Screening of Bioactive Phytocompounds in the Leaf of *Coldenia procumbens* Using Biochemical, FTIR and GCMS Analysis

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Abstract: *Coldenia procumbens* is a prominent medicinal plant used to treat several symptoms connected to sexually transmitted diseases in humans. Ancient Tamil literature contains abundant documentation on this subject. Rural siddha medical practitioners still utilise the *Coldenia procumbens* plant, relying on ancient literature and traditional knowledge, as an ingredient in various formulations to treat various sexually transmitted illnesses in humans. Hence, the present study has been programmed to Tidentify potential bioactive chemicals that may be used to treat different human ailments. This study included qualitative, quantitative, FTIR, and GCMS studies on the leaf of methanol extract from *Coldenia procumbens*. The leaf powder of *Coldenia procumbens* was subjected to maceration in ethanol and methanol, revealing a significant abundance of phenol, flavonoids, saponins, tannin, alkaloids, and a restricted quantity of terpenoids. The phenol content was quantitatively analysed and found to be 0.84±0.07mg GAE/g dry extract. The tannin content was determined to be 0.72±0.06 mg TAE/g dry extract. The alkaloid content was measured as 0.43±0.02mg/ATE/g dry extract. Lastly, the flavonoid content was determined to be 0.73±0.058 mg CHE equivalent/g dry extract. Analysed using FTIR, the methanolic leaf extract of *Coldenia procumbens* revealed the presence of nine bioactive compounds. The compounds mentioned consist of alkane, sulfonamide, aromatic ester, vinyl ether, sulfoxide, carboxylic acids, aromatics, alkenes, and halocompounds. The compounds were identified by detecting characteristic peaks at specific wavelengths: 2925.48 cm-1 (C-H stretching), 2853.17 cm-1 (C-H stretching), 1460.81 cm-1 (C-H bending), 1380.78 cm-1 (C-H bending), 1629.55 cm-1 and 717 cm-1 (C=C bending), 1709.59 cm-1 (C=O stretching), 3412.42 cm-1 (O-H stretching with hydrogen bonding), and 1087.66 cm-1 (C-O stretching). The ethanolic leaf extract of *Coldenia procumbens* was analysed using GCMS, which revealed the presence of seven bio-active phytocompounds. The identified compounds include 6-Octadecenoic Acid, (Z)-(27%), Ethyl Oleate (22%), 9-Borabicyclo[3.3.1]Nonane, 9-Decyl-(18%), N-Propionyl-D-Glucosamine (18%), N-Hexadecanoic Acid (9%), Heptanoic Acid, 2,6-Dimethyl-, Methyl Ester (4%), Trans-4-T-Pentylcyclohexanol (1%), and 2,5,8-Triphenyl Benzotristriazole (1%). The compounds were retained for the following durations: 17.970, 18.015, 19.136, 19.576, 19.626, 19.716, 27.064, and 19.841, respectively.

Key Words: FTIR, GCMS, Bioactive, Phytocompounds, Qualitative analysis, Quantitative analysis

1. Introduction

The use of herbal-based traditional medical treatments has been widespread in almost every country globally since ancient times [1] .Traditional medicine is widely employed and accounts for around 40% of the overall healthcare services [2]. Approximately 85% of conventional medicines are derived from botanical sources [3]. Various studies have shown that indigenous people make use of more than 25,000 formulations derived from plants [4]. In India, traditional medicine is not just employed by indigenous tribes, but also by the majority of the rural

populace. Millions of traditional practitioners employ this conventional therapeutic method [5]. Estimates suggest that almost 80% of the population in Africa and Asia predominantly depends on traditional medicine or cures rather than modern medicine for their primary healthcare needs [6]. The therapeutic characteristics of the majority of plants are ascribed to the existence of advantageous phytoconstituents in various parts of the plant, which enhance their capacity to alleviate and remedy diverse human ailments [7-8]. The botanical remedies derived from plants are considerably safer, exhibiting minimal or nonexistent adverse reactions when used to treat diverse medical conditions [9-10]. On the other hand, conventional herbs are regarded as eco-friendly and are becoming more and more employed to reduce the occurrence of adverse effects and improve overall health [11]. The pharmacological properties of diverse traditional medicines are being evaluated to determine their efficacy, which could potentially lead to the discovery and development of new drugs [12]. Therefore, the objective of this work was to analyse the phytochemicals found in the traditional medicinal herbs *Coldenia procumbens* Linn, as well as evaluate their antibacterial and free radical scavenging activities in order to determine their antioxidant qualities.

