarboxylic Acid, Mono (2-Ethylhexyl) Ester | $C_{16}H_{22}O_4$ |  | 278 | 5.38 |
| 5 | 26.52 | Hentriacontane | $C_{31}H_{64}$ |  | 436 | 4.78 |
| 6. | 29.58 | Cyclotrisiloxane, Hexamethyl- | $C_6H_{18}O_3Si_3$ |  | 222 | 6.38 |
| 7 | 30.19 | 2-oleanen-3-yl acetate,[3 alpha] | $C_{32}H_{52}O_2$ |  | 468 | 19.92 |
| 8 | 30.79 | 2R-acetoxymethyl-1,3,3-trimethyl-4T-(3-methyl-2-buten-1-yl)-1t-cyclohexanol | $C_{17}H_{30}O_3$ |  | 282 | 7.99 |

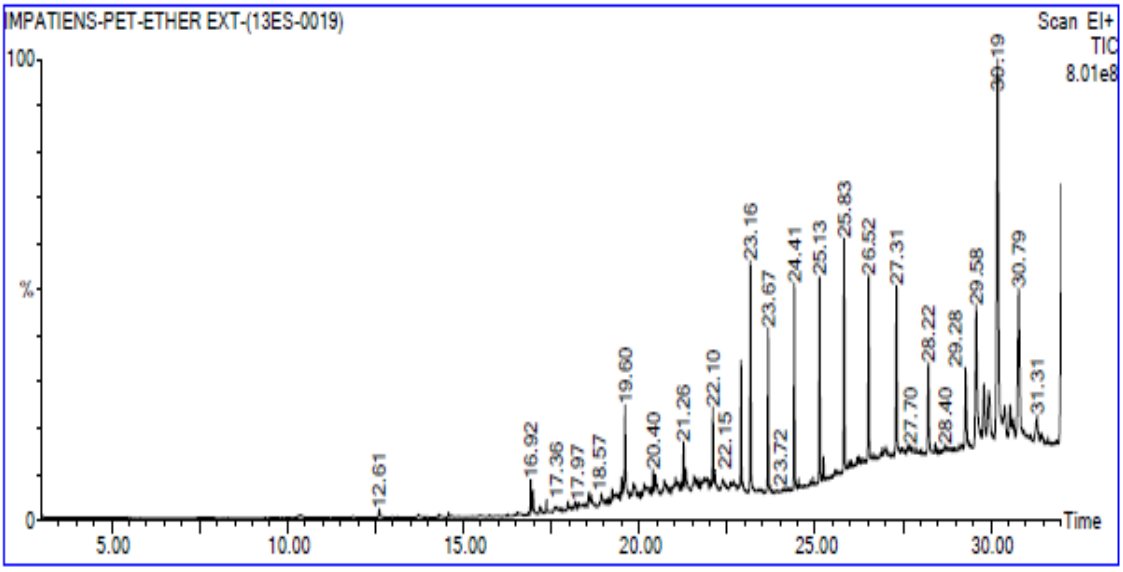



**Figure 2:** GC-MS investigation of petroleum ether extract of *I. minor.*

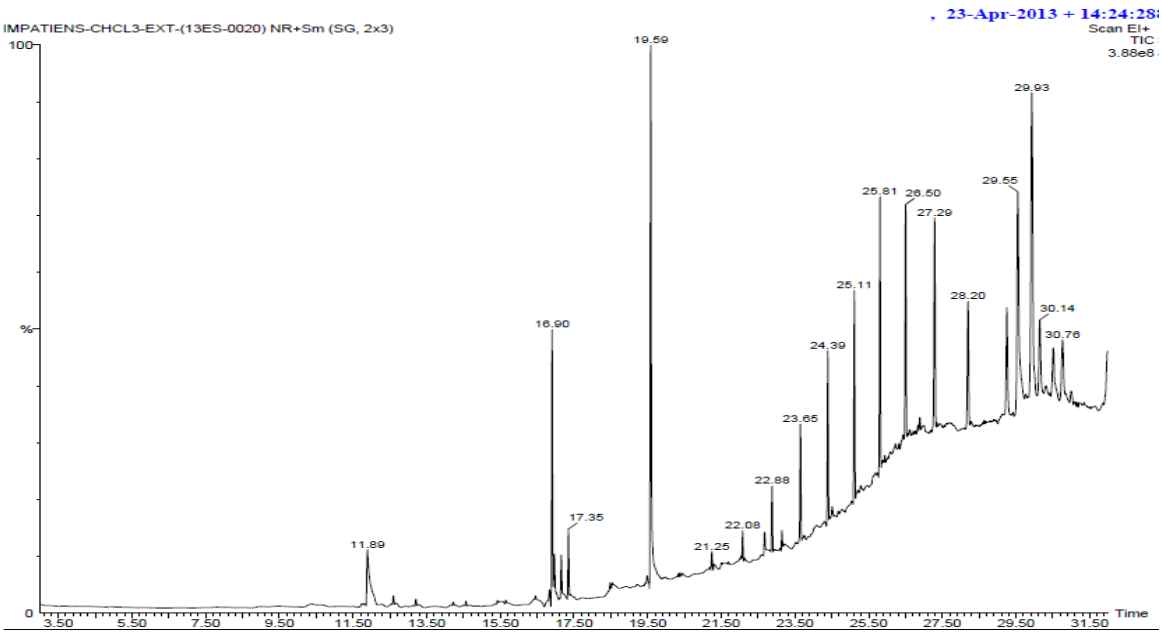
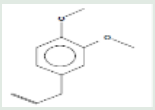
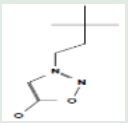
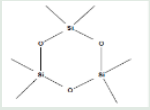



