# Gc-Ms Analysis And Antibacterial Activity Of *Dryopteris Hirtipes (Blumze) Kuntze Linn.*

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**Abstract**

**Objective:** To evaluate bioactive metabolites, antibacterial efficiency and GC-MS analysis of D. hirtipes.

**Methods**: Leaves of D. hirtipes was extracted with various organic solvents using soxhlet apparatus. Phytochemical analysis was carried out with standard protocols. GC-MS analysis was also carried out to identify the major chemical constituents present in the extracts. Antimicrobial activity of various extracts was done by agar well diffusion method.

**Results**: Various extracts of D.hirtipes was found to be rich in phytochemicals like Glycosides, Steroids, Alkaloids, Phenols, Terpenes, Flavanoids and Tannins. GC-MS analysis of various extracts showed the presence of major chemical components like n-Hexadecane, lupeol, Phytol, dioctyl phthalate, Neophytadiene and 1,3-Benzenedicarboxylic acid bis(2-ethylhexyl)ester were found based on their retention time and peak area with that of literature and by interpretation of mass spectra. Antibacterial activity of extracts at various concentrations (20, 40 and 60 µg/ml) was investigated to determine the minimum inhibitory efficacy against selected pathogens. In various extracts, tested against Pseudomonas aeruginosa, E.coli and Staphylococcus aureus. Pseudomonas aeruginosa was focused to be effective (6mm) against the higher concentration of (60µg/ml) ethyl acetate extract than others.

**Conclusion**: Ethyl acetate extract of D. hirtipes found to possess phytoconstituents in higher concentration which was found to be effective against human pathogens to treat infectitious diseases. GC-MS analysis revealed the major chemical compounds which could be responsible for its biological efficiency.

**Key words:** Dryopteris hirtipes, Phytoconstituents, Antibacterial activity and GC-MS analysis.

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## 1.Introduction

Herbal plants holds the effective resource of drugs that used in various ailments, used as food additives, pharmaceutical products that comes from natural or artificial processes to meet their human health care needs[1,2]. Medicinal plant therapy provides great attention of several plant researchers to discover the new biological compounds with remedies for various diseases[3,4].Medicinal plants produce various secondary metabolites namely flavanoids, steroids, alkaloids, phenols, triterpenes [5] and treated for illness. The continuous exploitation of the plants leads a scarcity and low availability of medicinal plants. The researchers found to rectify the problem to counteract the alternative source to use these ferns and fern allied species for future use .The ferns (pteridophyta) play a major role in important role in folklore medicine, also used in valuable food sources, fodder, and chemotherapeutants, prevents illness and maintenance of health.

Ferns, constitutes the major class of pteridophytes, are concluded for their medicinal property to treat against infections, trauma and cold and contain many activities such as anti-oxidant, antibacterial, antioxidant, anti-inflammatory, antitussive and anti-tumor properties[6]. Recently, ferns and fern allied species is reported to have a great economic potential due to some interesting pharmacological properties and different climatic conditions than the other primitive vascular plants [7].Researchers also concluded that the pteridophytes are not infected by pathogenic microorganisms and survival rate more than 350 million years [8] and aroused interest in bioactive compounds which may support as for making drugs. Nowadays, Peoples from medical grounds used to treat the serious infectious diseases from medicinal plants especially from ferns [9, 10].The phytoconstituents in ferns with adequate antimicrobial, antifungal, anti-helminthic, anti-inflammatory activities may be beneficial for the dreadful diseases [11]and intensive search of new alternative biocompounds to treat against the problem of multidrug resistance.

*Dryopteris* species (Dryopteridaceae) comprising approximately of more than 225 species occurs in tropical areas and also sub tropical regions [12]. In *Dryopteris* spp.,the small portion of tuber is used to treat rheumatoid arthritis, epilepsy and leprosy[13], crude galenicals is to treat snake bite, reduce pain, antifungal and insecticidal properties [14,15,16] .Young fronds *Dryopteris* species are edible which are used to cure anti-helminthic[17]. *Dryopteris hirtipes* leaf juice given in epilepsy and also used as antibiotics[18]. The genera of Dryopteris .*filix D. crassirhizoma* , *D. cochleata* , *D. chrysocoma*, and *D. syrmatica* possess good antimicrobial property[19,20] ·Among *Dryopteris* species, *D. cochleata* and *D. affinis* shows the strong activity on the phytoconstituents like phenols, tannins and quinines possess antimicrobial, antifungal [21,22] and also having more antioxidant scavenging properties[23].
GC-MS analysis helps to find out phytocompounds in herbal plants [24] and also in parts of storage organs. Considering the above information, plant investigation was done to analyze the presence of secondary metabolites, antibacterial effects against human pathogenic bacteria and GC-MS of *D.hirtipes*, a medicinal fern from Eastern Ghats of Tamil Nadu.

