SCREENING OF ANTIMICROBIAL ACTIVITY OF CONNARUS WIGHTII MEDICINAL PLANT AGAINST SOME HUMAN PATHOGENS

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Abstract

Biotechnology researchers are particularly interested in medicinal plants since they are used by the majority of the drug industry to produce pharmaceutical chemicals. Crude plant extracts which are made up of a complex combination of several phytochemical ingredients (plant secondary metabolites), are the primary ingredient in the majority of herbal medicines and their derivative products. These components' chemical characteristics vary greatly between species. The present investigation involves three different solvents of extract the leaves from Connarus wightii, the methanol extract of Connarus wightii shows the antibacterial activities in both Gram-positive (Staphylococcus aureus) and Gram-negative (Aeromonashydrophila) bacteria strain compared to other solvent extracts. In this studies the three different solvents of extract show high activity in 500 µg/ml concentration, the methanol, chloroform, and ethanol extracts of Connarus wightii exhibit greater inhibitory action against Staphylococcus aureus than Aeromonas hydrophila.Compare all of the extracts to the standard drug, fluconazole; the extracts had no effect against Aspergillus niger and very moderate efficacy against Candida albicans. As the concentration of extracts (µg/ml) the antifungal activity of the extracts also increased linearly. The results showed that when compared to typical medications, fungal activity from Candida albicans performed better than that of Aspergillus niger. GC-MS method used for the analysis of the obtained extracts can be an interesting tool for testing the amount of some active principles in herbs used in cosmetic, drugs, pharmaceutical.By using spectrophotometer was used to calculate the protein extracts from the C. wightii leaf sample's absorbance at 600 nm. Maximum protein content of 86.03 ± 0.008µg/ml The SDS-PAGE electrophoresis was carried out to estimate the molecular weight protein against the protein standard.The most abundant protein from powder and fresh leaves band occurs at 63kDa

Keywords: Antimicrobial Activity, Connarus Wightii, GC-MS Analysis, Protein Extraction

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1. Introduction

Since plants are a valuable source of novel medications, the quest for new drugs is warranted. For approximately 80% of people living in poor nations, plants constitute their only source of medicinal supplies. Therefore, people have utilised plants to cure common infectious ailments, and certain ancient medicines are still used to treat a variety of illnesses on a regular basis. (Abera., 2014).Millions of people's health has benefited from the use of plants that traditional healers have used to make cures. research on traditional knowledge, folk beliefs, skills, techniques, and procedures utilised in ethnomedicine to treat illnesses (Abdillahi et al., 2010). According to the World Health Organisation (WHO), traditional medicines made from medicinal plants continue to be beneficial for 80% of the poor world's population (Christenhusz et al., 2010).There are 28,187 medicinal species that people utilise, compared to an estimated 374,000 total number of plants. Over 20,000 species of medicinal plants have names assigned by WHO, which also lists them as possible sources of novel pharmaceuticals (Yadav et al., 2011). Regulations pertaining to therapeutic plants have been created in over 100 nations. In addition to approximately 30,000 antimicrobial molecules that have been identified from plants, there are about 1340 plants with known antibacterial activity. In addition, it has been calculated that 14–28% of higher plant species have therapeutic properties, and 74% of bioactive chemicals produced from plants were found through ethnomedical applications (Pandey et al., 2013).

Researchers are revisiting nature's pharmacopoeia in light of the growing threat of antibiotic resistance and other global health concerns, which have prompted them to look for alternative antimicrobial medicines. In this vast domain, therapeutic plants have surfaced as repositories of bioactive substances possessing exceptional antibacterial capabilities (Bereksi et al., 2018). The need to create novel antimicrobial agents that can both prevent the development of antibiotic resistance and reduce the usage of antibiotics is growing as a result. Given that almost 50% of today's medications and nutraceuticals are made from natural products or their derivatives, this has prompted researchers to seek for and extract novel bioactive compounds from plants in order to combat microbial resistance. Almost limitless supplies of bioactive chemicals are produced by medicinal plants, and their application as antimicrobial agents has been explored in a variety of methods. (Chavan et al.,2018).

Numerous investigations have demonstrated that coumarins, flavonoids, polyphenols, alkaloids, terpenoids, tannins, essential oils, polypeptides, and polyacetylenes are only a few of the phytochemical substances found in every medicinal plant. These bioactive chemicals can operate as a precursor to the development of antibiotics to treat infectious agents and can exhibit bactericidal or bacteriostatic actions on bacteria that are multidrug resistant pathogens (Gadisa et al., 2019). Throughout the world, antibiotics have been extremely beneficial, especially in poor nations with inadequate public health infrastructure, where they have reduced the rate of morbidity and mortality from bacterial infections in people. However, due to widespread abuse and misuse of antibiotics, a number of bacteria now have higher rates of antibiotic resistance (Aslam et al., 2018).

Antibiotic resistance is now acknowledged as posing a serious danger to world health in recent times. As a result, activities have been undertaken to combat the threat posed by antibiotic resistance while looking into alternate sources of antimicrobial compounds, such as medicinal plants (Rossolini et al., 2014). Connarus wightii, sometimes referred to as "Kuri White Hyena," is a plant species that has important therapeutic benefits. Connarus wightii, a member of the Connaraceae family, is native to India, specifically the Western Ghats area. Because of its many different pharmacological qualities, this plant has long been used in a variety of herbal treatments. Connarus wightii leaves, bark, and roots are among the components used for their therapeutic qualities in traditional Ayurvedic medicine.