2. Materials and Method

The whole plant and leaves of *Coldenia procumbens* were collected from the foot hills of Vallimalai, Eastern Ghats, in the Vellore District. The collected plants were photographed, and a herbarium was prepared. The photographs of live plants were sent to Dr. Rajendran, Professor, Thiagarjar College, Madurai, for identification. Then the fresh leaves of *Coldenia procumbens* were washed twice in tap water and double distilled water to remove the dust, small insects, eggs, and animal excreta adhered to them. Similarly, the infected leaves were also carefully removed by hand picking. All these healthy leaves were spread on the waste paper and kept in a shaded place until they were completely dried. Easy hand cursing of the leaves ensured complete drying. Thereafter, the dried leaves were milled in the mechanical grinder and made into a fine powder. The powder obtained was sieved to remove the coarse particles and stored in an airtight glass container.

2.1 Qualitative analysis of phytocompounds : In the present investigation, phytochemicals such as phenols, flavonoids, saponins, tannins, alkaloids, terpenoids, coumarins, anthocyanins, and anthraquinones were analysed by the following methods.

2.1.1 Test for phenols.

2.1.1.1 Ellagic acid test

In a well-cleaned test tube, one microliter of plant extract and a few drops of 5% glacial acetic acid were added. Then, 5% NaNO2 was also added to the above mixture. The appearance of a muddy brown colour indicates the presence of phenol[13] .

2.1.1.2 FeCl3 method

In a well-cleaned test tube, 1 mL of plant extract, 2 mL of distilled water, and a few drops of 10% FeCl3 were added. The formation of a blue-green colour was indicative of the presence of phenol.

2.1.2 Flavonoids test

2.1.2.1 Alkaline reagent test

In a well-cleaned test tube, 1 mL of plant extract and 2N NaOH in 1 mL were added. The appearance of a yellow colour indicates the presence of flavonoids.

2.1.2.2 FeCl3 test

In a well-cleaned test tube, 1 mL of plant extract was taken along with a few drops of FeCl3 solution. The formation of a blackish-red precipitate indicates the occurrence of flavonoids.

2.1.3 Test for seponin

2.1.3.1 Froth formation test for saponin analysis

In a well-cleaned test tube, 2 ml of extract and 2 ml of distilled water were added. This mixture was shaken well in a graduated cylinder lengthwise for 15 minutes. A layer of foam produced for 1 cm indicates the presence of saponins.

2.1.4 Test for tannins.

2.1.4.1Alkaline Reagent test for tannin Analysis

In a well-cleaned test tube, 2 ml of extract and 2 ml of NaOH were added. Changing the colour of the solution from yellow to red indicates the presence of tannins [14].

2.1.4.2 FeCl3 test for tannin analysis

In a well-cleaned test tube, 1 mL of plant extracts and 2 mL of 5% FeCl3 were added. The appearance of greenish black indicates the presence of tannins.

2.1.4.3 Salkowski test for tannin analysis

In a well-cleaned test tube, 1 mL of extract, 10 mL of chloroform, and 10 mL of concentrated sulfuric acid were added. The test tube containing the lower part of the solution shows a yellow colour with green fluorescence, and the upper part shows a red ring formation, which indicates the presence of steroids[14.]

2.1.4.4 Emulsion test for tannin analysis

In a well-cleaned test tube, 1 mL of plant extract and six drops of olive oil were added. This mixture was shaken well to form a stable froth. The formation of an emulsion revealed the presence of tannin [15].

2.1.5 Alkaloids analysis test

2.1.5.1 Hager test

The Hagers test was followed to trace out the presence of alkaloids in the plant. For this study, in a test tube, 2 mL of plant extract was taken along with Hagers reagent (dissolve 1 g of picric acid in 100 mL of distilled water). The formation of a yellow colour in the test tube indicates the presence of alkaloids.

2.1.5.2 Mayer's test

In a well-cleaned test tube, 2 mL of plant extract, 2 mL of HCl [conc.], and a few drops of Mayer's reagent were added and mixed thoroughly. White precipitate appeared in the test tube, which indicates the presence of alkaloids (Archana *et al.,* **2012**)

2.1.6 Test for terpenoids.

In a well-cleaned test tube, 2 mL of each extract and 1 mL of 1% HCl were added, and this was kept for 5–6 hours. After that, 1 ml of Trim-Hill reagent was added and then heated in a water bath to boiling temperature. The appearance of a bluish-green colour indicates the presence of terpenoids [13].