## 2.Materials and Methods

### *2.1. Phytochemical Analysis*

Various extracts were performed to identify the phytocomponents present in *D.hirtipes*[25].

### *2.2. GC-MS Analysis*

GC-MS analysis done in Clarus 680GC.GC-MS helps to find out the fragments depends on the molecular structure and mass based on the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns with the comparison of unknown compound with known compounds.

### Biological activity of *D.hirtipes* extracts

### *2.3. Preparation of Inoculums*

The antibacterial property of hexane, ethyl acetate and methanol extract of *D.hirtipes* were tested against *Staphylococcus aureus, E.coli* and *Pseudomonas aeruginosa* and cultured in Muller Hinton Broth (MHB) and

kept overnight in a rotary shaker at 37˚C and the prepared the inoculums for antibacterial test.

### *2.4. Antibacterial assay by Agar- Diffusion method*

Antibacterial activity of various extracts of *D.hirtipes* was evaluated. The pre-autoclaved Muller Hinton agar plates were inoculated with a $10^{-5}$ dilution of cultures namely *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *E.coli*, using sterile cotton swabs to attain uniform lawn of microbial growth and using sterile cork borer, wells made in the agar. Three different concentrations of crude extract were made in the concentrations of 20, 40, 60µg/ml, it was introduced through micro-pipette aseptically on the wells of inoculated agar plates. All cultured organisms were appropriately set at 37˚C for 18 to 24 hrs and this step was repeated thrice for bacterial sensitivity test and growth inhibition zone against the pathogenic bacteria was recorded[26] **.**

## 3.Results and Discussion

### *3.1.Phytochemical Analysis*

Phytochemical screening of *D.hirtipes* leaf extracts using five different solvents were confirmed the presence of major phytochemicals like polyphenolic derivatives [27] (flavanoids, tannins, phenols, triterpenes and alkaloids). The separation of biocom-pounds depends upon the polarities of the solvent to exhibit their nature. Alkaloids are naturally occurring nitrogenous chemical compounds and often have pharmacological effects like medications and drugs [28]. Flavanoids possess the antioxidant property and prevent cell damage[29, 30] .Researchers analyzed the flavanoid compound quercetin in various ferns growing in northern India especially the species of *D.cochleata, D. juxtaposita* contain a variable range of quercetin[31].Phenolic compounds shows the defense mechanism to counteract reactive oxygen species in order to survive and prevent from cell and molecular damage by microorganisms, insects and herbivores[32,33]. Compounds which possess phenolic character derivative of benzoic acid, caffeic acid, cinnamic acid and gallic acid[34].The natural activity of saponins act as a best defense mechanism for the ailment of fungal infections[35]and also to treat against microbial infections for humans and animals[36].

### *3.2.GC-MS Analysis*

The GC-MS of hexane extract from *D.hirtipes* identified the 4 major components by using NSIT Library. The compounds identified with their retention peaks were 1-Hexadecene (13.54), 3 –hydroxyl, (3α,5α), 2-Hexadecen-1-ol,3,7,11,15-tetramethyl (Phytol-18.96), Lupeol (21.53), 1,4-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester or dioctyl phthalate (24.27) depends on the relative peaks and relative compounds and the results were presented in Table 1 and Fig 1.

1-Hexadecene compound was reported in the hexane extract of marine fungi *Aspergillus ustus* and red algae and it concluded that possess effective antibacterial activity against *Pseudomonas aeruginosa* and also having antifungal, antioxidant activity have been reported by[37].[38] reported that *Hyptis verticillata* (hexane extract) contain more amount of 1-Hexadecene compound responsi-ble for antibacterial activity.[39] reported Lupeol was one of the triterpene found majorly in medicinal plants, fruits and vegetables and reported that better anti-inflammatory and anticancer activity against various cancer cells like lung carcinoma and cervical carcinoma and prostrate cancers,2-Hexadecen-1-ol, 3, 7, 11, 15-tetramethyl (Phytol),diterpene, alcohol isolated and responsible for antimicrobial, anti-inflammatory and anticancer activity from *L. cruciata* (Bryophytes) by[40]and also reported by[41]in the methanol leaf extract of *Phyllanthus debilis*. [42]isolated the Dioctyl phthalate(1,4-Benzenedicarboxylic acid ,bis (2-ethylhexyl ester) compound from endophytic fungi that posses antimicrobial activity in various extracts and showed better inhibitory effect against gram positive bacteria(*Staphylococcus aureus*) in *Cupressus torulosa*.