Connarus wightii is a plant that has strong anti-inflammatory properties. Herbal medicines made from this plant are used to treat inflammatory diseases. Traditionally, connarus wightii has been used as an antipyretic to lower fever. It is thought that plant extracts have wound-healing qualities that aid in the repair of injured tissues.Since antibiotic therapy has significantly reduced mortality, millions of lives have been spared. However, the growing resistance of bacteria to antibiotics is posing a threat to the efficacy of nearly all currently available medicines. The overuse and abuse of antibiotics, which places undue selection pressure on microorganisms, is the main source of this resistance (Paphitou,

2013). Due to this, diseases are now more difficult to cure, which raises the morbidity and death rates of illnesses that were formerly curable (Byarugaba, 2004).According to Graf and Martin (2000), infections brought on by microbes resistant to antibiotics are more likely to lengthen hospital stays, raise the risk of mortality, and necessitate the administration of more toxic and costly drugs.

Due to the inaccurate nature of diagnosis and dosage in traditional medicine, patients may get an excessive amount of medication—not only in developing nations, but globally as well. This is not exclusive to conventional medicine; contemporary medicine may also experience it (Kokwaro, 2009). The class and concentration of phytochemical elements in plants are thought to be responsible for their biological activity, which is why different plant extracts have different kinds of activities (Wang et al., 2010).Screening plant extracts using phytochemistry, particularly those which have been employed in conventional medicine is crucial for determining the phytochemical components of the plants that are in charge of a certain bioactivity. The current study focuses the quantitative phytochemical analysis and antibacterial activity of herbal plant connarus wightii that are historically utilized for various treatments.

2. Materials and Methods

Gathering Medicinal Plant Samples

In this study, fresh connarus wightii, a medicinal herbal plant, was gathered from several locations in the southern Tamilnadu Kanayakumari area, namely near Tholayavattam. To ensure thorough drying and preparation for grinding, the plant materials were subjected to shade drying. Verifying the plant samples was a taxonomist from Tamil Nadu Agricultural University (TNAU), Southern Circle, Coimbatore. After completely grinding the dried plant pieces into a fine powder with a mechanical blender, the mixture was put into labelled sealed containers.

Extraction of Connarus Wightii Extract by Soxhlet Extraction Process

Crude leaf extract was made by the Soxhlet extraction method. 250 ml of different solvents were extracted from the chosen medicinal plant after a constant 20 gram of powdered plant material was put in a thimble. The plant components were extracted using ethanol, chloroform, and methanol as three separate solvents. Until the solvent in the extractor's syphon tube turns colourless, the extraction procedure is carried out for five hours. The extract was thereafter put in a beaker and heated to between 30 and 40 degrees Celsius on a hot plate, or until the solvent was cooked off. In anticipation of their usage, the dried extract was stored in a refrigerator at 4ºC.

Maintainace and Preparation of Test Organism for Antimicrobial Activity

Gene Bank (MTCC), Chandigarh, India is the source of the standard microorganisms. The test microbes utilised in antibacterial analysis included the bacteria Aeromonas hydrophila and Staphylococcus aureus, as well as the fungus employed to investigate anti-fungicidal properties. Though fugal strains were produced in sabourad broth and kept on SDA agar slants at 4°C, Candida albicans and Aspergillus niger in the other hands the bacterial strains were cultivated in the nutrient broth and maintained on nutrient agar slants. The plate's pure culture was inoculated into a Nutrient Agar plate and subcultured for twenty-four hours at 37ºC. A bacterial suspension of 1.5×108 cfu/ml was obtained by aseptically introducing the new culture to a 2 ml sterile 0.145 mol/L saline tube, and then adjusting the cell density to meet the 0.5 McFarland turbidity that standardized inoculum was used for antimicrobial test.

Antibacterial Test for Antimicrobial Activity

In 1000 millilitres of distilled water, 38 grams of Mueller-Hinton Agar Medium (Hi Media) were dissolved to create the medium. The dissolved medium was autoclaved for 15 minutes at 121℃ and 15 pounds of pressure (pH 7.3). After cooling, thoroughly mixing, and pouring the autoclaved media into petriplates (25 ml per plate), the plates were swabbed with a pathogenic bacterial culture, namely Aeromonas hydrophila and Staphylococcus aureus. The bacterial culture's sensitivity determined the use of the usual medication Ciprofloxacin 5 mcg concentration disc for positive control, while an empty sterile disc was utilised for negative control. For 24 hours, the plates were incubated at 37°C. After that, the plates were incubated based on each organism's requirements for growth. The antibacterial activity of each sample was assessed in triplicate using a measurement and recording of the zones of inhibition in millimetres (mm).

Antifungal Assay by Disc Diffusion Method

The agar disc diffusion (Kirby-Bauer) technique was used to determine antibiotic susceptibility

testing. On SDA agar plates, sterilised cotton swabs were used to swab the fungus strains Candida albicans and Aspergillus niger. A sterile disc with 150 mcg of the standard medication fluconazole was utilised as the positive control, while an empty sterile disc served as the negative control.The plant extracts and protein extract was then allowed to diffuse for five minutes on the surface of the SDA medium, after which the disc was inserted there. The plates were then incubated for 48 hours at 28°C. Following the incubation period, inhibition zones surrounding the disc were measured using a clear ruler in millimetres.

Extraction of Protein from Leaves of Connarus Wightii

Using a pre-chilled mortar and pestle, 0.1 g of plant powder was dissolved in 2ml of PBS. Subsequently, the samples underwent three cycles of the freeze-thaw process, each lasting around twelve hours. The tubes were then centrifuged at 14000 rpm for 10 minutes at 4°C. The supernatant should be collected and stored at 4°C in a separate tube for quantification.Using Lowry's technique, the protein content was estimated. Polyacrylamide 11% slab gel electrophoresis using sodium dodecyl sulphate was used, according to Laemmli (1970). Samples were placed in sample buffer (25% 1 M Tris-HCl, pH 6.8), 4% SDS, 2% β-mercaptoethanol, and 5% glycerol), and heated for 3 minutes at 100°C with these ingredients. The gels were left at room temperature (30±2°C) and stained with a mixture of 0.05% coomassie blue R-250, 7% acetic acid, and 50% isopropyl alcohol. The gels were then destained using a mixture of 50% ethanol and 7% acetic acid in order to remove the stain.