2.1.7 Coumarins test

In a well-cleaned test tube, 1 mL of plant extract and 1 mL of NaOH (10%) solution were added. The appearance of a yellow colour confirmed the presence of coumarins[16].

2.1.8 Anthocyanin test

In a well-cleaned test tube, 1 mL of 2N NaOH was added along with 2 mL of plant extract, and it was left for 5 minutes at 100°C. The formation of a bluish-green colour indicates the presence of anthocyanin [17].

2.1.9 Anthraquinones test

In a well-cleaned test tube, 1 mL of extract, 1 mL of benzene, and 1 mL of ammonia solution (10%) were added. The formation of a red colour indicated the presence of anthraquinones. The addition of 2 ml of 2% HCl with plant extract gave a red precipitate, which indicates the presence of anthraquinones [18].

2.2 FTIR analysis

FTIR is the most powerful, easy, simple, fast, suitable, non-invasive, and cost-effective method for identifying the types of chemical bonds (functional groups) present in compounds. For this method, the dried plant samples were mixed with small quantities of potassium bromide (KBr) at a 1:100 ratio in a mortar and pestle and mixed well by squashing them. Finally, this mixture was poured into a die set and made into a pellet (FTIR grade) with the help of a 15T manual hydraulic pellet press at 100 psi for 5 minutes. The translucent pellet formed in this way was kept in a pellet holder and closed the chamber. This loaded pellet in the FTIR Spectroscope (Shimadzu, IR Affinity 1, Japan) was scanned in the range of 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.3 GCMS analysis

GCMS of the leaf extracts of plants *Andrographis paniculata, Acalypha indica, Stachytarpheta jamailensis, Wrightia tinctoria,* and *Coldenia procumbens* were performed using the TurboMass TM Spectrometer, a benchtop mass spectrometric detector (PerkinElme) by PerkinElme Instruments. The carrier gas used is helium at a flow rate of 1.0 ml/min. The initial temperature is 60°C for 2.50 minutes; ramp 10°C/m to 300°C hold brin. The injector is auto and was operated at 260 °C. The solvent delay is -2.50 min, and the transfer temperature is 200°C. Scan 40 to 600DA. Colum 30.00 to 250µ. The software present in the GCMS compared the retention indices of the instruments with Willey and NIST libraries attached to the GC-MS instrument, and the identification of bioactive phytocompounds was done.

3. Results and Discussion

The aqueous solvent maceration of *Coldenia procumbens* leaf powder showed a low concentration of phenols, flavonoids, saponins, and tannins. However, it did not include any alkaloids, terpenoids, coumarins, anthocyanins, or anthraquinones. The ethanol maceration of the leaf powder has a significant quantity of flavonoids, saponins, alkaloids, and terpenoids. It has a moderate concentration of tannins and a low amount of coumarins and anthraquinone. The maceration process using acetone solvent revealed a considerable presence of phenols and tannins, as well as a low concentration of flavonoids, saponins, alkaloids, and terpenoids. However, no coumarins, anthocyanins, or anthraquinones were detected. The leaf powder, when soaked in ethyl acetate, contains a significant quantity of phenols, a moderate amount of phenols, flavonoids, saponins, tannins, alkaloids, and terpenoids, a low amount of anthocyanin and anthraquinone, and none of coumarins. The petroleum ether maceration of leaf powder showed a low presence of phenols and alkaloids, with no detection of any additional chemicals. The hexane maceration of the leaf powder exhibited minimal phenolic content and no presence of additional chemicals.The qualitative phytochemical examination of *Coldenia procumbens* leaf extracts verifies the existence of several phytochemicals including phenols, flavonoids, saponins, tannins, alkaloids, terpenoids, coumarins, anthocyanins, and anthroquinone in specific quantities.Their quantities were roughly verified based on the colour intensity. The chromatic intensity of all phytocompounds varied across various solvents. Phenolic compounds are widely found in all plants and have various biological effects, such as antioxidant and free radical scavenging abilities. They also have anti-inflammatory and anti-carcinogenic properties. As a result, these compounds may contribute to the prevention of several chronic diseases, including cardiovascular disease, cancer, diabetes, bacterial infections, and parasitic infections [19]. Flavonoids have the ability to hinder the beginning, advancement, and advancement of tumours [20]. Consuming flavonoids can decrease the occurrence of coronary heart disease [21]. The flavonoids present in these five plants exhibit inhibitory effects on xanthine oxidase, peroxidase, and nitric oxide synthase enzymes. This activity allows them to scavenge free radicals and reduce oxidative damage to macromolecules [22]. Additionally, these flavonoids have the potential to prevent platelet aggregation, combat microorganisms, hepatotoxins, viruses, tumours, ulcers, inflammation, and allergies [23]. Saponin, found in all plant species, possesses lipid-lowering properties and exhibits anthelminthic, antibacterial, cytotoxic, and expectorant effects by stimulating the reflex of the upper digestive tract [23]. It is also utilised in the treatment of yeast and fungal infections [24]. Tannins are employed as astringents, antioxidants, and free radical scavengers. They also aid in wound healing and the treatment of peptic ulcers [25]. Terpenoids, on the other hand, are utilised for their cardio-protective and antioxidant properties [26]. Steroids are commonly employed as biological signalling agents and have the effect of reducing membrane fluidity [27]. The coumarins are employed for their antimalarial and antiplasmodial activities [28] . Anthocyanin possesses anti-obesity, anti-inflammatory, anticancer, and neuroprotective effects [29]. Anthraquinones found in plants regulate immunity and have therapeutic effects in autoimmune diabetes [30].The quantitative analysis of phytocompounds in the leaf of *Coldenia procumbens* showed phenols,