**Figure 3:** GC-MS scrutiny of chloroform extract of the *I. minor.*

**Table 3: Compounds identified in the GC-MS assessment of chloroform extract of *I. minor.***

| Sl.No. | Retention time | Name of the compound | Mol. Formula | Structure | Mol. Wt. | Peak Area % |
|---|---|---|---|---|---|---|
| 1. | 11.89 | Benzene, 1,2-Dimethoxy-4-(2-Propenyl) | $C_{11}H_{14}O2$ |  | 178 | 3.73 |
| 2 | 16.97 | Sydnone, 3-(3,3-Dimethylbutyl)- | $C_8H_{14}O_2N_2$ |  | 170 | 1.19 |
| 3. | 19.58 | Z,Z-6,2 8-Heptatriactontadien-2-one | $C_{37}H_{70}O$ |  | 530 | 11.64 |
| 4. | 25.11 | Hentriacontane | $C_{31}H_{64}$ |  | 436 | 4.44 |
| 5 | 26.50 | Silane, Trichlorooctadecyl | $C_{18}H_{37}C_{l3}Si$ |  | 386 | 5.16 |
| 6. | 29.93 | Cyclotrisiloxane, Hexamethyl- | $C_6H_{18}O_3Si_3$ |  | 222 | 12.54 |

area to the total area. Turbo Mass Ver5.4.2 software was used to handle the mass spectra and chromatograms.[18]

## RESULTS

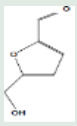
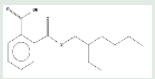
### Preliminary Phytochemical Screening

Results of preliminary qualitative phytochemical screening of petroleum ether, chloroform and ethanol extract of whole plant of *I. minor* is shown in Table 1. Alkaloids was absent in the tested extracts. The ethanol extract showed the presence of glycosides, phenolic compounds, flavonoids and carbohydrates. Terpenoids was found in all the tested petroleum ether, chloroform and ethanol extract. The presence of sterols was detected in the petroleum ether and chloroform extract.

### GC-MS analysis

Spectrums obtained in the GC-MS analysis of all the prepared extracts viz., petroleum ether, chloroform and ethanol extract of the whole plant *I. minor* were matched with the spectra available in the spectral library of NIST version 2.1 to identify the chemical constituents present in the extracts. Peak area, molecular weight and retention time also helped in the identification. Presence of

**Table 4: Compounds showed by GC-MS analysis of ethanol extract of *I. minor*.**

| Sl.No. | RT | Name of the compound | Mol. Formula | Structure | Mol. Wt. | Peak Area % |
|---|---|---|---|---|---|---|
| 1 | 6.54 | Butane, 1, 4-bis(methylthio)- | $C_6H_{14}S_2$ |  | 150 | 12.33 |
| 2. | 9.56 | 2-Furan carboxaldehyde,5-(hydroxymethyl)- | $C_6H_6O_3$ |  | 126 | 17.50 |
| 3. | 13.39 | Sedoheptulosan | $C_7H_{12}O_6$ |  | 196 | 11.32 |
| 4. | 16.90 | Z,Z-6,2 8-Heptatriactontadien-2 -one | $C_{37}H_{70}O$ |  | 530 | 3.05 |
| 5. | 17.15 | Trans,cis-1,8 -dimethylspiro[4.5] decane | $C_{12}H_{22}$ |  | 166 | 1.16 |
| 6. | 17.34 | Cyclohexanol,5-Methyl-2-(1-Methylethyl)- ,(1α,2β,5α)- | $C_{10}H_{20}O$ |  | 156 | 2.17 |
| 7. | 19.59 | Chloroacetic Acid, Tetradecyl Ester | $C_{16}H_{31}O_2Cl$ |  | 290 | 2.42 |
| 8 | 22.87 | Hentriacontane | $C_{31}H_{64}$ |  | 436 | 2.039 |
| 9. | 23.14 | 1,2-Benzenedicarboxylic Acid, Mono(2-Ethylhexyl) Ester | $C_{16}H_{22}O_4$ |  | 278 | 1.75 |
| 10. | 25.10 | Heneicosane, 3-Methyl | $C_{22}H_{46}$ |  | 310 | 1.36 |
| 11. | 25.80 | Silane, Trichlorooctadecyl | $C_{18}H_{37}C_{l3}Si$ |  | 386 | 1.50 |
| 12. | 29.52 | Cyclotrisiloxane, Hexamethyl- | $C_6H_{18}O_3Si_3$ |  | 222 | 6.06 |

eight compounds was found in the GC-MS analysis of petroleum ether extract (Figure 2 and Table 2). Presence of six compounds was identified in the chloroform extract (Figure 3 and Table 3) and the ethanol extract showed the presence of twelve compounds (Figure 4 and Table 4).