GC-MS-Based Quantitative Phytochemical Screening

In the current investigation, The Connarus wightii extracted were using ethanol, chloroform, and methanol as three separate solvents. The three extracts were used for phytochemicals identified and quantified by GC-MS. Using a 5975C mass selective sensor with an HP5 MS low bleed capillary column, a 7890 A Gas-Chromatograph was connected. The operational parameters of the mass spectrometer were as follows: relative detector gain mode; 70eV ionisation energy; 3.3 min filament delay time; 1666μ/sec scan speed; 40–550 m/z scan range; 230 °C ion source temperature; and 180 °C quadrupole temperature. A steady flow of 1.25 millilitres per minute of 99.9% helium carrier gas was employed. 200 °C was the mass transfer temperature established when the injector line transfer was occurring.

3. Result and Discussion

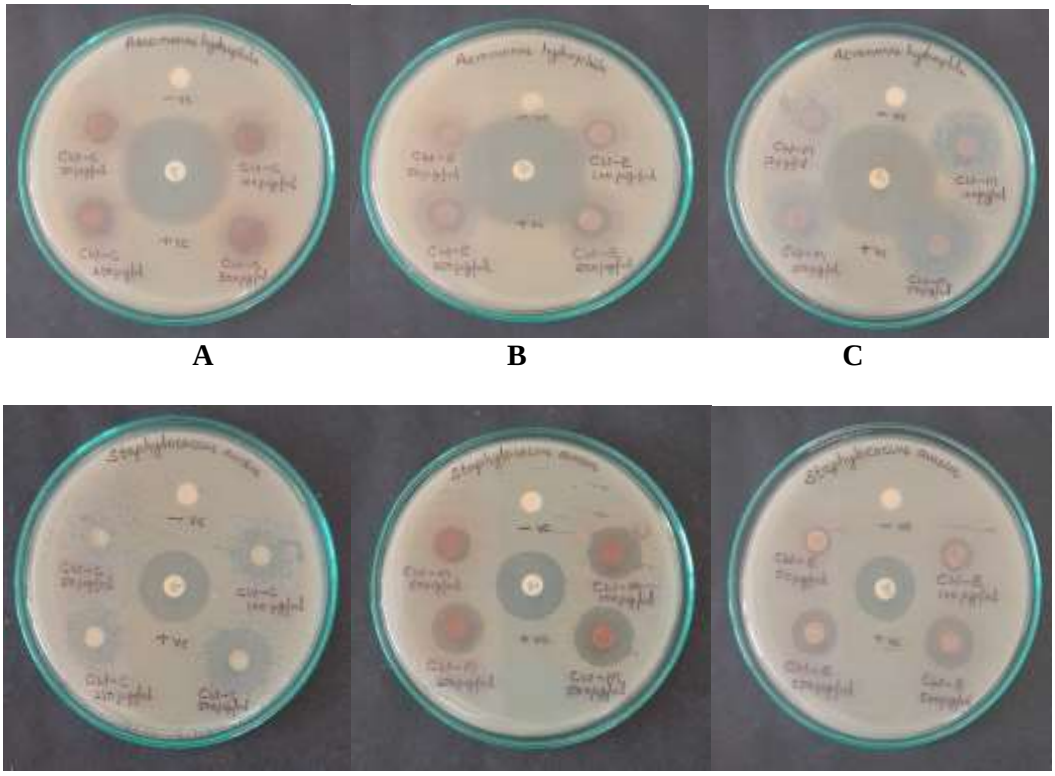
Antimicrobial Activity of Connarus Wightii

As research has shown the potential advantages of chemicals originating from plants, the scientific and pharmaceutical communities have recently developed a greater interest in medicinal plants. Since ancient times, a variety of medical applications, such as pharmaceuticals, complementary and alternative treatments, and natural medicines, have been based on the therapeutic qualities of plant extracts (Biswas et al. 2005). Additionally, a lot of plant materials are employed in traditional medicine, especially in developing countries where they are the primary readily available resource for treating a variety of diseases and illnesses in rural communities (Karimi and Jaafar 2011). Plants are a rich source of secondary metabolites and a significant source of chemical diversity, which means they can produce novel human medicines.Three different solvents are used to extract the leaves from Connarus wightii: methanol, ethanol, and chloroform. Crude extracts and protein of Connarus wightiiwere tested for their antimicrobial effects on Aeromonashydrophila, Staphylococcus aureus, Candida albicans, and Aspergillus niger using the well and disc diffusion methods, respectively. For bacterial and fungal research, respectively, streptomycin and fluconazole were used as standard drugs. The present investigation involves three different solvents of extract the leaves from Connarus wightii,the methanol extract of Connarus wightiishows the antibacterial activities in both Gram-positive (Staphylococcus aureus) and Gram-negative (Aeromonashydrophila) bacteria strain compared to other solvent extracts. In this studies the three different solvents of extract show high activity in 500 μg/ml concentration, the methanol, chloroform, and ethanol extracts of Connarus wightiiexhibit greater inhibitory action against Staphylococcus aureus than Aeromonashydrophila; The results showed that when the activity of crude extracts was compared to that of standard streptomycin (15.25±0.35 mm), the methanol extracts (14.5± 0.35 mm) almost had the same activity.Figure-1 andTable 1 lists the width of the inhibitory zone in response to extract concentration of different bacterial strains. Pathogenic bacteria are susceptible to the effects of many chemicals (Cowan, 1999; Awouafack et al., 2013; Tsopmo et al., 2013; Erfan and Marouf, 2019). Such metabolites' presence in the examined

plant extracts can provide a rough explanation for their antibacterial and antimicrobial properties. Variations were noted in the antimicrobial properties of the extracts. These could result from variations in both their chemical makeup and the way that their bioactive components work (Cowan, 1999). Secondary metabolites are abundant in all of the extracts; nevertheless, their presence and potential interactions with other components, in addition to their quantity, are also factors that affect the extracts' ability to perform, as noted by (Dzotam et al 2016).