tannins, alkaloids and flavonoids levels were .84±0.07mgGAEequiv/g dry extract,0.72±0.06mgTAEequiv/g dry extract, 0.43±0.02 ATEmgequiv/g dry extract and 0.73±0.058 mgCHEequiv/g dry extract respectively.The phytochemical analysis of ethanol extract of leaf of *Coldenia procumbens* by FTIR showed the medium spectral peaks at 2925.48 (C-H stretch), 2853.17 (C-H stretch), 1460.81 (C-H bends), 1380.78 (C-H bending) indicate the presence of alkanes. The same medium and strong peaks at 1629.55 and 717 with C=C bending revealed the presence of alkenes. The characteristics strong peak at 1709.59 with C=O stretching specified the presence of carboxylic acids. Prominent strong broad peaks at 3412.42 with O-H stretch H bonded demonstrate the presence of alcohols and phenols. The unique strong peaks at 1087.66 with C-O stretch revealed the existence of secondary alcohol.The aforementioned results confirmed through the FTIR analysis in the *Coldenia procumbens* leaf extracts revealed the existence of bioactive compounds such as alkanes, alkenes, carboxylic acid, phenols, secondary alcohol, and alcohol. The bioactive phyto-compounds presents in the ethanolic leaf extract of *Coldenia procumbens* anlysed by *GCMS* showed 7 peaks at retention time 17.970, 18.015, 19.136, 19.576, 19.626, 19.716, 27.064 and 19.841 indicate the presence of 7 bio-active phytocompounds which were recorded as 6-Octadecenoic Acid, (Z)-(27 %), Ethyl Oleate (22 %), 9-Borabicyclo[3.3.1]Nonane, 9-Decyl-(18 %) and N-Propionyl-D Glucosamine (18 %), N-Hexadecanoic Acid (9%), Heptanoic Acid, 2,6-Dimethyl-, Methyl Ester (4 %), Trans-4-T-Pentylcyclohexanol (1 %) and 2,5,8-Triphenyl Benzotristriazole (1%).These findings suggest that *Coldenia procumbens* possesses potent medicinal properties that can effectively treat various diseases and disorders in humans. Therefore, *Coldenia procumbens* Linn. is utilised for many therapeutic applications. The topical use of a concoction made from the freshly harvested leaves of *C. procumbens Linn.* is used to treat inflammation caused by rheumatism [31]. A desiccated botanical specimen utilised as part of a medicinal blend to induce the formation of pus in boils [32]. *Coldenia procumbens* has various medicinal uses, including the treatment of fever, piles, leucorrhoea, and menorrhagia [33.] Additionally, it has been traditionally used by the Yanadi tribes in South India as an antidote for snake poison [34]. Research has shown that the aqueous and ethanolic extract of *Coldenia procumbens* leaves exhibit antibacterial activity against *Staphylococcus aureus, Streptococcus pyrogenus, Salmonella typhi, Escherichia coli,* and the fungus *Candida albicans* [35].