GC-MS analysis of ethyl acetate extract of *D.hirtipes* show 4 main compounds identified as 1-Hexadecene(5.3),2-Hexadecen-1-ol,3, 7, 11,15-tetramethyl or 3,7,11,15-tetramethyl 2-Hexadecen-1-ol (Phytol-18.96), 1,3-Benzenedicarboxylic acid bis (2-ethylhexyl)

ester-(24.28), Neophytadiene (13. 55) compound possess the various biological activities**.** [43] reported that Hexadecane responsible for the antibacterial and antioxidant effects. [44]concluded that 3, 7, 11, 15 tetra methyl 2-Hexadecen-1-ol is otherwise known as 'Phytol', a bioactive compound found in terpenes family expressed an effective antibacterial activity against microbial pathogens. 1, 3-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester and it derivatives were identified by [45, 46] reported that *Dryopteris ryo-itoana* , and leaf parts *D.sublaeta*, possess better anticancer activity. [47] investigated that Neophytadiene possess the excellent antioxidant and antimicrobial activity reported in the *Ophiorrhiza rugosa*.

### *3.3. Antibacterial Activity:*

Antibacterial activity of hexane extract of *D.hirtipes* were carried out against the bacterial pathogens (*Pseudomonas aeruginosa*, *Staphylococcus aureus* and *E.coli* ) .The hexane extract showed moderate inhibitory activity(2mm) in the concentration of 60 µg/ml against *Pseudomonas aeruginosa* and least inhibition (0.5mm) was noted in two different concentrations (20 and 40µg / ml). [48] reported in hexane fraction shown a least activity against the tested pathogens except *Pseudomonas aeruginosa*.

Whereas Gram positive bacteria *Staphylococcus aureus* showed the mild inhibition (1mm)in the concentration of 60µg/ml and least inhibition was noted in varying concentrations of 20 and 40µg/ml. [49]reported the role of secondary metabolites from endophytic fungus *Pestalotiopsis neglecta* and concluded that the presence of dioctyl phthalate compound responsible for inhibiting the gram positive bacteria (*Staphylococcus aureus*) showed the minimum inhibitory effect (1mm) against the extract tested.

The ethyl acetate extract of *D.hirtipes* showed active against some bacterial pathogens and control (streptomycin antibiotic) and the results were presented in Table 5. In this research findings, ethyl acetate extract has the ability in controlling the bacterial strains and the results showed maximum activity in the concentration of 60 µg/ml and showed minimum inhibitory concentration(MIC) of about 6mm against *Pseudomonas aeruginosa*. [50] reported that the antibacterial effect of *D.hirtipes* due to the presence of bioactive compounds in the ferns (like flavanoids and polyphenolics) showed the better antibacterial action. The results were presented in Table 2 and Fig 2 a, b and c.

## 4.Conclusion

The present study confirms the report on phytoconstituents, present in the ethyl acetate extract of *D.hirtipes* shows the significant antimicrobial activity found to be treating against human pathogens to cure infectitious diseases. GC-MS analysis revealed the major chemical compounds which could be responsible for its biological efficiency and which it recommends further research needed for the active isolation of bioactive components for future use.