Table-1 Diameter zone of inhibition of three solvents extracts of Connarus wightii

Sl. No.	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1	Aeromonashydrophila	Chloroform	14±0.35	13±0.35	12±0.350	10±0.35	11.5±0.7
	Staphylococcus aureus		20±0.35	18±0.35	15±0.350	9 ±0.35	15.25±0.35
2	Aeromonashydrophila	Ethanol	13±0.35	12 ±0.35	10±0.350	8 ±0.35	14.5±0.7
	Staphylococcus aureus		16 ±0.7	14±0.35	10 ±0.35	9 ±0.350	13.25±0.35
3	Aeromonashydrophila	Methanol	21±0.35	16 ±0.35	14 ±0.35	11±0.350	13.25±0.35
	Staphylococcus aureus		18 ±0.7	17±0.35	16 ±0.35	11±0.350	15.25±0.35



A **B** **C**

D **E** **F**

Figure-1 The inhibition zone (mm) of Chloroform (A), Ethanolic extracts (B) , Methanolic extract (C) of Connarus wightii against Aeromonas hydrophila and The inhibition zone (mm) of Chloroform (D), Ethanolic extracts (E) , Methanolic extract (F) of Connarus wightii against Staphylococcus aureus

Antifungal Activity of Three Solvent Extracts of Connarus Wightii

The efficiency of these medicinal plants' metabolites against human infections may be assessed by evaluating the clinical isolates' sensitivity to antibiotics Assessing the antifungal properties of herbal plants against prevalent invasive species of Candida is crucial because there is insufficient data on their in vitro efficaciousness against Candida species (Mahmood et al 2015). The antifungal properties of the Connarus wightii leaf extract are the main subject of this study. Using methanol, chloroform, and ethanol as the three different solvents, the Soxhlet extraction method was employed to produce crude leaf extract. Compare all of the extracts to the standard drug, fluconazole; the extracts had no effect against Aspergillus niger and very moderate efficacy against Candida albicans. As the concentration of extracts (µg/ml)

the antifungal activity of the extracts also increased linearly. The results showed that when compared to typical medications, fungal activity from Candida albicans performed better than that of Aspergillus niger. For fungal strains, the growth inhibition zone measurements varied from 7 to 15 mm. Figure-2 andTable -2 list out the inhibitory zone width in response to various fungal extract concentrations.

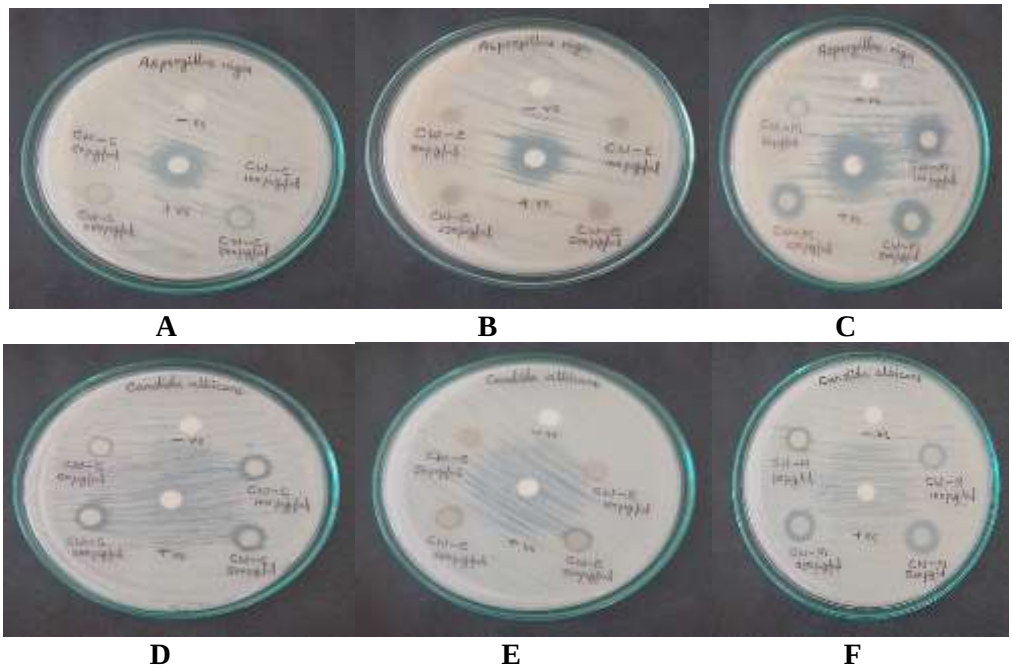


Figure-2 The inhibition zone (mm) of Chloroform (A), Ethanolic extracts (B) , Methanolic extract (C) of Connarus wightii against Aspergillus niger and The inhibition zone (mm) of Chloroform (D), Ethanolic extracts (E) , Methanolic extract (F) of Connarus wightii against Candida albicans