Table.1 Qualitative determination of phenols, flavanoids, saponins, tannins, alkaloids, terpenoids, coumarins, anthocyanin, and anthroquinone from different solvent extraction of leaf of *Coldenia procumbens*

Solvent / **phytocompounds**	Water	Ethanol	Methanol	Acetone	Ethyl acetate	Petroleum ether	Hexane
Phenols	+	+++	+++	++	+++	+	+
Flavonoids	+	+++	+++	+	++	-	-
Saponins	+	++	+++	+	++	-	-
Tannins	+	+++	++	++	++	-	-
Alkaloids	-	++	+++	+	++	+	-
Terpenoids	-	++	+++	+	++	-	-
Coumarins	-	-	+	-	-	-	-
Anthocyanin	-	-	-	-	+	-	-
Anthraquinone	-	-	-	-	+	-	-

+++= high amount ++= Moderate amount += low amount - = Nil amount

Figure: 1 FTIR spectrum for methanolic leaf extract of *Coldenia procumbens*

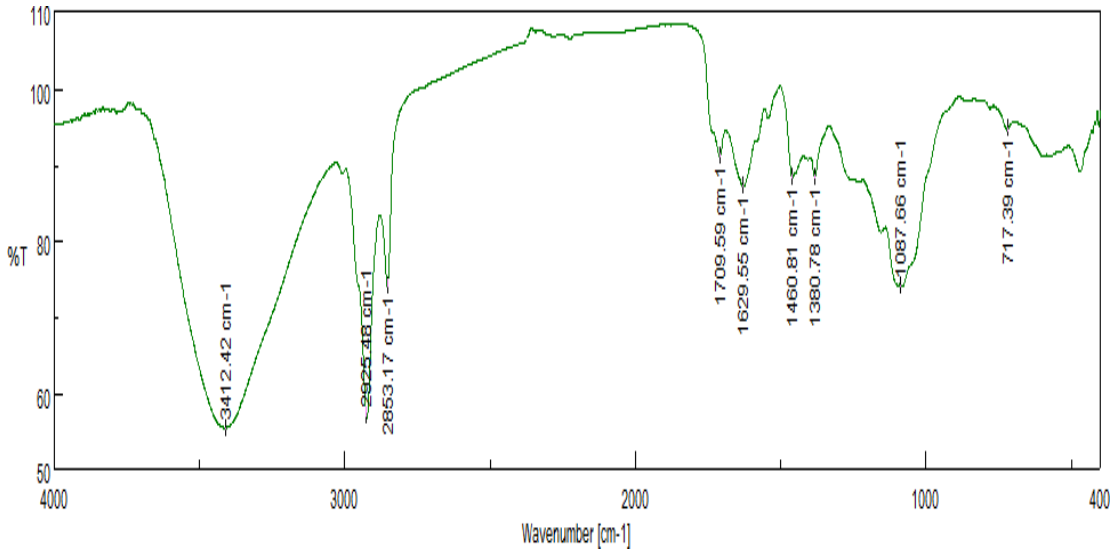


Table: 2 FTIR spectral peak values, reference ranges, intensity, functional group assigned and phytocompounds identified for ethanolic leaf extract of *Coldenia procumbens*

S.No	Wave number cm^{-1} (reference article)	Wave number (cm^{-1}) (Test Sample)	Functional group assignment	Intensity	Phytocompound identified
1.	3500–3200	3412.42	O–H stretch, H–bonded	strong, broad	alcohols, phenols
2.	3000–2850	2925.48	C–H stretch	Medium	alkanes
3.	3000–2850	2853.17	C–H stretch	Medium	alkanes
4.	1720-1706	1709.59	C=O stretching	Strong	Carboxylic acid
5.	1662-1626	1629.55	C=C stretching	Medium	Alkene
6.	1470–1450	1460.81	C–H bend	Medium	Alkanes
7.	1385-1380	1380.78	C-H bending	Medium	Alkane
8.	1124-1087	1087.66	C-O stretching	Strong	Secondary alcohol
9.	730-665	717.39	C=C bending	Strong	Alkene

Table: 3 Bioactive phyto-compounds identified for the ethanolic leaf extract of *Coldenia procumbens*