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## TABLES AND FIGURES

**Table 1. GC-MS analysis in the various extract of *D.hirtipes***

| S.No | Extract | RT | Name of the compound | Molecular Formula | Area % | Medicinal Property |
|---|---|---|---|---|---|---|
| 1 | Hexane | 13.547 | 1-Hexadecene | $C_{16}H_{32}$ | 2.28 | Antibacterial and Antioxidant property |
| 2 | | 18.96 | 2-Hexadecen-1-ol,3,7,11,15-tetramethyl (Phytol) | $C_{20}H_{40}O$ | 3.52 | Antibacterial property |
| 3 | | 21.53 | Lupeol | $C_{30}H_{50}O$ | 17.22 | Anti-inflammatory Activity, Antioxidant and Anticancer property |
| 4 | | 24.27 | 1,4-Benzenedicarboxylic acid ,bis(2-ethylhexyl)ester (dioctyl phthalate) | $C_{24}H_{38}O_4$ | 7.16 | Antibacterial Activity |
| 1 | Ethyl acetate | 5.31 | 1-Hexadecene | $C_{16}H_{32}$ | 2.28 | Antibacterial and Antioxidant property |
| 2 | | 18.964 | 2-Hexadecen-1-ol,3,7,11,15-tetramethyl(Phytol) | $C_{20}H_{40}O$ | 22.76 | Antibacterial property |
| 3 | | 24.28 | 1,3-Benzenedicarboxylic acid ,bis(2-ethylhexyl)ester | $C_{24}H_{38}O_4$ | 13.54 | Anticancer activity |
| 4 | | 13.551 | Neophytadiene | $C_{20}H_{38}$ | 19.63 | Analgesic, antipyretic Anti-inflammatory, antioxidant and antimicrobial activity |

**Figure1. GC-MS analysis of hexane (A) and ethyl acetate (B) extracts of *D.hirtipes***

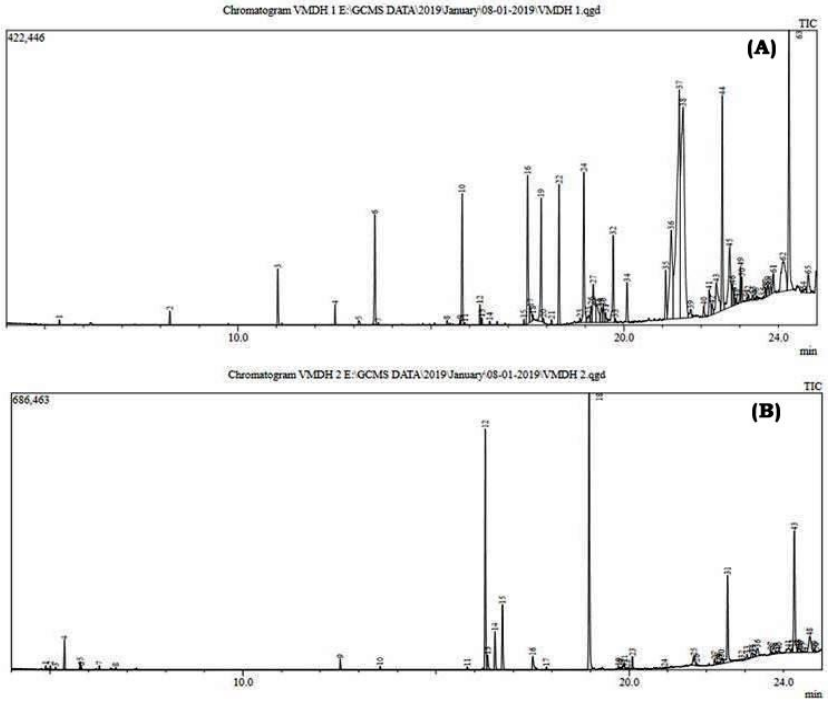


**Table 2..Antibacterial Activity of various extracts of *D.hirtipes***

| ***Dryopteris hirtipes*** | **Concentration in µg/ml** | **Diameter of Inhibition Zone (mm)** | | |
|---|---|---|---|---|
| | | ***E.coli (mm)*** | ***P.aeruginosa (mm))*** | ***S.aureus (mm)*** |
| Hexane | 60 | 0.5 | 2 | 1 |
| | 40 | 0.5 | 0.5 | 0.5 |
| | 20 | 0.5 | - | 0.5 |
| Ethylacetate | 60 | 2 | 6 | 2 |
| | 40 | 1 | 4 | 1 |
| | 20 | 1 | 2 | 1 |
| control (Streptomycin) | 20 | 1 | 1 | 1 |

**Fig.2 a. Antibacterial Activity of crude Ethyl acetate extract from *D.hirtipes***

a) Staphylococcus aureus b) Pseudomonas aeruginosa c) E.coli

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**Fig.2 b Antibacterial Activity of crude Hexane extract from D.hirtipes**

a) Staphylococcus aureus b) Pseudomonas aeruginosa c) E.coli

**Fig.2 c. Antibacterial Activity of crude methanol extract from *D.hirtipes***

a) Staphylococcus aureus b) Pseudomonas aeruginosa c) E.coli

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