Table-2 Antifungal activity of three solvents extracts of Connarus wightii

Sl. No.	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1	Candida albicans	Chloroform	12 ±0.35	11 ±0.35	10±0.35	7 ±0.35	14.25±0.35
	Aspergillus niger		9 ±0.35	8 ±0.35	7 ±0.35	NZ	7.5±0.7
2	Candida albicans	Ethanol	9 ±0.7	7 ±0.35	NZ	NZ	13.5±0.7
	Aspergillus niger		NZ	NZ	NZ	NZ	13.25±0.7
3	Candida albicans	Methanol	13 ±0.35	12 ±0.35	11 ±0.35	10 ±0.35	14.5±0.7
	Aspergillus niger		15 ±0.35	14 ±0.35	13 ±0.35	7 ±0.35	14.25±0.35

Protein Estmation of Connarus Wightii

Using Lowry's technique, the protein content was determined following the extraction of protein extracts from the Connarus wightii leaf sample. The absorbance at 600 nm of the protein extracts from the Connarus wightii leaf sample was measured with a spectrophotometer. The maximal protein levels were 86.03 ± 0.008 µg/ml. Since the Lowry technique is straightforward and readily available, it has been used extensively for many years to determine protein. But the Folin-Ciocalteu reagent interacts with a variety of different substances in addition to aromatic amino acids (Everette et al 2010).To determine the protein content and molecular weight of the Connarus wightii leaf extract. The molecular weight protein was assessed using SDS-PAGE electrophoresis in relation to the protein standard. Along with minor bands found at 245, 180, 135, and 100 kDa, the most abundant protein from the powder and fresh leaf band is found at 63 kDa was shown in theFigure-3.

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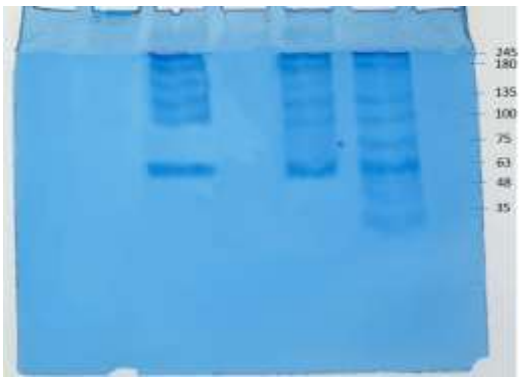


Figure-3 SDS-PAGE profiles of protein of C. wightii; protein from powder leaf (1st well from left), protein from fresh leaf (2nd well), marker(3rd well)

GCMS (Gas Chromatography- Mass Spectroscopy)

New medications are derived from medicinal plants. A large number of contemporary medications are derived indirectly from therapeutic herbs. They have made several essential contributions to combat a wide range of illnesses and ailments. Herbal formulations are developed, modernised, and quality-controlled in large part by the examination and extraction of plant material. Understanding plant toxicity and assisting in the defence of humans and animals against natural toxins are two additional benefits of studying therapeutic plants. Therefore, the goal of the current work is to use mass spectroscopy and gas chromatography to identify the bioactive chemicals found in the three different solvents According to the (methanol, ethanol, and chloroform) of extract the leaves from Connarus wightii. Table -3,4,5 and Figure-4,5,6 provide the active principles together with their retention time (RT), molecular formula, molecular weight (MW), and concentration (peak area %). Among the phytocompounds that have been discovered, squalene, piperine, tetradecanoic acid, and hexadecanoic acid have been shown to possess antioxidant, 5-alpha-reductase inhibitor, hemolytic, antifibrinolytic, and antibacterial properties (Abirami and Rajendran 2011; Kala et al. 2011). Previous studies have revealed the anti-inflammatory, hypocholesterolemic, and antiarthritic properties of octadecadienoic acid (Z, Z) (Rani et al. 2009; Ponnamma and Manjunath 2012; Uma et al. 2009). Moreover, naphthalene has strong antibacterial action (Uma et al. 2009).Figures 4.6 to 4.8 below show the GCMS chromatogram for the Connarus wightii extract. According to Table-3, 4, and 5, the active components analysis of the Connarus wightii leaves revealed the existence of chemical compounds that may contribute to the plant's therapeutic capabilities.

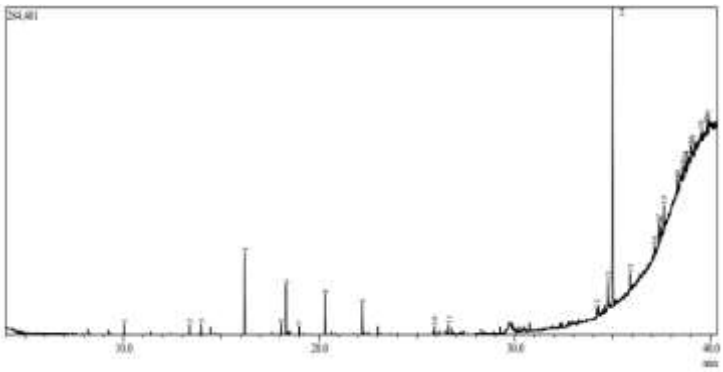
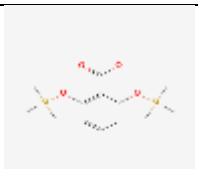
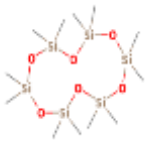
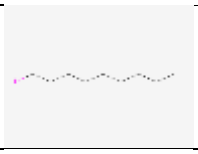
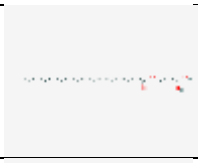
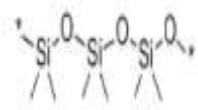
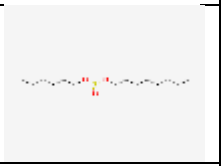
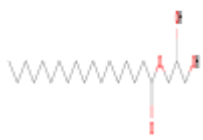
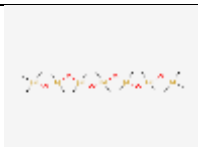
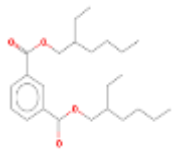
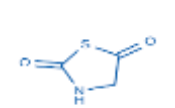
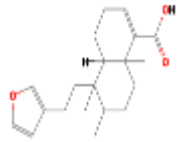
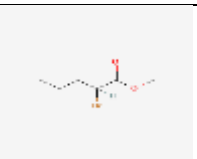
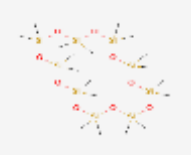
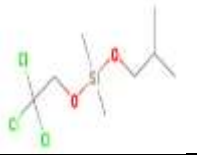
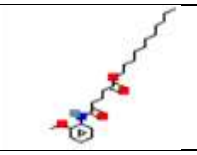