## DISCUSSION

Summarily, result of preliminary qualitative phytochemical evaluation of *I. minor* extracts revealed the presence of various important phytochemicals with significant medicinal values. For example, glycosides are reported to have analgesic and anti-inflammatory, cardio-tonic, anticancer and antimicrobial such as antibacterial, antifungal and antiviral effects. Likewise, the phenolic compounds and tannins are well known for their antimicrobial, anti-parasitic, antiviral, antioxidant, anti-inflammatory, immunomodulation etc. Terpenoids are renowned for their anti-microbial, anti-allergic, anti-inflammatory, anti-hyperglycemic, anti-spasmodic, and immunomodulatory properties. The previous studies have documented the presence of cardiotonic, insecticidal, and anti-microbial properties in sterols.

Notably, several of the compounds identified in the GC-MS analysis of the present study have been documented to possess antioxidant, anti-inflammatory, and anticancer properties. For example, Hentriacontane was the major component found in the petroleum ether, chloroform, and ethanol extract of *I. minor* was reported to have anti-inflammatory, antimicrobial, antioxidant

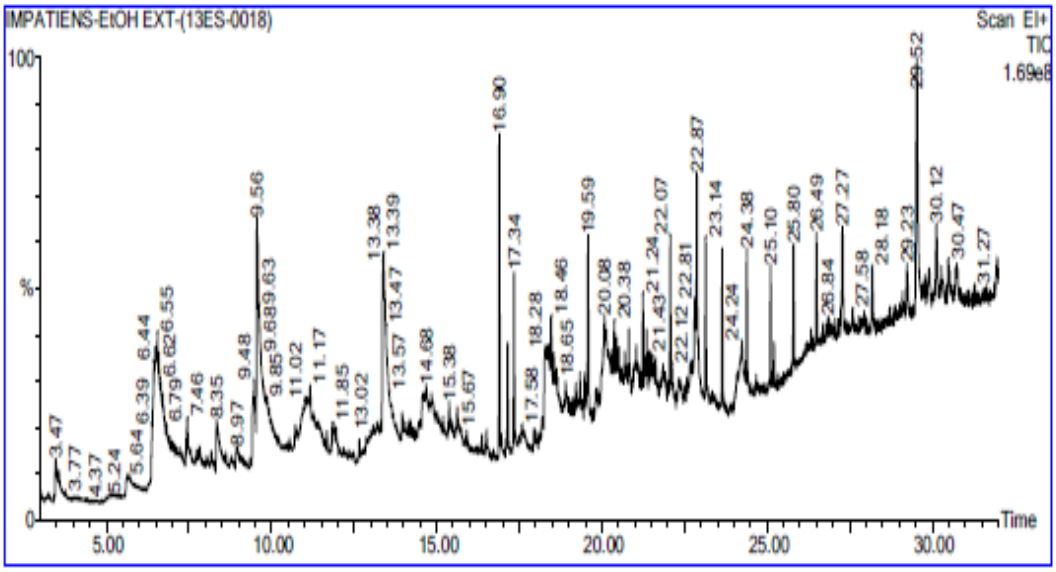



**Figure 4:** GC-MS examination of ethanol extract of *I. minor.*

and anticancer property.[19] 2-Furan carboxaldehyde,5-(hydroxymethyl)- present in ethanol extract also documented to have antioxidant and anticancer property. Phytol and 12-oleanen-3-yl acetate, [3 alpha] present in petroleum ether was documented to have anti-inflammatory, antioxidant and anticancer activities.[20,21]

## CONCLUSION

In this study, the whole plant *I. minor* was collected, authenticated, dried and made in to coarse powder. The plant material in powder form was extracted through Soxhlation method, utilizing various solvents such as petroleum ether, chloroform, and ethanol. The dried extract thus obtained was subjected to preliminary phytochemical evaluation and GC-MS investigation. The preliminary phytochemical evaluation, disclosed the absence of alkaloids in all the tested extracts, while the ethanol extract showed the presence of glycosides, phenolic compounds, flavonoids and carbohydrates. Terpenoids was found in all the tested petroleum ether, chloroform and ethanol extract. Petroleum ether and chloroform extract showed the presence of sterols. GC-MS investigation indicated the presence of eight major compounds in the petroleum ether extract, while six major compounds were found in the chloroform extract. Presences of 12 major compounds were found in the ethanol extract of *I. minor*. Summarily, results of the present study revealed the presence of various important phytochemicals with significant medicinal values in the extracts of *I. minor*. This particular GC-MS analysis serves as the initial stage in comprehending the characteristics of active components in this medicinal plant, paving the way for more in-depth research in the future.

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We would like to thank our guide Dr. (Sr) Molly Mathew and to Malik Deenar College of Pharmacy, Seethangoli, Kasaragod, Kerala for granting the facilities to carry out this research.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**GC-MS:** Gas Chromatography-Mass Spectrometry; **NIST:** National Institute of Science and Technology.

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