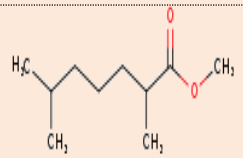
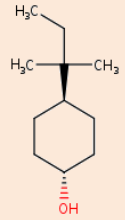
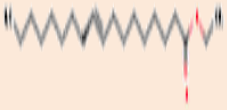
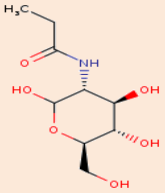
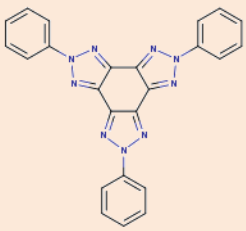
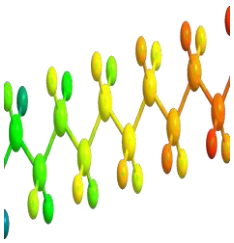
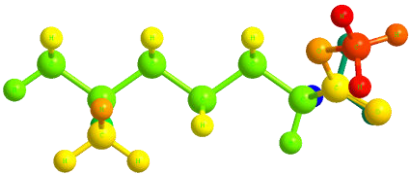
S.NO	R/T	NAME OF THE COMPOUND	MOLECULAR FORMULA	M.W	STRUCTURE
1.	17.970	N-HEXADECANOIC ACID	C16H32O2	256	
2.	18.015	HEPTANOIC ACID, 2,6-DIMETHYL-, METHYL ESTER	C10H20O2	172	
3.	19.136	TRANS-4-T-PENTYLCYCLOHEXANOL	C11H22O	170	
4.	19.576	9-BORABICYCLO[3.3.1]NONANE , 9-DECYL-	C18H35B	262	
5.	19.626	ETHYL OLEATE	C20H38O2	310	
6.	19.716	6-OCTADECENOIC ACID, (Z)-	C18H34O2	282	
7.	19.841	N-PROPIONYL-D-GLUCOSAMINE	C9H17NO6	235	
8.	27.064	2,5,8-TRIPHENYL BENZOTRISTRIAZOLE	C24H15N9	429	

Figure:2. 3D structure of bioactive phyto-compounds identified from the ethanolic leaf extract of *Coldenia procumbens*

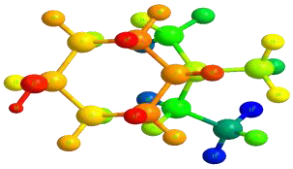
N-Hexadecanoic Acid



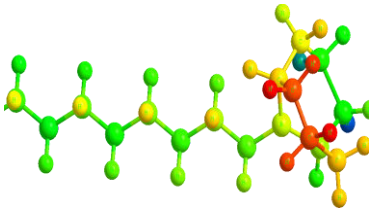
Heptanoic Acid, 2,6-Dimethyl-,Methyl Ester



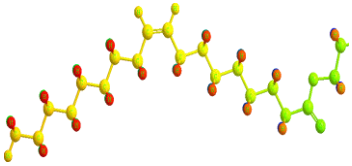
Trans-4-T-Pentylcyclohexanol



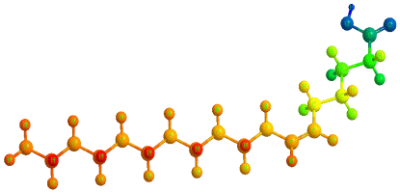
9-Borabicyclo[3.3.1]Nonane, 9-Decyl-



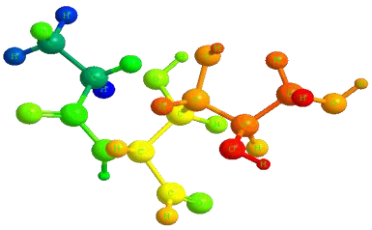
Ethyl Oleate



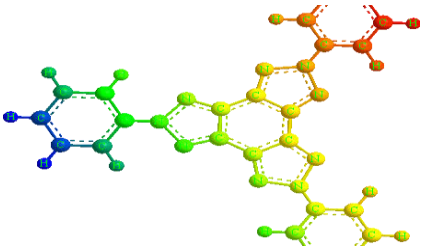
6-Octadecenoic Acid



N-Propionyl-D-Glucosamine



2,5,8-Triphenyl Benzotristriazole



4. Conclusion

The plant *Coldenia procumbens* is commonly utilized in the Siddha and Ayurvedic traditional medicine for its therapeutic properties. However, there is a scarcity of scientific research on the separation, identification, and recording of the plant's phytochemical constituents. Therefore, further scientific investigation is required on this species.

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