Figure-4. GC-MS chromatogram of chloroform extract of Connarus wightii

Table-3 Compounds identified in the chloroform extract of Connarus wightii in GC-MS

Sl No	Name	Retention time	Molecular formula	Mol. Wt.	Area %	Structure
1	Benzoicacid,2,6-bis(trimethylsiloxy)-,trimethylsilylester	10.05	$C_{16}H_{30}O_4Si_3$	370	1.08	
2	Cyclohexasiloxane,dodecamethyl-	13.983	$C_{12}H_{36}O_6Si_6$	444	0.93	
3	Octadecane	16.197	$C_{18}H_{38}$	254	8.02	
4	Nonane,1-iodo-	18.988	$C_9H_{19}I$	254	0.81	
5	1-(2-Hydroxyethoxy) tridecane	25.895	$C_{15}H_{32}O_2$	244	0.84	
6	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethylester	34.743	$C_{19}H_{38}O_4$	330	3.11	
7	Bis(2-ethylhexyl) phthalate	34.99	$C_{24}H_{38}O_4$	390	35.42	
8	Siliconeoil	35.88	$C_6H_{18}OSi_2$	162.38	2.08	
9	Sulfurous acid,hexyloctylester	37.118	$C_{14}H_{30}O_3S$	278	1.86	
10	Octadecanoic acid,2,3-dihydroxypropyl ester	37.348	$C_{21}H_{42}O_4$	358	3.68	
11	Heptasiloxane,hexadecamethyl-	37.472	$C_{16}H_{48}O_6Si_7$	532	2.59	

12	1,3-Benzenedicarboxylicacid,bis(2-ethylhexyl) ester	37.615	$C_{24}H_{38}O_4$	390	3.3	
13	Sulfurous acid,pentadecyl2-propylester	38.305	$C_{18}H_{38}O_3S$	334	1.33	
14	Thiazolidine-2,5-dione	38.625	$C_3H_3NO_2S$	117	2.93	
15	(4aS,5R,6S,8aS)-5-(2-(Furan-3-yl)ethyl)-5,6,8a-trimethyl-3,4,4a,5,6,7,8,8a-octahy	38.745	$C_{20}H_{28}O_3$	316	1.78	
16	Methyl2-bromopentanoate	38.835	$C_6H_{11}BrO_2$	194	1.14	
17	Cyclononasiloxane,octadecamethyl-	39.015	$C_{18}H_{54}O_9Si_9$	666	3.35	
18	Silane,dimethyl(2,2,2-trichloroethoxy)isobutoxy-	39.185	$C_8H_{17}Cl_3O_2Si$	278	1.23	
19	1-OctanoL,2,2-dimethyl-	39.549	$C_{10}H_{22}O$	158	1.01	
20	Glutaric acid,2-methoxyphenyl undecylester	39.825	$C_{23}H_{37}NO_4$	391.5	1.5	

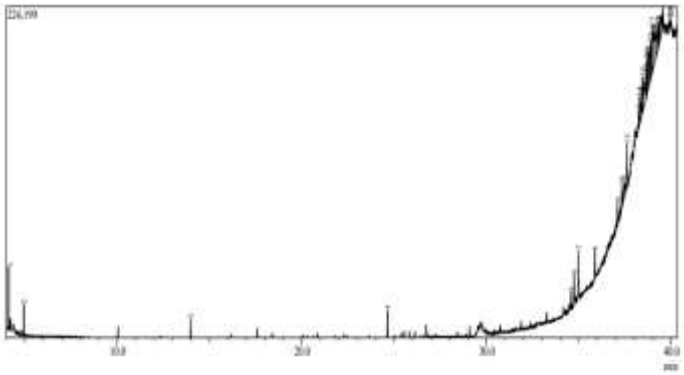
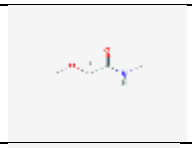
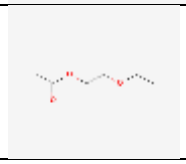
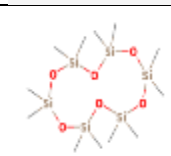
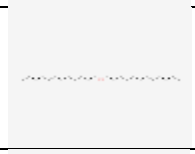
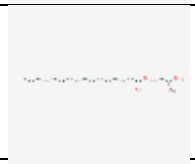
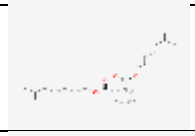
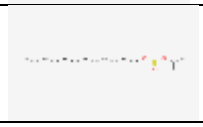
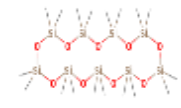
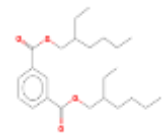
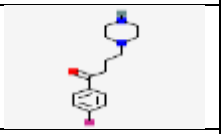
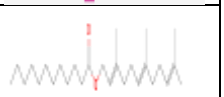
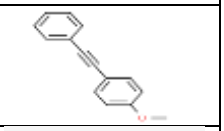
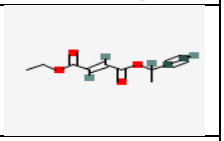
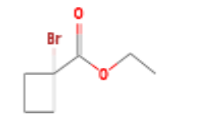
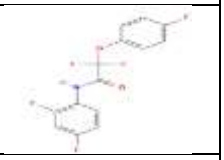
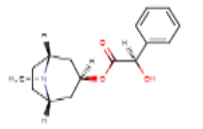
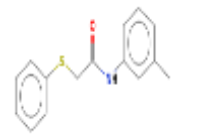
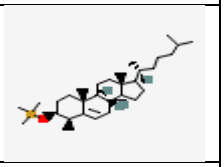


Figure-5 GC-MS chromatogram of ethanol extract of Connarus wightii

Table-4 Compounds Identified in the Ethanol Extract of Connarus Wightii in GC-MS

Sl No	Name	Retention time	Molecular formula	Mol.Wt	Area %	Structure
1	Propanamide,2-methoxy-N-methyl-	4.132	$C_5H_{11}NO_2$	117	3.56	
2	2-Ethoxyethyl acetate	4.942	$C_6H_{12}O_3$	132	1.72	
3	Cyclohexasiloxane,dodecamethyl-	13.98	$C_{12}H_{36}O_6Si_6$	444	1.14	
4	6-octen-1-ol,3,7-dimethyl-,propanoate	24.642	$C_{13}H_{24}O_2$	212	1.94	
5	Dodecane,1,1'-oxybis-	34.592	$C_{24}H_{50}O$	354	1.35	
6	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethylester	34.737	$C_{19}H_{38}O_4$	330	2.94	
7	Diisooctyl-phthalate	34.984	$C_{24}H_{38}O_4$	390	3.93	
8	Sulfurous acid,decyl2-propylester	35.871	$C_{13}H_{28}O_3$	264	2.33	
9	Decanedioic acid,didecylester	37.108	$C_{30}H_{58}O_4$	482	2.2	
10	Octadecanoic acid,2,3-dihydroxypropyl ester	37.343	$C_{21}H_{42}O$	358	1.56	
11	Cyclononasiloxane,octadecamethyl-	37.45	$C_{18}H_{54}O_9Si_9$	666	2.98	
12	1,3-Benzenedicarboxylicacid,bis(2-ethylhexyl) ester	37.61	$C_{24}H_{38}O_4$	390	3.61	
13	16-Trimethylsilyloxy-9-octadecenoic acid, methyl ester	38.25	$C_{22}H_{44}O_3Si$	384	0.69	

14	Triacontane, 1-bromo-	38.299	$C_{30}H_{61}Br$	500	2.97	
15	1-Butanone,1-(4-fluorophenyl)-4-(1-piperazinyl)-	38.41	$C_{14}H_{19}FN_2O$	250	3.16	
16	(2E,6E)-3,7,11-Trimethyldodeca-2,6,10-trien-1-yldodecanoate	38.445	$C_{27}H_{48}O_2$	404	3.26	
17	Anisole,O-(phenylethynyl)-	38.51	$C_{15}H_{12}O$	208	6.32	
18	Fumaricacid,but-3-yn-2-yl ethylester	38.745	$C_{10}H_{12}O$	196	5.08	
19	Ethyl.alpha.-bromocyclobutanecarboxylate	38.815	$C_7H_{11}BrO$	206	5.54	
20	2-(2,4-DifluorophenoXY)-N-(4-fluorophenyl) acetamide	38.9	$C_{14}H_8F_5NO_2$	317.21	4.17	
21	8-Methyl-8-azabicyclo[3.2.1]oct-3-ylhydroxy(phenyl) acetate	39.174	$C_{16}H_{21}NO_3$	275	1.55	
22	Acetamide,N-(3-methylphenyl)-2-phenylthio-	39.22	$C_{15}H_{15}NOS$	257	4.24	
23	Silane,trimethyl[(4.beta.-methylcholesteryl)oxy]-	39.464	$C_{31}H_{56}OSi$	472	4.4	
24	2-Methylhexacosane	39.539	$C_{27}H_{56}$	380	2.8	

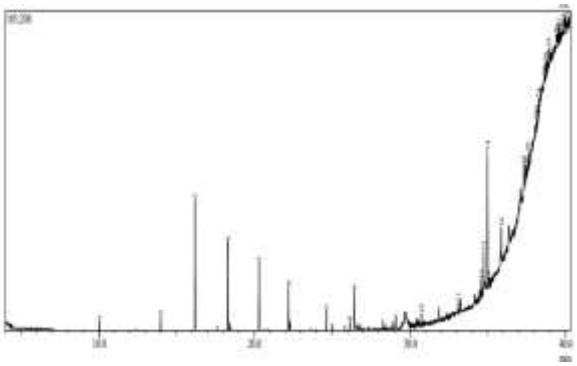
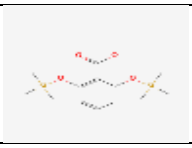
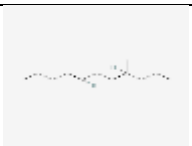
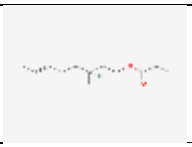
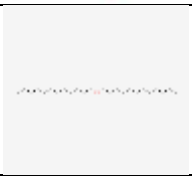
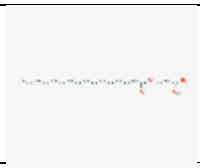
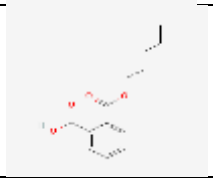
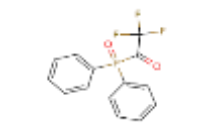
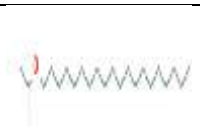
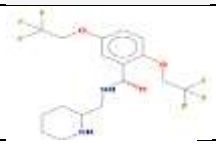
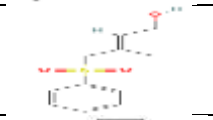
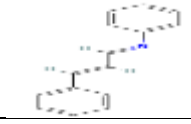
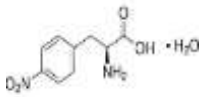
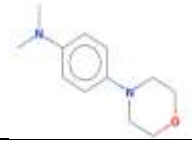
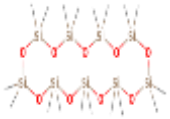
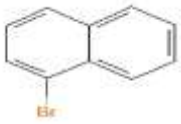
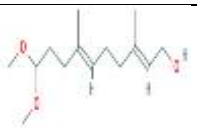
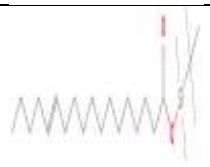


Figure-6s. GC-MS chromatogram of methanol extract of Connarus wightii

Table-5. Compounds identified in the methanol extract of Connarus wightii in GC-MS

Sl No	Name	Retention time	Molecular formula	Mol. weight	Area %	Structure
1	Benzoicacid,2,6-bis(trimethylsiloxy)-,trimethylsilylester	10.045	$C_{16}H_{30}O_4Si_3$	370	0.98	
2	Cyclohexasiloxane,dodecamethyl-	13.98	$C_{12}H_{36}O_6Si_6$	444	1.24	
3	Undecane,4,7-dimethyl-	16.193	$C_{13}H_{28}$	184	12.05	
4	Octadecane	18.306	$C_{18}H_{38}$	254	8.28	
5	6-Octen-1-OL,3,7-dimethyl-,propanoate	24.643	$C_{13}H_{24}O_2$	212	1.68	
6	Methyl6-hydroxyhexanoate	26.132	$C_7H_{14}O_3$	146	0.83	
7	2H-[1,2,3]Triazole, 4-nitro-	30.747	$C_2H_2N_4O_2$	114	1.17	
8	Adipic acid,di(2,4-dimethylpent-3-yl) ester	33.094	$C_{17}H_{32}O_4$	300.43	0.94	
9	Dodecane,1,1'-oxybis-	34.593	$C_{24}H_{50}O$	354	1.75	

10	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester	34.738	$C_{19}H_{38}O_4$	330	4.22	
11	1,2-benzenedicarboxylic acid	34.986	$C_{24}H_{38}O_4$	390	16.72	
12	Phosphineoxide,diphenyl(trifluoroacetyl)-	35.07	$C_{14}H_{10}F_3O_2P$	298	0.48	
13	Eicosylisopropylether	35.871	$C_{23}H_{48}O$	340	2.78	
14	Octadecanoic acid,2,3-dihydroxypropyl ester	37.334	$C_{21}H_{42}O_4$	358	3.46	
15	Heptasiloxane,hexadecamethyl-	37.45	$C_{16}H_{48}O_6Si_7$	532	2.69	
16	Benzamide,N-(2-piperidinylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)-	37.57	$C_{17}H_{20}F_6N_2O_3$	414	0.32	
17	1,4-Benzenedicarboxylicacid,bis(2-ethylhexyl) ester	37.605	$C_{24}H_{38}O_4$	390	1.64	
18	(2E)-3-Methyl-4-(phenylsulfanyl)-2-buten-1-ol	38.13	$C_{11}H_{14}O_3S$	194	0.78	
19	1H-pyrrole-1-propanoic acid,2,5-dihydro-2,5-dioxo-	38.185	$C_7H_7NO_4$	169	0.69	
20	Propane,1,2-dibromo-	38.225	$C_3H_6Br_2$	200	1.5	
21	4(3H)-pyrimidinone,6-amino-2-methyl-	38.299	$C_5H_7N_3O$	125	0.66	
22	Aniline,N-cinnamylidene-	38.665	$C_{15}H_{13}N$	207	0.57	

23	Thiazolidine-2,5-dione	38.7	$C_3H_3NO_2S$	117	1.13	
24	4-Nitro-l-phenylalanine	38.758	$C_9H_{10}N_2O_4$	210	1.72	
25	Benzeneamine,4-(1-morpholinyl)-N,N'-dimethyl-	38.83	$C_{12}H_{18}N_2O$	206	0.69	
26	Cyclononasiloxane,octadecamethyl-	38.97	$C_{18}H_{54}O_9Si_9$	666	2.16	
27	Naphthalene, 1-bromo-	39.549	$C_{10}H_7Br$	206	1.39	
28	10,10-Dimethoxy-3,7-dimethyl-deca-2,6-dien-1-ol	39.674	$C_{14}H_{26}O_3$	242	1.9	
29	13-Octadecenoic acid,(E)-,TBDMSderivative	39.87	$C_{24}H_{48}O_2Si$	396.72	2.58	
30	6-Dimethyl(chloromethyl)silyloxytetradecane	39.957	$C_{19}H_{44}OSi2$	320	4.28	

4. Conclusion

Medicinal plants, the foundation of traditional medicine, have been the focus of intense pharmacological research in recent decades due to their recognition as potential sources of lead compounds for drug development as well as new compounds with therapeutic value. Thus, GC-MS analysis was used to identify the bioactive molecule in Connarus wightii and the results indicate the existence of 20 compounds. Among the substances that were found, piperine, squalene, n-hexadecanoic acid, and octadecanoic acid had antibacterial, antioxidant, anticancer, and anti-inflammatory properties. The results of this study suggest that the existence of these phytochemicals in Connarus wightii may make it a new potential source of medicinal products.

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